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**Marine invasion by non-indigenous benthic foraminifera
in the Sicily Channel (Central Mediterranean Sea):
distribution, dynamics and impact**

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INDEX

ABSTRACT

1. INTRODUCTION.....	1
1.1 Topic of the research.....	1
<i>1.1.1 Non-indigenous species in the Mediterranean Sea.....</i>	<i>1</i>
<i>1.1.2 Amphistegina lobifera and some cryptogenic species of benthic foraminifera...3</i>	<i>3</i>
1.2 Aims of the PhD project.....	7
1.3 Novelty of the research.....	9
1.4 Thesis outline.....	10
2. MATERIALS AND METHODS.....	11
2.1 Study area.....	11
2.2 Sample collection.....	14
<i>2.2.1 Bottom samples: sediments and algae.....</i>	<i>14</i>
<i>2.2.2 Sediment cores.....</i>	<i>16</i>
<i>2.2.3 Rocky samples from outcrops.....</i>	<i>18</i>
2.3 Sample preparation.....	21
<i>2.3.1 Washed residues of sediment and rocky samples.....</i>	<i>21</i>
<i>2.3.2 Algae.....</i>	<i>22</i>
<i>2.3.3 Thin sections.....</i>	<i>22</i>
2.4 Analysis of the foraminiferal content.....	23
<i>2.4.1 Counting of modern foraminiferal assemblages from sea bottom sediment and algal samples.....</i>	<i>23</i>
<i>2.4.2 Counting of fossil foraminiferal assemblages from Miocene rocky samples...24</i>	<i>24</i>
<i>2.4.3 Counting of reworked fossil foraminifera within sea bottom sediment samples.....</i>	<i>25</i>
2.5 Other laboratory preparations and analyses.....	25
<i>2.5.1 Grain-size analysis.....</i>	<i>25</i>
<i>2.5.2 Radiometric dating.....</i>	<i>26</i>
2.6 Statistical analyses.....	28

3. SPREADING OF NON-INDIGENOUS AND CRYPTOGENIC FORAMINIFERA IN THE CENTRAL MEDITERRANEAN SEA.....	30
3.1 Introduction.....	30
3.2 Materials and methods.....	31
3.2.1 <i>Study areas and sampling strategy.....</i>	31
3.2.2 <i>Sampling processing and foraminiferal analyses.....</i>	32
3.2.3 <i>Species distribution models (SDMs).....</i>	33
3.3 Results.....	34
3.3.1 <i>Maltese Islands.....</i>	36
3.3.2 <i>Southern Sicily.....</i>	37
3.3.3 <i>Pantelleria and Favignana islands and northern Sicily.....</i>	39
3.3.4 <i>New distribution models.....</i>	40
3.4 Discussion.....	43
3.4.1 <i>Spatial and temporal patterns and possible spreading vectors.....</i>	43
3.4.2 <i>Present and future ranges: implications from SDM for Amphistegina in the Mediterranean Sea.....</i>	46
3.5 Final remarks.....	47
4. INVASION HISTORY OF AMPHISTEGINA SPP. IN MALTA ASSESSED FROM SEDIMENT CORES.....	48
4.1 Introduction.....	48
4.2 Materials and methods.....	49
4.2.1 <i>Study area and sample collection.....</i>	49
4.2.2 <i>Grain-size, radiometric and micropaleontological analyses.....</i>	50
4.2.3 <i>Sea Surface Temperature (SST) dataset.....</i>	51
4.3 Results.....	52
4.3.1 <i>Granulometric curves and calcareous nannoplankton content down core.....</i>	52
4.3.2 <i>Radiometric dating.....</i>	52
4.3.3 <i>Amphistegina spp. content down core.....</i>	54
4.3.4 <i>Sequential stages of invasion and comparison with SST trends.....</i>	55
4.4 Discussion.....	59
4.4.1 <i>The response of Amphistegina spp. to warming trends.....</i>	59

4.4.2 The micropaleontological approach as a reliable procedure in studies of invasion dynamics.....	62
4.5 Final remarks.....	63
5. OCCURRENCE OF <i>A. LOBIFERA</i> IN A TSUNAMITE: A CASE OF “FAILED INVASION”.....	64
5.1 Introduction.....	64
5.1.1 The problem of the ‘early failed invasions’.....	64
5.1.2 The 1908 Messina tsunami and the effects in the Maltese Islands.....	65
5.2 Materials and methods.....	66
5.2.1 Sampling site and core collection.....	66
5.2.2 Sedimentological, radiometric and micropaleontological analyses.....	67
5.3 Results.....	68
5.3.1 Sedimentological signatures.....	68
5.3.2 ²¹⁰ Pb and ¹³⁷ Cs chronologies.....	69
5.3.3 Foraminiferal content down core.....	69
5.3.4 Calcareous nannoplankton content down core.....	74
5.3.5 Cumulative evidences of a sudden high energy depositional event.....	75
5.4 Discussion.....	76
5.4.1 The historical tsunami generated by the 1908 Messina earthquake.....	76
5.4.2 Triggering mechanisms for the early failed invasion of <i>Amphistegina</i> spp.....	78
5.5 Final remarks.....	78
6. THE POTENTIAL IMPACT OF <i>A. LOBIFERA</i> ON NATIVE FORAMINIFERAL ASSEMBLAGES OF THE SICILY CHANNEL.....	81
6.1 Introduction.....	81
6.2 Materials and methods.....	83
6.2.1 Preparatory analyses and experimental design.....	83
6.2.2 Sampling processing and foraminiferal analysis.....	84
6.2.3 Statistical analyses.....	84
6.3 Results.....	85
6.3.1 The native assemblages of the Sicily Channel.....	85
6.3.2 Comparison between depths in the Maltese Islands.....	86

6.3.3 Comparison across the three stages of invasion.....	92
6.4 Discussion.....	99
6.4.1 The potential impact of <i>A. lobifera</i> colonization in the Maltese Islands and Sicily.....	99
6.5 Final remarks.....	101
7. SUMMARY OF MAIN RESULTS AND FUTURE RESEARCH DIRECTIONS.....	103
7.1 Distribution of benthic foraminiferal NIS in the Sicily Channel.....	103
7.1.1 Present patterns of fora-NIS in the Central Mediterranean.....	103
7.1.2 Range expansion of <i>Amphistegina</i> in the Mediterranean Sea: present and future.....	104
7.2 Invasion dynamics of <i>Amphistegina</i> spp. in Malta Island.....	105
7.2.1 The 70 year-long invasion history of <i>Amphistegina</i> under climate change...	105
7.2.2 The unexpected 'early failed invasion' of <i>A. lobifera</i>	106
7.3 Impact of <i>A. lobifera</i> colonization on native assemblages of benthic foraminifera.....	108
7.4 Open questions.....	109
8. THE STATE-OF-THE-ART BEFORE AND AFTER THE PHD PROJECT.....	111
9. REFERENCES.....	114
ACKNOWLEDGMENTS.....	134
ELECTRONIC APPENDIX (https://drive.google.com/drive/folders/16x64Vjl9BfarlvIUZk-VRUbNBVoKawRd?usp=sharing)	
Appendix 1: Details of the sampling sites	
Appendix 2: Foraminiferal species list and range charts	
Appendix 3: Sea Surface Temperature dataset	
Appendix 4: ²¹⁰ Pb activity along sediment cores	

ABSTRACT

Amphistegina lobifera, a large symbiont-bearing taxon of benthic foraminifera native to the Red Sea, entered the Eastern Mediterranean through the Suez Canal, an artificial waterway opened in 1869. This thermophilic non-indigenous species (NIS) has already successfully colonized the Levantine basin, where it is known to have altered the benthic foraminiferal community structure and to have modified native habitats. More recently, *A. lobifera* has been reported from the Maltese and Pelagian Islands within the Sicily Channel and in the southern Adriatic Sea (Central Mediterranean), where, however, very little is known about the current distribution and establishment status of this alien species and of other benthic foraminifera of cryptogenic origin, as well as its possible impact on native benthic communities.

This PhD project aims to fill part of these gaps on knowledge, focusing on the marine invasion by the non-indigenous *A. lobifera* and the congeneric cryptogenic *A. lessonii* in the Sicily Channel (Central Mediterranean), which represents a natural corridor connecting the eastern to the western basin. In particular, my PhD project addresses three specific research questions corresponding to three main aims: Aim 1) evaluating the current distribution and establishment status of *A. lobifera* and other cryptogenic benthic foraminifera in the Sicily Channel; Aim 2) reconstructing the invasion dynamics of *A. lobifera* and *A. lessonii* in the Maltese Islands; Aim 3) assessing the potential impact of the highly invasive *A. lobifera* on native foraminiferal assemblages from the Maltese Islands, Southern Sicily and Southeastern Sicily. This PhD project is a pioneer study that applies an unprecedented multidisciplinary approach, which combines for the first time methods commonly used in Marine Ecology with those used in Micropaleontology for the investigation of marine bioinvasions.

In order to address Aim 1, the current distribution, range expansion and establishment status of the non-indigenous *A. lobifera*, the congeneric cryptogenic *A. lessonii* and other cryptogenic foraminifera in the Sicily Channel were investigated. To this purpose, in 2017 and 2018, a total of 98 sediment and 51 algal samples were collected at different depths (between 0 to ~ 25 m) from 56 sites located in four different areas of the Sicily Channel (Maltese Islands, Southern Sicily, Pantelleria and Favignana islands) and one area of the Western Mediterranean (Northwestern Sicily). Two or three replicates were collected from each site by using a Van Veen grab or a small corer operated by a diver. After collection, samples were immediately treated with buffered Rose Bengal dye, following the standard procedures suggested by the FOBIMO protocol. Several new records of both amphisteginid species were achieved: *A. lobifera* was documented for the first time in Southern Sicily along the coast between Ragusa and Siracusa and in Pantelleria and Favignana

islands; *A. lessonii* was recorded for the first time in the Maltese archipelago and Pantelleria and Favignana islands. Analysis of the foraminiferal assemblages showed that *A. lobifera* was widely established around Malta and Pantelleria islands, where it occurs with high abundances, whereas in Southern and Southeastern Sicily, it displayed lower abundances. The present finding of *A. lobifera* in Favignana Island, although represented by only a few individuals, at present represents the westernmost limit of its distribution range in the Mediterranean basin and documents that this NIS has reached the oceanographic boundary between the Central and Western Mediterranean. The collected results also showed that amphisteginids have been rapidly spreading northwestward within the Sicily Channel towards the Tyrrhenian Sea and into the Adriatic Sea and allowed for updating the species distribution model for *A. lobifera*, which is expected to colonize wide areas in the Adriatic Sea, Central and Western Mediterranean in the next 50 and 100 years, favoured by the ongoing sea warming.

In order to address Aim 2, the invasion dynamics of both *A. lobifera* and *A. lessonii* were reconstructed in one selected locality of Malta, also relating the recorded abundance patterns with the temporal trends of sea surface temperature (SST) measured in the area since 1950. To this purpose, a ‘micropaleontological approach’ was applied to study bioinvasions for the first time, through the analysis of the benthic foraminiferal content along two sediment cores chronologically constrained through radiometric dating. Two sediment cores were collected a few meters away from each other using a hand-corer operated by a SCUBA diver in Marsamxett Harbour (Malta): the first one (CORE18) was sampled at 16 m depth in May 2018, and the second one (CORE19) was sampled at 17 m depth on September 2019. The sampling site in the natural bay of Marsamxett was accurately selected within the entire archipelago in order to satisfy all the stringent conditions of sediment grain-size, shelter, depth and absence of human activities, which were mandatory for the reliability of this study. Both sediment cores were chronologically constrained by measuring activities of ^{210}Pb and ^{137}Cs isotopes along the sedimentary record. Results showed that the current invasion of both *A. lobifera* and *A. lessonii* in Malta started at the beginning of the 1940s and that both species remained undetected for about 60 years. Data also evidenced a clear positive correlation between the abundance increase of *Amphistegina* spp. and the SST increase recorded in the Central Mediterranean, corroborating the hypothesis that the current global warming has been driving the colonization success of tropical species in the Mediterranean basin. Moreover, this study allowed to document an early invasion of *Amphistegina* spp. in Malta at the beginning of the twentieth century, which failed due to the tsunami wave generated by the 1908 Messina earthquake. This unexpected finding probably makes *A. lobifera* one of most precocious NIS of Indo-Pacific

origin in the Mediterranean Sea and, consequently, may lead to reconsideration of its colonizing capabilities, which appear to be much higher than estimated so far.

Finally, in order to address Aim 3, the potential impact of the highly invasive *A. lobifera* on native benthic foraminiferal assemblages in the Sicily Channel (Central Mediterranean) was evaluated. To this purpose, a nested sampling design was applied through the comparison of benthic foraminiferal community structure across three areas that, through the results of Aim 1, are known to be at advanced, medium and early stages of invasion, namely the Maltese Islands, Southern Sicily and Southeastern Sicily, respectively. The designed experimental plan included, for each study area, three different sampling sites (9 sites in total) and two different bathymetries (2-5 m and 15-20 m depth), collecting four random replicates from the uppermost part of the sea-bottom using a small corer operated by a SCUBA diver according to the FOBIMO recommendations. At each sampled bathymetry, three replicates were analysed in benthic foraminiferal content (the fourth replicate was taken for grain-size analysis) in order to evaluate differences across areas, sites and depths, using community structure variables (species composition, richness, diversity, evenness) as response variables. Collected data documented a severe loss of biodiversity in the Maltese Islands, where *A. lobifera* was highly abundant. On the contrary, this phenomenon seems to be only at the beginning in Southern and Southeastern Sicily, where *A. lobifera* displayed lower abundances and the community structure was more diversified. These results suggested that both diversity and richness of benthic foraminiferal community from the Maltese Islands were strongly modified by the occurrence of *A. lobifera* and allowed for predicting what could happen in the next future in the whole Sicily Channel, as well as in the rest of the Mediterranean Sea.

In conclusion, this thesis provides relevant advancements in the knowledge of the spatial distribution, temporal dynamics and ecological impacts of a small-sized and poorly known marine NIS, also revealing unexpected facts (such as a precocious invasion stopped by a tsunami wave). Yet, the current findings also open novel research questions that should be addressed in the future, especially regarding the ecology and habitat preference of *A. lobifera* in its invaded range.

CHAPTER 1

INTRODUCTION

1.1 Topic of the research

1.1.1 Non-indigenous species in the Mediterranean Sea

Biological invasions constitute one of the most significant components of global change and are considered one of the major threats to biodiversity and ecosystem functioning, impairing the associated services, with often harmful effects on economy and human health (Butchart et al., 2010; Early et al., 2016). Non-indigenous species (NIS) are species directly or indirectly introduced in a new area outside their native range (present and past) by human activities. In some cases, NIS can become so abundant to negatively affect native communities and to generate significant change of local ecosystems; when this happens, NIS are considered “invasive alien species” (Occhipinti-Ambrogi & Galil, 2004). Particularly, in marine environment, the dispersal of NIS is redefining the biogeography of the oceans and continental seas (Occhipinti-Ambrogi, 2007). The introduction of NIS can be intentional (e.g. aquaculture) or unintentional, when the species are inadvertently transported by humans (e.g. in ballast waters) from a basin to another. One of the most important vectors of NIS introduction in the marine environment is represented by commercial shipping (EC, 2015). In addition, the opening of artificial canals and new gateways (e.g. Panama and Suez Canals, and the eastern European waterways connecting the Ponto-Caspian region to the Baltic Sea), used for maritime trades, has created new connections between basins previously separated, allowing NIS to pass by natural spread or facilitated also by shipping traffic. Furthermore, the globalization of maritime trade in the last decades has exponentially accelerated this phenomenon (Hulme, 2009; Seebens et al., 2013).

The Mediterranean Sea is a semi-enclosed basin highly impacted by humans and strongly affected by NIS introductions, which have been preponderant in the eastern area, namely the Levantine Sea (Galil et al., 2018). The excavation of the Suez Canal, an artificial waterway completed in 1869 by the engineer Ferdinand de Lesseps, has in fact led to an unprecedented biogeographic phenomenon called ‘Lessepsian invasion’: the massive entry of Red Sea and Indo-Pacific marine species into the Mediterranean, causing a dramatic shift of marine communities and making the Mediterranean Sea a hotspot for marine bioinvasion studies (Givan et al., 2018 and references therein). The entry of Lessepsian species has not always occurred at the same rate. When the Suez Canal was opened, it was 163 km long, 59-98 m wide (as total surface) and had a navigational depth of 8 m. Successive works made the canal significantly wider and deeper (~ 30 m depth and 350 m wide) (El-Serehy et

al., 2013). The enlargement of the canal, combined with the construction of a second navigation lane (completed in 2015) and the absence of physiographic barriers, facilitated the entrance of Indo-Pacific species in the Mediterranean Sea (Arndt & Schembri, 2015; Galil et al., 2015). Additionally, along its way the Suez Canal crosses three different lakes: the Timsah Lake, the Great Bitter Lake and the Little Bitter Lake. The last two lakes, in particular, derived the name by their hyperaline waters that acted as natural barrier for marine species coming from the Red Sea, mainly during the early years from the Suez Canal opening. However, the continuous coming and going of commercial ships has contributed to mix the Bitter Lake waters, causing a marked salinity decrease. Finally, the construction of the Aswan dam (completed in 1970) along the Nile has strongly reduced the influx of freshwater and of transported sediments to the Mediterranean entry of the Suez Canal at Port Said; in turn, this has changed the nutrient flux, inducing hyper-oligotrophic conditions that are more suitable for many tropical species, thus removing the last ecological barrier to Lessepsian invaders (Hassan et al., 2003; Hyams-Kaphzan et al., 2008). As a result, in the last decades, the phenomenon has dramatically increased. Furthermore, the current global warming seems to favour the spread of tropical Indo-Pacific NIS into the Mediterranean basin, driving the so-called ‘tropicalization’ of the Mediterranean Sea (Lejeusne et al., 2010; Hiddink et al., 2012; Bianchi et al., 2013; Galil et al., 2018). Recent data show a clear northward spread of Suez immigrants within the Mediterranean, as well as thermophilic native species that were previously confined to the southern sectors of the basin (phenomenon known as ‘meridionalization’; Boero et al., 2008), and tropical Atlantic species that are expanding their range from the western coasts of Africa into the Mediterranean through the Gibraltar Strait. All these species are likely favoured by the ongoing climate change and consequent rising of sea surface temperature (SST), which is driving both ‘tropicalization’ and ‘meridionalization’ of the Mediterranean Sea leading to, on the one hand, an increase in the arrival, establishment and range extension of thermophilic NIS and, on the other hand, to a northward extension and increase in abundance of native thermophilic species (Boero et al., 2008; Lejeusne et al., 2010; Hiddink et al., 2012; Bianchi et al., 2013; Galil et al., 2018).

Despite the increasing efforts of Mediterranean scientists and countries to assess the phenomenon, several gaps of knowledge exist. First, the ‘non-indigenous’ *versus* ‘native’ status of several marine species occurring in the Mediterranean is uncertain. Ecologists routinely categorise indigenous or endemic species as ‘native’, including prehistorical invasions, and taxa resulting from recent human-mediated introductions as ‘non-indigenous’ (Carlton, 1996a). However, due to the historical global transport of plants and animals by humans, combined with a lack of marine biodiversity data, it is impossible to distinguish for many species their actual native origin from their introduced range. Species that cannot be assigned to either native or non-indigenous categories are called

‘cryptogenic species’ (crypt-, Greek, *kryptos*, hidden away; -genic, New Latin, *genesis*, origin) (Carlton, 1996a). Cryptogenic species are remarkably common and, as result, their abundance has important consequences for understanding biological invasions, leading to a serious underestimation of the impact on native communities. Secondly, the number of new introductions is likely to be grossly underestimated and the available data are presumably accurate only for the largest species or those that are more easily detectable and recognizable. Small-sized marine NIS, particularly unicellular taxa, are largely overlooked and unrecognized, and their identity and origin are in doubt and subject to continuous revisions (Galil et al., 2018; Gomez, 2019). As a result, several cryptic, taxonomically challenging or small-sized NIS remained undetected for years, sometimes decades, and their records are scant and affected by uncertainty. Furthermore, targeted surveys are subject to both spatial and temporal limitations. Consequently, the first record of small-sized taxa in a new area generally occurs only when such NIS have already generated well-established and dense populations or have negatively affected native communities, and this prevents correct and efficient management. The lag time between a new introduction and its first record may span from years to decades (Carlton, 2009), and the real first arrival remains unknown in most cases. These data gaps limit our ability to correctly quantify marine invasions and to collect accurate information on the temporal occurrence, distribution and impact of small-sized NIS on native communities.

The present PhD project aims to fill part of these gaps of knowledge, with a specific focus on non-indigenous and cryptogenic species of benthic foraminifera (fora-NIS). Due to their small size (< 2 mm), this unicellular group of protists, living in the water column like plankton or above and within the sea bottom like benthos (e.g. Murray, 2006), is so far poorly studied and often goes overlooked in routine monitoring of marine organisms.

1.1.2 *Amphistegina lobifera* and some cryptogenic species of benthic foraminifera

The most widespread and successful fora-NIS in the Mediterranean is the large algal symbiont-bearing genus *Amphistegina*, represented by the Indo-Pacific species *A. lobifera* Larsen 1976, and its congeneric *A. lessonii* d’Orbigny 1826. While *A. lobifera* is a Lessepsian immigrant, *A. lessonii* is a cryptogenic species. The status of these two species, as well as other foraminiferal species, has long been controversial with consequent confusion and contradictory assessments in the literature, where particularly *A. lobifera* has been reported as either a non-indigenous or cryptogenic species (Meriç et al., 2008; Koukousioura et al., 2010; Zenetos et al., 2012; Meriç et al., 2016; Zenetos et al., 2018 and references therein). The mistake is mostly due to the probable uncorrected dating of Pleistocene records from Turkey that contain specimens of *A. lobifera* (Meriç et al., 2016; 2018). In

particular, according to the authors, modern populations of *A. lobifera* in the Mediterranean Sea may have originated from ancient introductions occurred through some natural waterway connecting the Indo-Pacific to the Eastern Mediterranean during the Pleistocene. However, several evidences suggest otherwise: I) as supported by geological data from the Eastern Mediterranean outcrops, a possible connection between the two basins persisted only until the Gelasian (early Pleistocene; Popov et al., 2006); II) *A. lobifera* has never been found in the Mediterranean Plio-Pleistocene record (Di Bella et al., 2005); and III) rDNA analyses, recently performed on extant specimens collected from the Mediterranean and the Red Seas and from the Indian and Pacific Oceans, have shown genetic dissimilarity between Pacific Ocean and Red Sea populations, but extremely high similarity between Red Sea and Mediterranean populations (Schmidt et al., 2016; Prazeres et al., 2020). These evidences confirm that the population of *A. lobifera* currently spreading in the Mediterranean Sea cannot represent the survival of an independent lineage of Plio-Pleistocene or Tethyan relicts, but is a true Lessepsian invader resulting from a recent introduction from the Red Sea. Following these last updates on the status of *A. lobifera*, it has been included in the list of marine invasive alien species (IAS) of the European Seas, where it is now considered as one of the species with the highest potential impact (Tsiamis et al., 2019; Stulpinaite et al., 2020).

On the contrary, the status of *A. lessonii* in the Mediterranean Sea is still unresolved. This is because during the Pliocene-early Pleistocene time (until 2.1 Ma), *A. lessonii* was the most abundant colonizer of Atlantic origin in the Paleo-Mediterranean. Its carbonate tests were so abundant when accumulated on the sea floor that they contributed to the formation of biogenic rocks known as *Amphistegina*-rich beds, distributed all over the Mediterranean basin (Di Bella et al., 2005 and references therein). The abrupt climate cooling, culminating in the mid-Pleistocene, drove Mediterranean amphisteginids and other warm water taxa to extinction (Roveri & Taviani, 2003; Di Bella et al., 2005). Consequently, the origin of modern *A. lessonii* populations is uncertain: on the one hand, this species could have re-entered the Mediterranean Sea by natural spread from the Strait of Gibraltar, either recently or during Pleistocene warmer interglacial and then survived in ecological oasis until today; on the other hand, it could have passed through the Suez Canal since its opening in 1869 as a Lessepsian colonizer, along with *A. lobifera*. Only future genetic analyses will definitively assess the status of modern Mediterranean *A. lessonii* populations.

In the Indo-Pacific region, *A. lobifera* proliferates at tropical and subtropical latitudes, living on hard and vegetated substrata at shallow depth < 20 m (Langer & Hottinger, 2000; Triantaphyllou et al., 2012 and references therein). Since an excess of sun irradiation may damage the algal symbiont housed within the test, *A. lobifera* usually seeks protection from direct solar radiation under reef

rubble, algal thalli or within reef ravines (Mateu-Vicens et al., 2009 and reference therein). Recent studies from the Maldives in the Indian Ocean, have documented bleaching in *Amphistegina* spp. living on coral reefs when specimens were exposed to high temperatures, especially during times of maximum solar irradiance ($> 30\text{ }^{\circ}\text{C}$) and water transparency (Spezzaferri et al., 2018). In the Mediterranean Sea, only limited studies on the ecology of *A. lobifera* have been conducted.

Recent data on the distribution of *A. lobifera* have shown that this non-indigenous species has already colonized the eastern Mediterranean basin and is now particularly abundant and well-established along the coasts of Israel, Turkey and Greece (Fig. 1.1), where it is known to have modified the native habitats and to have altered the community structure (Hyams et al., 2002; Meriç et al., 2008; Triantaphyllou et al., 2009; Çinar et al., 2011; Langer et al., 2012; Schmidt et al., 2015). Amphisteginids have a calcite test that persists after their death, forming biogenic sands. In the Eastern Mediterranean, their mass deposits have modified the coastal structure, locally covering rocky substrates with a thick layer of carbonate sand, and altering diversity of benthic assemblages, with subsequent major changes in ecosystem structure and functioning (Langer et al., 2012). Nevertheless, while *A. lobifera* is a successful invader, the congeneric *A. lessonii* seems to occur more rarely and often with smaller-sized specimens (Titelboim et al., 2019). As a consequence, less is known about the current spreading process of *A. lessonii* in the Mediterranean Sea, also considering that the source of the current Mediterranean populations is still unknown. Probably favoured by the transport of their planktonic propagules by shallow currents, amphisteginids have been rapidly expanding northwestward in the Mediterranean Sea. More recently, *A. lobifera* has been recorded in the Central Mediterranean, within the Sicily Channel, along the coasts of the Maltese and Pelagian Islands (Yokes et al., 2007; Caruso & Cosentino, 2014) and in the southern Adriatic Sea, in Albania (Langer & Mouanga, 2016), but the real status of invasion in the Central Mediterranean basin is still poorly known (Fig. 1.1). Lack of historical records makes it impossible to recognize the timing of first occurrence and to understand the spreading dynamics of both *A. lobifera* and *A. lessonii* in the Central Mediterranean.



Fig. 1.1: Current biogeographic distribution of *Amphistegina* spp. in the Mediterranean Sea and the northern Red Sea. The orange area indicates the sectors colonized by amphisteginids in both Eastern (Egypt, Gaza Strip, Israel, Syria, Lebanon, Turkey, Greece and Albania) and Central Mediterranean (Libya, Tunisia and Maltese Islands). The winter isotherm of 14 °C, considered a limit threshold for amphisteginids, is also indicated. Modified from Langer et al. (2012) and Langer & Mouanga (2016).

Additionally, the available knowledge is also insufficient to clearly assess the current colonization of the Mediterranean basin by other small-sized forams and cryptogenic foraminifera. Some species are already present in the Levantine basin and, more recently, have also been found in the Sicily Channel (Meriç et al., 2008, 2013; Merkado et al., 2012; Caruso and Cosentino, 2014 and references therein; Schmidt et al., 2015; Ounifi-Ben Amor et al., 2016). They are: *Amphisorus hemprichii* Ehrenberg, 1839, *Coscinospira arietina* Batsch, 1791, *Coscinospira hemprichii* Ehrenberg, 1839, *Cymbaloporella squamosa* d'Orbigny, 1839, *Heterostegina depressa* d'Orbigny, 1826, *Pseudolachlanella slitella* Langer, 1992, *Sorites orbiculus* Forskal, 1775, and *Sorites variabilis* Lacroix, 1941. The rDNA analyses, conducted on specimens of *S. orbiculus* collected from the Gulf of Eilat (northern Red Sea) and from the northern coasts of Israel, have shown that these two populations are genetically identical, suggesting that *S. orbiculus* recorded in the Eastern Mediterranean is a non-indigenous species from the Red Sea (Merkado et al., 2013). However, *S. orbiculus* in the Central Mediterranean is considered cryptogenic because: I) there are no molecular evidences that could definitively address the status of the Central Mediterranean populations; II) the Atlantic Ocean populations are morphologically identical to Red Sea

populations and, consequently, it is very difficult to distinguish the Lessepsian specimens from the Atlantic ones that could have passed through the Strait of Gibraltar and could co-occur in the Central Mediterranean with the populations of Indo-Pacific origin. Clarifying the status of these species is problematic because most of them are rare in foraminiferal assemblages.

1.2 Aims of the PhD project

This PhD project aims at studying the marine invasion by non-indigenous and cryptogenic species of benthic foraminifera in the Sicily Channel (Central Mediterranean), which represents a natural corridor for bioinvasions, with a particular focus on the Lessepsian *Amphistegina lobifera* and the congeneric cryptogenic *A. lessonii*.

My PhD project addresses three specific research questions that correspond to three main aims (Fig.1.2):

- AIM 1) evaluating the current distribution and establishment status of fora-NIS in the Sicily Channel, through the analysis of living and dead foraminiferal assemblages preserved in bottom sediment and algal samples.
- AIM 2) reconstructing the dynamics of invasion of *A. lobifera* and *A. lessonii* in the Maltese Islands, through the analysis of historical records preserved within two sediment cores, temporally constrained by radiometric dating (^{210}Pb and ^{137}Cs chronologies).
- AIM 3) assessing the potential impact of the highly invasive *A. lobifera* on native foraminiferal assemblages, comparing the effects on community structure among three areas at different stages of invasion: Maltese Islands (advanced stage), Southern Sicily (medium stage) and Southeastern Sicily (early stage).

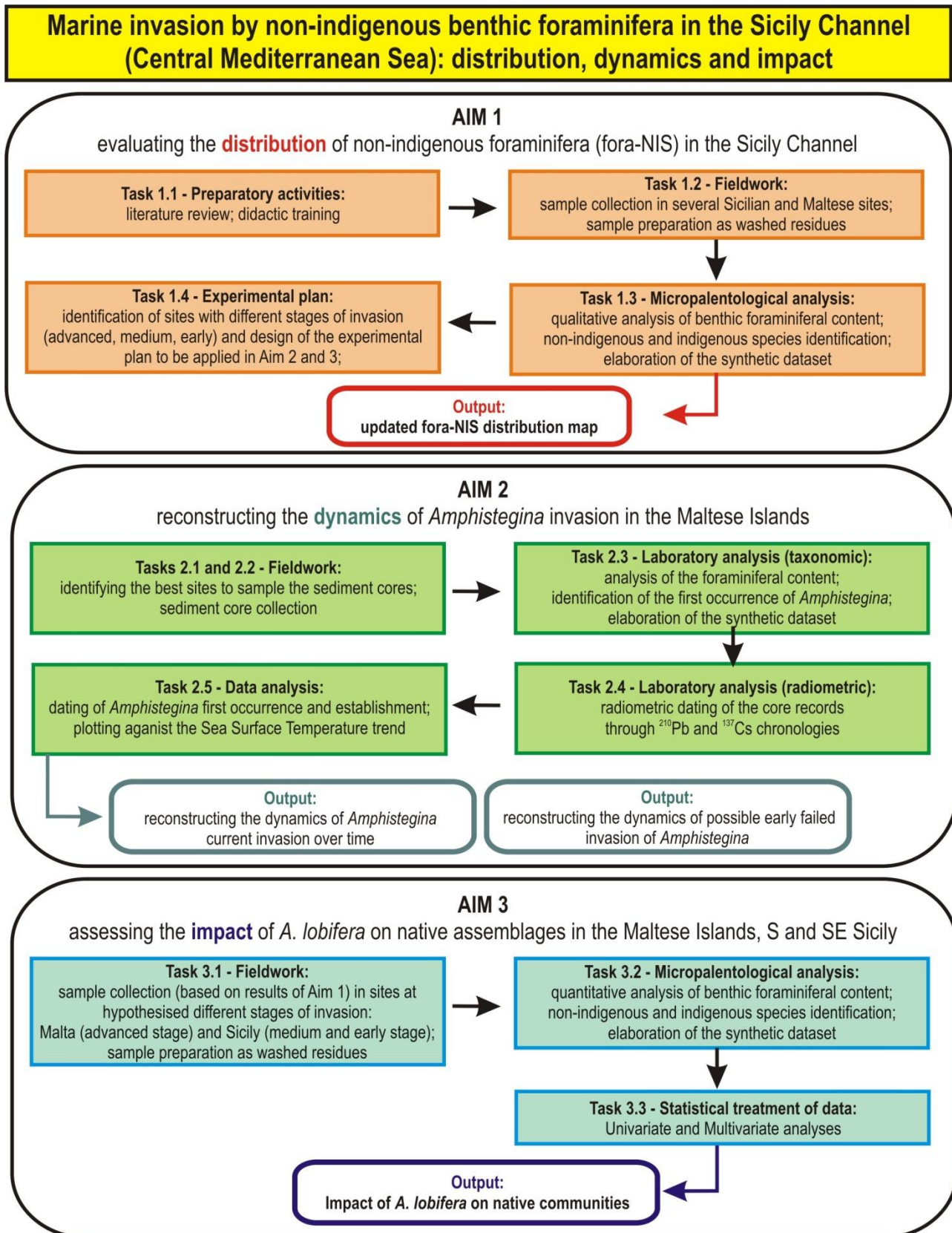


Fig. 1.2: Summary scheme of the three main aims of the PhD project with the relative tasks and final outputs.

1.3 Novelty of the research

The novelty of my PhD research regards the unprecedented multidisciplinary approach to the study of marine bioinvasions: this work in fact combines for the first time methods commonly used in Marine Ecology with those used in Earth Sciences (particularly in Micropaleontology), also using oceanography temporal series for the interpretation of the results.

The ‘ecological approach’ was used following the standard procedures suggested by the FORaminiferal BIO-MONitoring group (FOBIMO; Schönfeld et al., 2012), with the following purposes: I) to study the spatial distribution of fora-NIS in the Central Mediterranean in order to address Aim 1 (see **Chapter 3**), and II) to assess the impact of *A. lobifera* on native assemblages of Maltese Islands, Southern Sicily and Southeastern Sicily in order to address Aim 3 (see **Chapter 6**). In particular, sediment and algal samples were collected at different depths from several localities within the Sicily Channel; samples were stained with a Rose Bengal solution in order to distinguish living from dead foraminifera. The collected data allowed to update the current distribution of fora-NIS within the Sicily Channel and to design an experimental plan that was used to assess the impact of the highly invasive *A. lobifera* on benthic foraminiferal communities.

The ‘micropaleontological approach’ was utilized with the purpose of reconstructing the invasion dynamics of *Amphistegina* spp. in one locality of the Maltese Islands in order to address Aim 2 (see **Chapter 4** and **Chapter 5**). This method is based on the analysis of historical records preserved in two sediment cores, chronologically constrained through radiometric dating, which is commonly utilized for recent sediment cores in order to furnish good estimates of the age of sediments, their accumulation rate and deposition processes. The most common technique to date recent sediments (up to 100-150 years) applies the decay of ^{210}Pb isotope, a natural radio-nuclide (Abril et al., 2014; Kosnik et al., 2015; Incarbona et al., 2016) and ^{137}Cs , an artificial radio-nuclide introduced in atmosphere after the nuclear tests around the world starting from the 1950s. In this PhD project, for the first time, ^{210}Pb and ^{137}Cs chronologies are used to date two sediment cores containing fora-NIS, particularly the Lessepsian species *A. lobifera* and the cryptogenic *A. lessonii*, in order to reconstruct through time their colonization history (for several decades, from their first arrival to the current population status). The advantages of this approach include the possibility to identify early stages of invasion, that commonly go overlooked for a long time, and to directly explore response of the NIS colonization to environmental parameters.

Finally, the population dynamics of the target species have been related to environmental factors, considering both extreme oceanographic events (namely, an earthquake-induced tsunami) and

continuous temporal patterns of Sea Surface Temperature (SST), under the current global warming scenario. The outcome of this novel integration of approaches has led to some unexpected scientific discoveries.

1.4 Thesis outline

After an introduction on the topic of the PhD research described in **Chapter 1**, the subsequent **Chapter 2** provides a description of the study area, including a brief historical framework of the Mediterranean Sea and the oceanographic setting of the Sicily Channel, and a detailed description of the sampling design, along with an overview of the materials and methods employed in the field and the laboratory. **Chapter 3** focuses on the current distribution and relative abundances of the genus *Amphistegina* and other cryptogenic species of benthic foraminifera recorded in five areas of the Sicily Channel, within the Central Mediterranean, as output of Aim 1. **Chapter 4** reconstructs the current invasion dynamics of *A. lobifera* and *A. lessonii* in one locality of the Maltese islands, as first output of Aim 2. **Chapter 5** contains the reconstruction of an early failed invasion recorded within a tsunamite preserved in the cores collected in the same locality mentioned above, as second output of Aim 2. **Chapter 6** explores the possible impact of the highly invasive *A. lobifera* on native foraminiferal assemblages at two different depths in nine localities, respectively, three in the Maltese Islands, three in Southern Sicily and three in Southeastern Sicily, as output of Aim 3. **Chapter 7** summarizes the main results of this thesis and reports the open questions that still need to be addressed. **Chapter 8** evidences the general conclusions with the state-of-the-art before and after the research project. **Chapter 9** is the reference list.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study area

The Mediterranean Sea is one of the world's 'biodiversity hotspots' due to both its turbulent geological history during the Tertiary and the climatic fluctuations of the Quaternary (Bianchi et al., 2012 and references therein), which have allowed species with different biogeographic origins and affinities to colonise the basin. The Mediterranean is a semi-enclosed basin that, at present, extends for about 2,505,000 km² among southern Europe, northern Africa and western Asia, and can be considered as a relict portion of the wider Tethys Ocean that formed since the late Paleozoic (~ 250 millions of years ago – Ma). For a long time, the basin was connected to the Red Sea and Indo-Pacific region as well as to the Atlantic Ocean (Reuter et al., 2009); its marine fauna and flora still partially mirror this past. During Neogene, due to the rotation of the African plate towards Asia, the eastern connection between the Mediterranean basin and the Indo-Pacific Ocean was interrupted (Popov et al., 2006), hence separating the subsequent evolutionary history of their biota (BouDagher-Fadel, 2008; Mateu-Vicens et al., 2009). During the Messinian (about 7-6 Ma), the western connection with the Atlantic Ocean was also temporally interrupted as a consequence of a tectonic uplift of the area adjacent the modern Gibraltar Strait (Kouwenhoven et al., 2003). This prolonged isolation from the Atlantic Ocean caused a massive evaporation that, in turn, led to the desiccation of the basin, known as the 'Messinian Salinity Crisis' (Manzi et al., 2005; Roveri et al., 2014). The increased salinity induced the deposition of a thick evaporitic succession in the whole Mediterranean and caused a massive extinction of most marine organisms, including benthic foraminifera (Kennett et al., 1977; Wright, 1979; Kouwenhoven et al., 2006; Rouchy et al., 2007). The subsequent re-colonization, which abruptly occurred at the beginning of the Pliocene ~ 5.3 Ma, was exclusively by Atlantic species entering the Mediterranean through the Strait of Gibraltar (Langer and Schmidt-Sinns, 2006, Hayward et al., 2009 and references therein).

Climate also strongly influenced the biogeographical origins of Mediterranean organisms. After the opening of the Strait of Gibraltar, the warm Pliocene climatic phase favoured in the Mediterranean Sea the proliferation and spread of warm-water taxa typical of subtropical and tropical contexts of the Atlantic Ocean. This warm phase was followed by a climatic deterioration since ~ 2.6 Ma, which culminated with the intensification and duration of glacial periods in the mid-Pleistocene (Lisiecki & Raymo, 2005; 2007; Bianchi et al., 2012) and the onset of major glaciations in the Alpine region (e.g. Muttoni et al., 2003). This climatic change led to the so-called 'Gelasian crisis' that caused the extinction of most warm-water taxa and stenothermal organisms living in the

Mediterranean and, as a result, much of the pre-existing biota went lost again (e.g. Monegatti & Raffi, 2001; Por, 2009). However, the Pleistocene was characterised by the alternation between glacial and interglacial periods, which caused the penetration (and the following extinction when the climatic conditions reversed) into the Mediterranean Sea of species with boreo-arctic affinities (the so-called ‘Northern guests’) and species with (sub)tropical affinities (the so called ‘Senegalese guests’), respectively (Garilli, 2011 and reference therein; Bianchi et al., 2012). As a result, the present marine Mediterranean fauna was influenced by the dramatic climatic events of the Quaternary, which acted as a sort of ‘biodiversity pump’ (Bianchi & Morri, 2000) and drove several waves of immigration/extinction of both temperate and tropical taxa, concerning especially the shallow-water biota (Bianchi et al., 2012).

The excavation of the Suez Canal in 1869 artificially recreated a connection between the Mediterranean and the Indo-Pacific area, which had been interrupted for millions of years, allowing tropical NIS coming from the Red Sea to colonize again the Mediterranean basin during the last 150 years. This was the case of our target species *Amphistegina lobifera* and possibly *Amphistegina lessonii*, even if its Lessepsian origin is strongly debated and, at present, this species is considered cryptogenic given its cosmopolitan distribution (Parker & Gischler, 2011; Prazeres et al., 2012).

The Sicily Channel is located in the central Mediterranean Sea, representing the oceanographic boundary between the colder western and warmer and saltier eastern waters, and acting as a natural bio-geographical corridor for Atlantic species spreading eastward, as well as for Lessepsian immigrants, expanding westward (Azzurro et al., 2014; Di Lorenzo et al., 2017 and reference therein; Evans et al., 2020) (Fig. 2.1). The surface circulation of the Sicily Channel is characterized by currents of the Modified Atlantic Water (MAW), which form two wide counterclockwise vortices within the channel: the Adventure Bank Vortex (ABV), located east of Pantelleria Island, and the Ionian Shelf break Vortex (ISV), located off the southernmost tip of Sicily (Fig. 2.1). The MAW derives from the Atlantic Ocean surface waters that enter the Mediterranean through the Strait of Gibraltar. After entering the Western Mediterranean, the MAW flows eastward reaching the northwestern coast of Sicily, where it divides into two branches: the first one flows northward into the Tyrrhenian Sea and the second one continues eastward into the Sicily Channel and the Central Mediterranean (Fig. 2.1A). This last branch reaches the Levantine basin in the Eastern Mediterranean, where it progressively increases its salinity thanks to the strong evaporation coupled to a very reduced fresh-water input, hence becoming denser and eventually sinking to 200-600 m depth as Levantine Intermediate Water (LIW) (Drago et al., 2010). The LIW flows below the MAW in the opposite direction, reaching the Western Mediterranean and entering the Atlantic Ocean

through the Strait of Gibraltar. This circulation scheme likely affects the spreading pattern of planktonic taxa, benthic taxa with planktonic life cycle stages, as well as organisms passively transported by floating algae or debris (Alve & Goldstein, 2003, 2010; Weinmann & Goldstein, 2017; Finger, 2018).

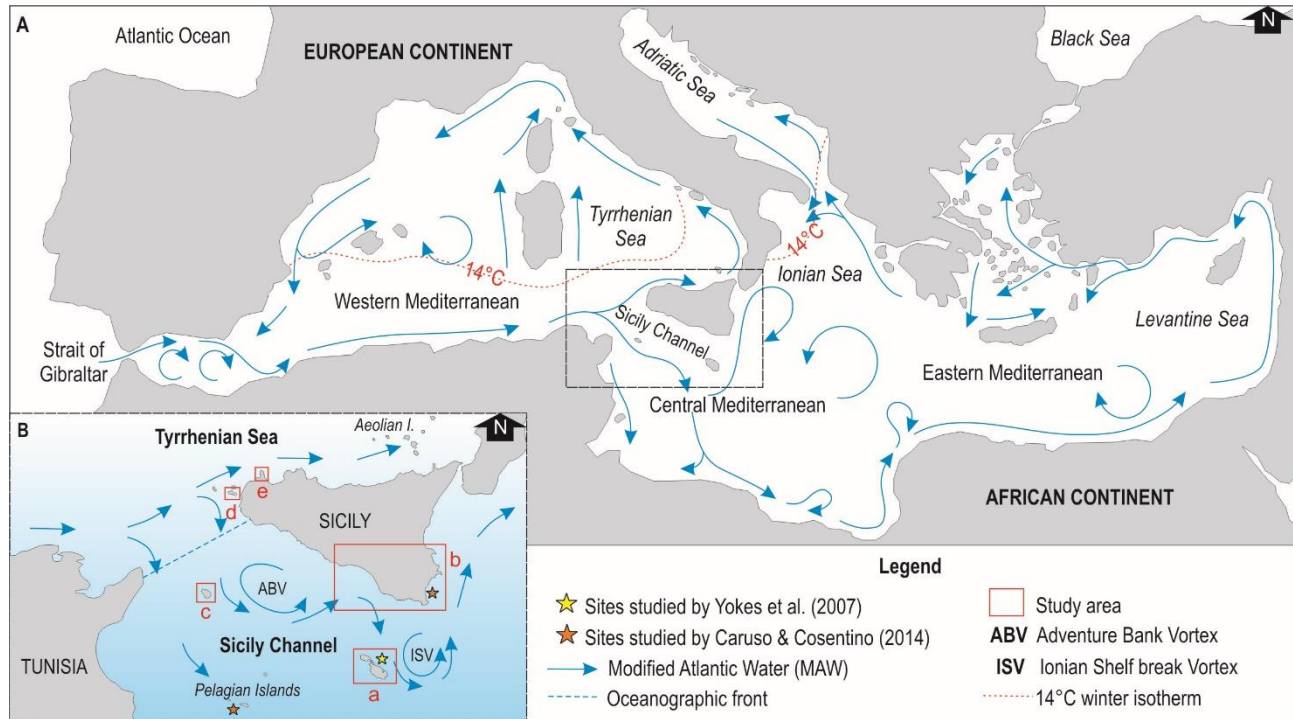


Fig. 2.1: A) Scheme of the surface circulation with the 14 °C winter isotherm in the Mediterranean Sea (redrawn from Pascual et al., 2017); B) The Sicily Channel with the location of the five sampling areas: a) Maltese Islands, b) Southeastern Sicily, c) Pantelleria Island, d) Favignana Island, e) Northwestern Sicily. The sites previously studied by Yokes et al. (2007) and Caruso & Cosentino (2014) are also indicated. Modified from Drago et al. (2010) and from Pasqual et al. (2017).

In the Sicily Channel, the Sea Surface Temperature (SST) follows the main pattern of the surface current flow (NW-SE), with an increasing gradient between the northern and southern portion of the channel. During the summer, the SST varies between 24 °C and 28 °C and during the winter between 15 °C and 18 °C, from the north to the south, respectively (Sorgente et al., 2003). The absence of SST values below 14 °C during the winter season allows the survival of thermophilic NIS in the Sicily Channel (Zmiri et al., 1974; Langer & Hottinger, 2000).

For the present PhD research, five different areas within the Sicily Channel were sampled and analysed (Fig. 2.1B a-e). They are: Maltese Islands, southeastern coast of Sicily, Pantelleria Island, Favignana Island and northwestern coast of Sicily (this last area within the western Mediterranean basin).

2.2 Sample collection

2.2.1 Bottom samples: sediments and algae

In order to investigate the spatial distribution of *Amphistegina* spp. and other forams in the Sicily Channel (Aim 1 in Fig. 1.2), several sediment and algal samples were collected from a total of 56 sites distributed within the five areas reported above (Fig. 2.2). Details on sampling localities, including geographical coordinates, sampling depth, type of substratum, number of replicates and other information are synthesized in the electronic appendix of this Thesis (Appendix 1).

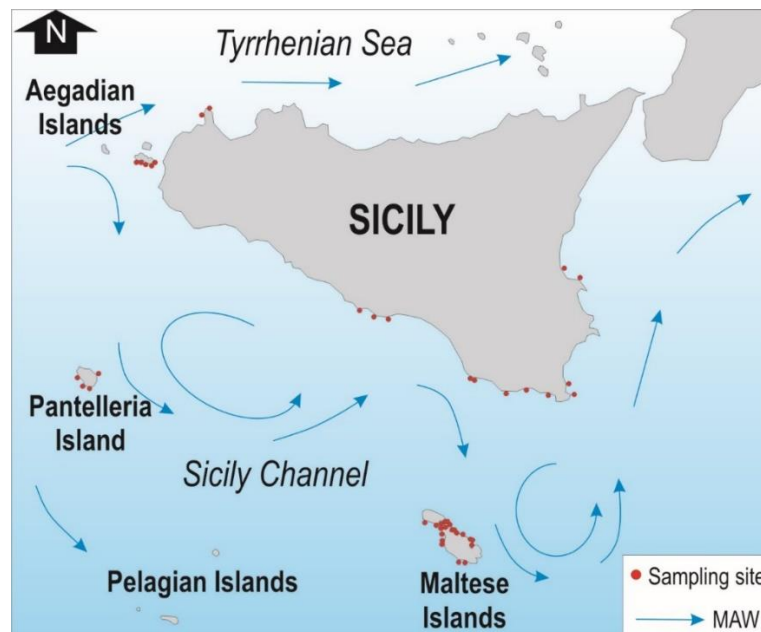


Fig. 2.2: Sampling sites from the five study areas within the Sicily Channel and the Tyrrhenian Sea: Maltese Islands, Southeastern Sicily, Pantelleria Island, Favignana Island and Northwestern Sicily. The surface circulation (MAW) is also reported.

Sample collection was conducted in the framework of various independent surveys during 2017 and 2018 (Appendix 1), some of which had been carried out before the current PhD project and included in process. Therefore, this first step of my work followed an ‘opportunistic’ experimental plan. Moreover, both sediments and algae were collected, because our target taxon is known to live on both types of substratum (Hallock, 1999; Langer & Hottinger, 2000; Hohenegger, 2011). Sediment samples were collected following the standard procedures suggested by the FOBIMO protocol (Schönfeld et al., 2012). A total of 98 sediment samples were picked at different depths, from the shoreline to a depth of ~ 20 m, depending on the local conditions of the substratum. Two or three replicates were sampled from each locality, collecting the upper 2 cm of the sea-bottom using a diver-operated core or a small Van Veen grab (Fig. 2.3A). A total of 51 algal samples were also hand-collected by a scuba diver at depths shallower than 6 m (Appendix 1).

After collection, both sediment and algal samples were stored in polyethylene bottles and treated with buffered Rose Bengal dye (Fig. 2.3B-C), previously prepared by dissolving ~ 2 g of Rose Bengal in 1 L of ethanol, for at least 14 days in order to distinguish living (stained) from dead (unstained) specimens of benthic foraminifera. Due to the peculiar conditions of some sampling surveys, the staining treatment was not applied to the samples collected from Pantelleria Island in summer 2017 and from northwestern Sicily in spring 2018.



Fig. 2.3: A) Collection of sediment samples from the uppermost portion of the sea-bottom by a scuba diver; B) Rose Bengal treatment on algal sample; C) Storage of sediment samples with Rose Bengal dye in polyethylene bottles for at least of 14 days.

After this first step focused on Aim 1, an experimental plan was defined and applied to collect bottom sediment samples and sediment cores, in order to assess the impact of the highly invasive *Amphistegina lobifera* on native benthic foraminiferal assemblages (Aim 3) and to reconstruct the population dynamics of both *A. lobifera* and *A. lessonii* in Malta (Aim 2), respectively. According to the designed experimental plan (Fig. 2.4) based on results derived from Aim 1 (Fig. 1.2), the Sicily Channel was subdivided in three areas at different stages of invasion by the genus *Amphistegina*: Maltese Islands – advanced stage; southern Sicily – medium stage; and southeastern Sicily – early stage. Again, sample collection followed the guidelines of the FOBIMO group (Schönfeld et al., 2012) and was undertaken during two surveys: in May 2018 in the Maltese Islands and in July 2018 in southern and southeastern Sicily. For each area, three different localities were sampled (for a total of nine sampling sites) at two different bathymetries (2-5 m depth and 15-20 m depth), taking four replicates from the uppermost part of the sea-bottom using a small corer operated by a scuba-diver. For each sampling site, three replicates were used for the micropaleontological analysis and one replicate was used for the granulometric analysis (Fig. 2.4). All the collected sediment samples were stored in polyethylene bottles and treated with buffered Rose Bengal dye for at least 14 days. Details of the sampling sites are synthesized in Appendix 1.

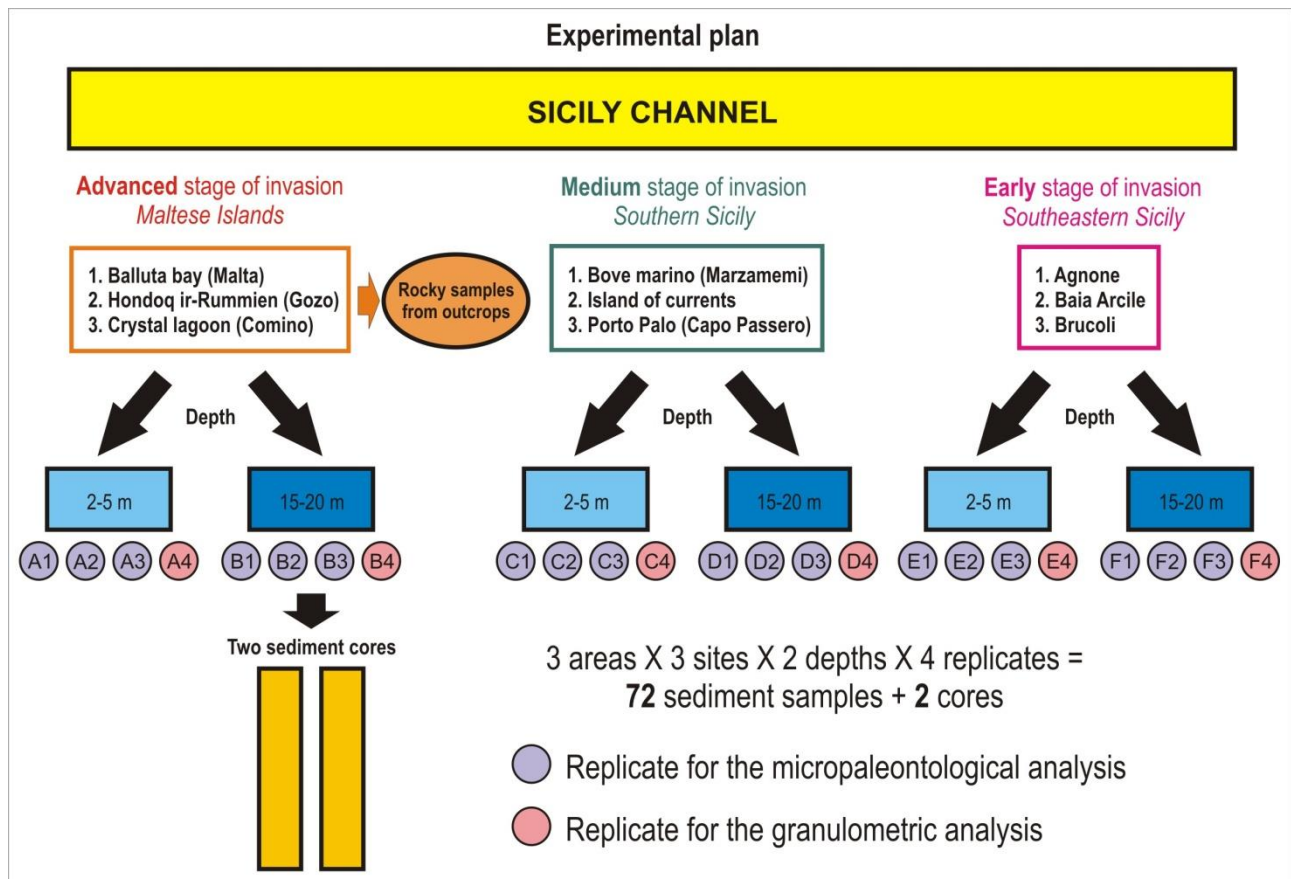


Fig. 2.4: The experimental plan applied to address Aims 2 and 3, as described in Fig. 1.2.

2.2.2 Sediment cores

Two sediment cores were collected in one locality of the Maltese Islands as designed in the experimental plan (Fig. 2.4). Core samples reflect the sedimentation pattern on the sea floor that occurred in the past decades, thus recording the colonization history of non-indigenous species in the area. However, two stringent conditions have to be satisfied: sediments have to be fine-grained and the core record cannot be vertically mixed (i.e. must be undisturbed). In order to satisfy the two conditions and other fundamental requirements, the identification of suitable sites for collecting the sediment cores was carried out complying the following criteria: I) occurrence of a well-established population of *A. lobifera* and subordinately of the congeneric *A. lessonii*; II) absence of reworked fossil amphisteginids derived from the neighbouring outcropping rocks and mixed together with the current foraminiferal assemblages in marine sediments; III) proximity to one of the four sites where *A. lobifera* was recorded for the first time in Maltese Islands by Yokes et al. (2007), in order to have a temporal key-point to reconstruct the population dynamics; IV) dominance of fine-grained sediments on the sea-bottom, necessary to carry out the radiometric dating of sediment cores through ^{210}Pb decay (Tylmann et al., 2016; Andersen, 2017); V) location within an enclosed bay, protected from storm waves and littoral currents that would alter the normal sedimentation process;

VI) absence of dredging or beach nourishment operations in the area; VII) adequate range of bathymetry, with depth > 10 m to avoid sediment mixing by sea waves and < 20 m, since *A. lobifera* is a shallow water taxon with the optimum depth for the development of dense populations at around 10-15 m (Hallock, 1984; Hohenegger, 1995). Along the entire Maltese Islands, just one site satisfied all the stringent conditions required for the cores collection: Marsamxett harbour, located in a natural bay in Malta. Despite very careful searches, no other sites were identified to replicate the analysis elsewhere.

The first sediment core (named CORE18) was collected at 16 m depth on 8th May 2018, while the second one (CORE19) was collected at 17 m depth on 4th September 2019, just a few meters away from the first one. CORE18 was collected as a ‘pilot’ sample in order to evaluate if all the stringent conditions listed above could be satisfied, while CORE19 was collected in order to have a replicate that could confirm the data provided by the first core. Both cores were collected using a hand-corer operated by a scuba diver (Fig. 2.5A). CORE18 is 41 cm long and CORE19 is 50 cm long, both of them mostly made up by fine-grained sediments (fine sands, silts and clays). After collection, both cores were longitudinally sectioned in two halves (Fig. 2.5B), photographed, lithologically described and finally crosscut at each centimetre, thus obtaining 41 sediment samples from CORE18 (Fig. 2.5C) and 50 sediment samples from CORE19 (Fig. 2.5D).



Fig. 2.5: A) Hand-corer used to collect the sediment cores; B) Instrument used for core sectioning at the University of Palermo; C-D) Images of the sectioned CORE18 and CORE19, respectively.

2.2.3 Rocky samples from outcrops

A total of eight rocky samples were collected from outcrops of the Maltese islands, which are entirely covered of limestones with subsidiary marls and clays, late Oligocene (Chattian) to late Miocene (early Messinian) in age (Magri, 2006). Quaternary deposits, mostly Pleistocene in age, are limited to few localities and include cliff breccias, cave and valley loams, sands and gravels. Deposition occurred in the following stratigraphic succession (from base to top): Lower Coralline Limestone Formation, *Globigerina* Limestone Fm., Blue Clay Fm., Greensand Fm. and Upper Coralline Limestone Fm. (Fig. 2.6).

The Lower Coralline Limestone Formation is the oldest unit (Oligocene) and is characterized by massive limestones and mudstones particularly rich in coralline algae, macrofossil remains (mostly corals, molluscs and echinoids) and larger benthic foraminifera (*Lepidocyclina*) indicative of a shallow-water environment of the inner shelf (Magri, 2006). The *Globigerina* Limestone Formation (Aquitania – early Langhian) is the most extensive unit on islands and is mostly composed of cream coloured limestones and grey marls, particularly rich in planktonic foraminifera (genera *Globigerina* and *Globigerinoides*), indicative of pelagic contexts, typical of the outer shelf to upper slope environments with a bathymetry around 150-200 m depth (Magri, 2006; Baldassini & Di Stefano, 2017). The Blue Clay Formation (Langhian – Tortonian) consists of grey marls rich in planktonic foraminifera typical of a pelagic context as the underlying *Globigerina* Limestone Fm. (Magri, 2006). This unit is overlaid by the Greensand Formation (Tortonian), much thinner than the other four formations, that is characterized by friable glauconite-rich sands above a marked erosion surface truncating the Blue Clay Formation. Finally, the Upper Coralline Limestone Formation is the youngest unit (late Tortonian – early Messinian) and is very similar in many aspects to the Lower Coralline Limestone Fm., especially in colour and coralline algal content. The formation is characterized by massive limestone and mudstone rich in invertebrate fossils and larger benthic foraminifera (e.g. *Heterostegina*) indicative of an inner shelf environment (Magri, 2006; Baldassini & Di Stefano, 2017).

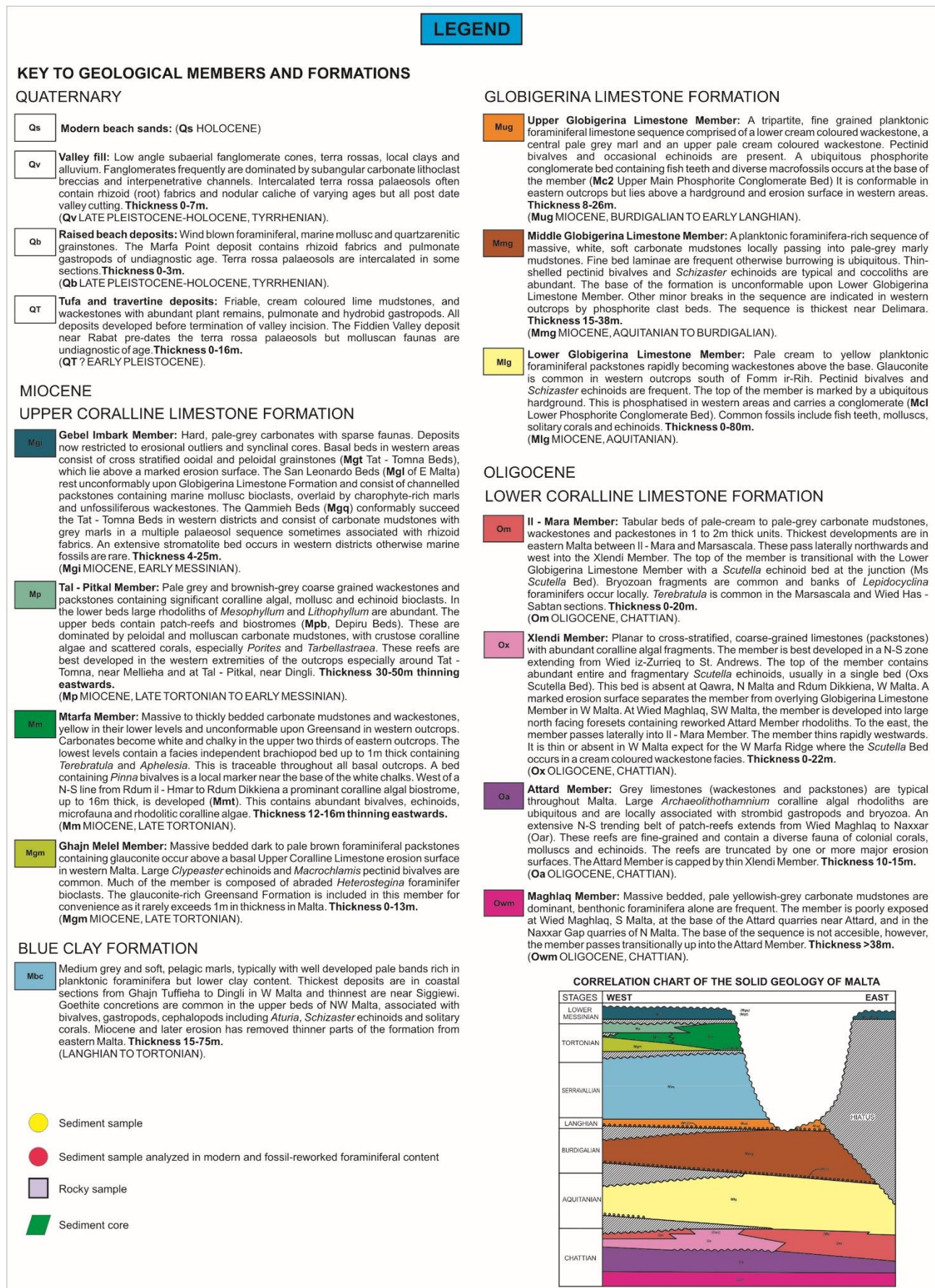


Fig. 2.6: Legend of the geological units cropping out on the Maltese Islands. The image is provided by the source: Continental Shelf Department of Malta (www.continentalshelf.gov.mt; Accessed on September 30, 2018).

For the present study, three rocky samples were collected from the *Globigerina* Limestone Fm. (Fig. 2.7, samples GL-1 – GL-3), three samples from the Upper Coralline Limestone Fm. (Fig. 2.7, samples UC-1 – UC-3) and two samples from the Lower Coralline Limestone Fm. (Fig. 2.8, samples Hon-A and Hon-B). The rocky samples were used to evaluate the fossil foraminiferal content that could be reworked within the sediments covering the sea floor and within the sediment record of the two collected cores.

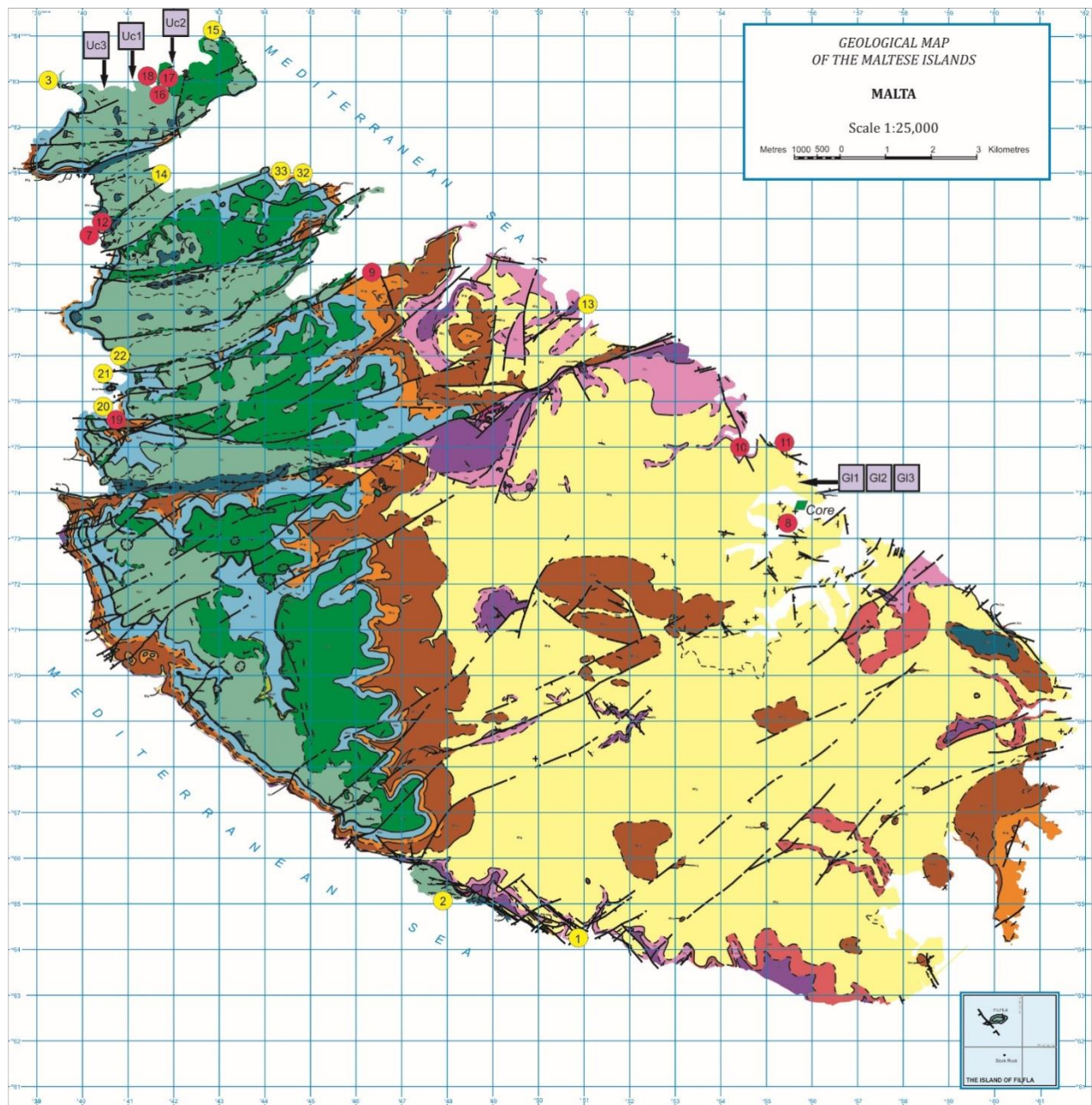


Fig. 2.7: Geological map of Malta Island. White circles indicate the bottom sediment samples collected for Aim 1, red circles indicate the sediment samples used to estimate the reworking degree of fossil foraminifera and violet squares indicate the six rocky samples collected from outcrops in order to evaluate the fossil foraminiferal assemblages. The map is provided by the source: Continental Shelf Department of Malta (www.continentalshelf.gov.mt; Accessed on September 30, 2018).

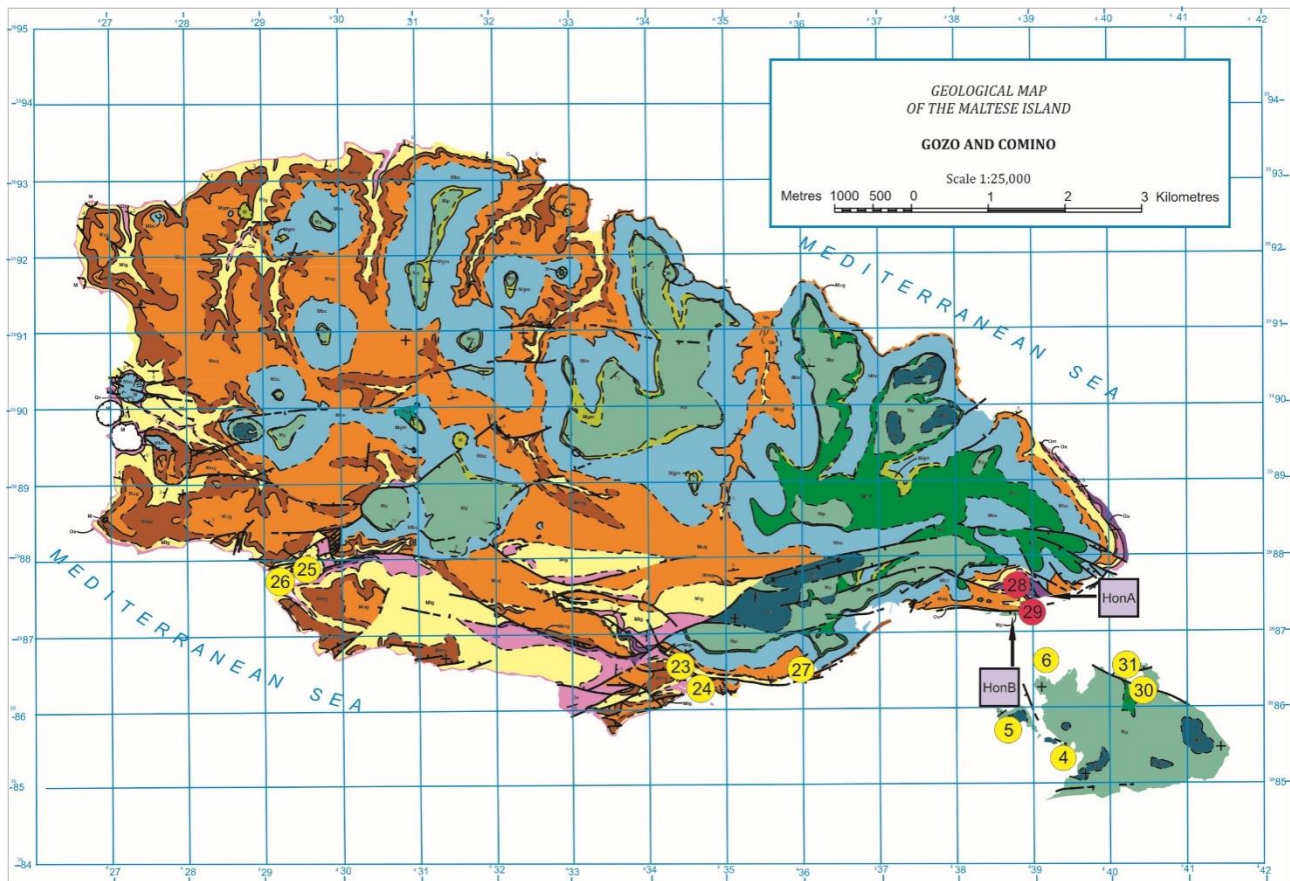


Fig. 2.8: Geological map of Gozo Island and Comino Island. White circles indicate the bottom sediment samples collected for Aim 1, red circles indicate the sediment samples used to estimate the reworking degree of fossil foraminifera and violet squares indicate the two rocky samples collected from outcrops in order to evaluate the fossil foraminiferal assemblages. The map is provided by the source: Continental Shelf Department of Malta (www.continentalshef.gov.mt; Accessed on September 30, 2018).

2.3 Sample preparation

2.3.1 Washed residues of sediment and rocky samples

For foraminiferal analyses, sediment samples were prepared as washed residues following the standard procedures suggested by the FOBIMO protocol (Schönfeld et al., 2012). Samples were oven-dried at 40 °C for one day, weighed, washed over a 63 µm sieve and then oven-dried again. When necessary for the analysis at the stereomicroscope, the obtained washed residues were separated into discrete aliquots using a precision micro-splitter.

Regarding the rocky samples, only the marls collected from the *Globigerina* Limestone Fm. (samples GL-1, GL-2 and GL-3) were prepared as washed residues. Samples were disaggregated in a mortar (Fig. 2.9A), adding two spoons of hydrogen peroxide (130 volumes), then wet sieved using

three overlapped sieves (meshes of 425 μm , 150 μm and 63 μm , respectively; Fig. 2.9B) and finally oven-dried at 40 °C. After preparation, washed residues were stored in plastic bags.

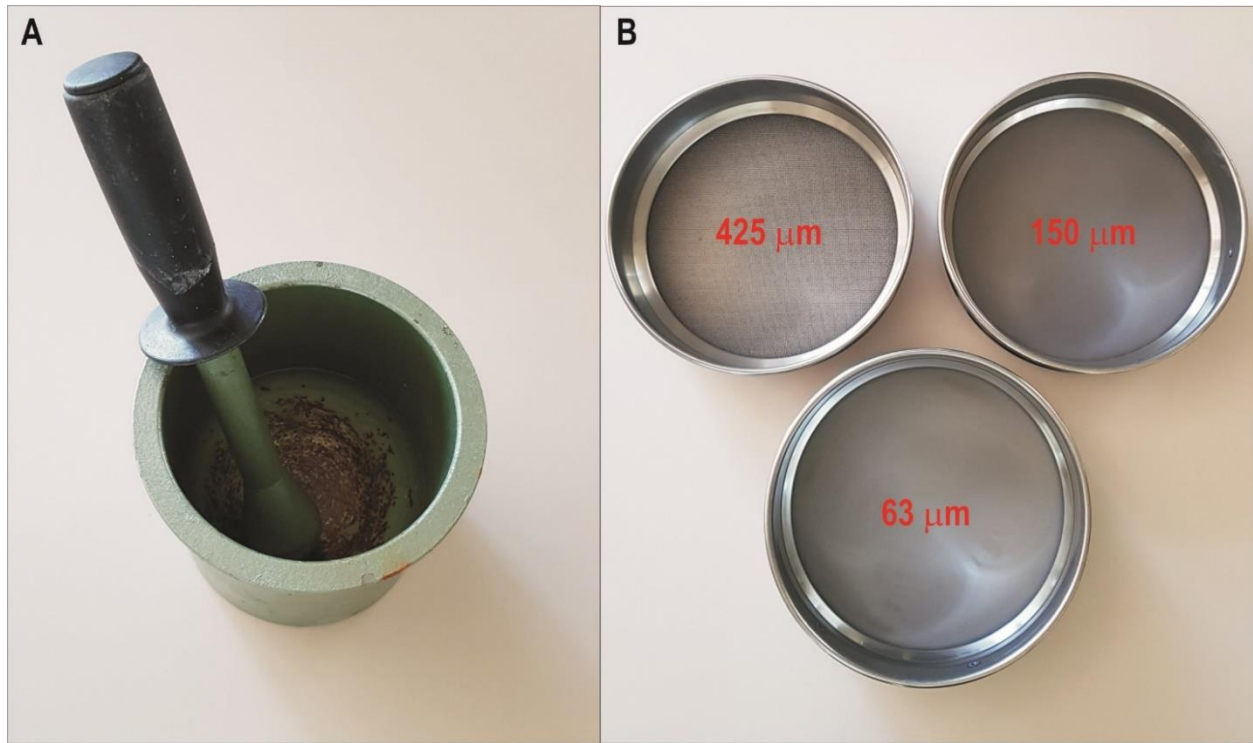


Fig. 2.9: A) Mortar used to disaggregate the rocky samples from the *Globigerina* Limestone Fm.; B) sieves used to prepare the washed residues.

2.3.2 Algae

Algal samples were prepared following Wilson (1998): algae were oven-dried at 40 °C until they crumbled and then passed through a 63 μm mesh size sieve, in order to separate epiphytic foraminiferal tests from algal remains. Because some foraminiferal tests could persist attached to algal remains after the preparation, the latter were also preserved to be further analysed under the stereomicroscope.

2.3.3 Thin sections

Hard rocky samples collected from the Lower and Upper Coralline Limestone Formations (UC-1, UC-2, UC-3, Hon-A and Hon-B) were prepared as thin section, which is a 30 μm thick slice of rock attached to a glass slide with epoxy (Fig. 2.10). This technique involved the following steps: sectioning a rock slab using a blade; pasting of the rock slice on a glass slide and subsequent dressing using an abrasive rotating disk, in order to mechanically grind the rock slice surface and progressively reduce the thickness to $\sim 30 \mu\text{m}$; polishing of the sectioned surface and pasting of a transparent cover slip with epoxy.

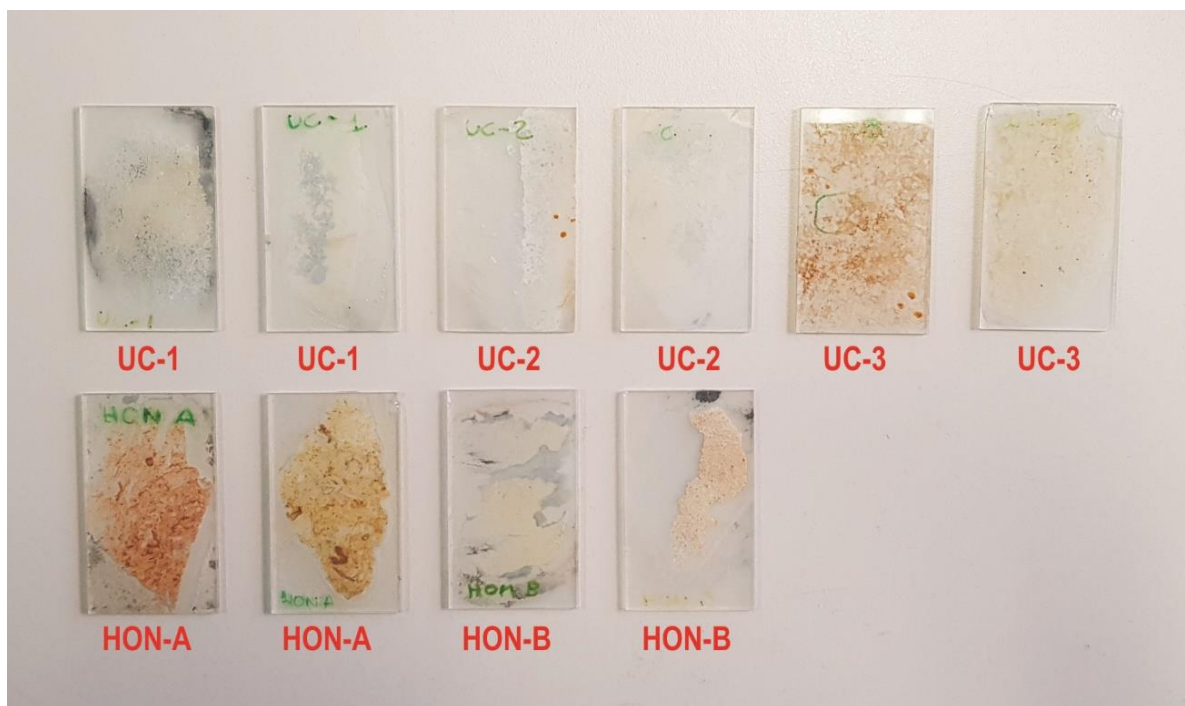


Fig. 2.10: Thin sections of rocky samples collected from the Lower and Upper Coralline Limestone Formations.

2.4 Analysis of the foraminiferal content

2.4.1 Counting of modern foraminiferal assemblages from sea bottom sediment and algal samples

Modern foraminiferal assemblages were analysed under a stereomicroscope (Fig. 2.11) counting about 300 specimens when possible (otherwise, the whole residue was counted) and distinguishing between stained and unstained specimens for the samples treated with Rose Bengal dye as suggested by the FOBIMO protocol (Schönfeld et al., 2012). The largest algal remains were also examined at the stereomicroscope in order to quantify the content of permanently attached foraminifera. All foraminiferal specimens were identified to species level (when possible) following Loeblich & Tappan (1987), Cimerman & Langer (1991), Hottinger et al. (1993) and Milker and Schmiel (2012). Those specimens that were particularly well preserved were also placed on specific slides, photographed using a digital camera and preserved in the micropaleontological collection at the Department of Earth and Environmental Sciences of the University of Pavia. Foraminiferal census counts were used to calculate both relative abundances, as percentage of the total number of counted specimens (%), and absolute abundances, as number of specimens recorded per gram of dry sediment ($N\ g^{-1}$). Quantitative data from all the analysed washed residues are synthesised in Appendix 2.



Fig. 2.11: Stereomicroscope used for the micropaleontological analyses.

2.4.2 Counting of fossil foraminiferal assemblages from Miocene rocky samples

Washed residues obtained from rocky samples belonging to the *Globigerina* Limestone Fm. were analysed under a stereomicroscope following the same procedure as above. The coarser washed fractions (425 μm and 150 μm) were quantitatively analysed counting about 300 specimens of both benthic and planktonic foraminifera, while the smaller fraction (63 μm) was qualitatively analysed estimating the relative abundance of each taxon as class of frequency (very rare: 1-3 specimens; rare: 4-8; frequent: 9-14; very frequent: 15-30; common: 31-50; very common: > 50). Taxonomical attributions of Miocene planktonic species follow Bolli & Saunders (1985), while fossil species of benthic foraminifera were identified by using the Agip Atlas published in 1982.

Thin sections were observed under a polarized light microscope at low magnifications (10X) and classified according to Dunam's classification (1962), which is mainly based on depositional texture of carbonatic rocks. For each thin section, the following steps were performed:

- 1) **analysis of the grain composition**, distinguishing between grains with a carbonatic origin (ooliths, peloids, other carbonatic particles, etc.) and grains with a biogenic origin (remains of invertebrates, algae and larger benthic foraminifera). When possible, the larger benthic foraminifera were taxonomically determined (at least at the genus level) in order to date the sample and to infer information about the depositional environment.
- 2) **analysis of the matrix**, evaluating if it was fine-grained homogeneous or carbonate peloidal and if it contained microfossils (mainly planktonic microorganisms).

- 3) **analysis of the cement**, evaluating its composition (calcite or dolomite) and microstructure (e.g. pervasive or partial recrystallization, crystal growth in micro cavities, etc.).

2.4.3 Counting of reworked fossil foraminifera within sea bottom sediment samples

The content of reworked fossil foraminifera was quantified in 12 sea bottom sediment samples collected from eight localities of the Maltese Islands nearby the collection sites of rocky samples (Figs. 2.6, 2.7 and 2.8, red circles). A total of 300 specimens of benthic foraminifera were counted distinguishing between modern species (live plus dead specimens) and fossil Miocene species. Relative abundance of reworked fossil foraminifera was calculated as percentage (%) of the total number of counted specimens, in order to obtain the reworking degree for each sampled site. Quantitative data are reported in Appendix 2.

2.5 Other laboratory preparations and analyses

2.5.1 Grain-size analysis

Granulometric analyses were performed on a total of 109 sediment samples (18 sea-bottom samples and 91 core samples). Each sample was oven-dried at 40 °C for one day, weighed and separated by wet sieving using five overlapped sieves (Fig. 2.12), with meshes respectively of 1 mm, 500 µm, 250 µm, 125 µm and 63 µm, as indicated in Blott & Pye (2012). The fraction retained on each sieve was oven-dried and weighed again, obtaining weight percentage data (in the form of pie charts or cumulative curves along the core records, respectively), distinguishing between very coarse, coarse, medium, fine and very fine sands. The weight of the indistinct mud fraction, comprising silts and clays, was calculated by difference with the total weight.

The granulometric analysis of bottom sediment samples provides various information about the deposition system, such as the type of substratum present at the sea floor and the hydrodynamism of the sampling area. These factors, together with other environmental parameters (e.g. nutrient supply, light, oxygen content etc.), control the distribution and abundance of benthic species, including benthic foraminifera (e.g. Murray, 2006). The understanding of deposition dynamics allows to correctly evaluate if and how native assemblages of benthic foraminifera change during the colonization by non-indigenous species, especially in the case of our target taxon, the highly invasive *Amphistegina lobifera*.

Additionally, the granulometric analysis of sediment cores provides further information: first of all, whether the preserved sedimentary records are continuous and undisturbed (not vertically mixed by waves) and if the energy at the sampling site is low enough to permit deposition of fine-grained

particles. Both conditions have to be satisfied in order to correctly apply the ^{210}Pb -radiometric dating and, in turn, chronologically constrain the sediment core (Andersen, 2017). On the one hand, storm waves and littoral currents could alter the normal sedimentation process, causing vertical mixing or removing part of the depositional succession and, consequently, making the radiometric dating unusable; on the other hand, sediments have to be fine-grained because ^{210}Pb isotope sinks at the sea floor associated with fine-grained particles (see the next paragraph for more information on ^{210}Pb sources in marine environments).

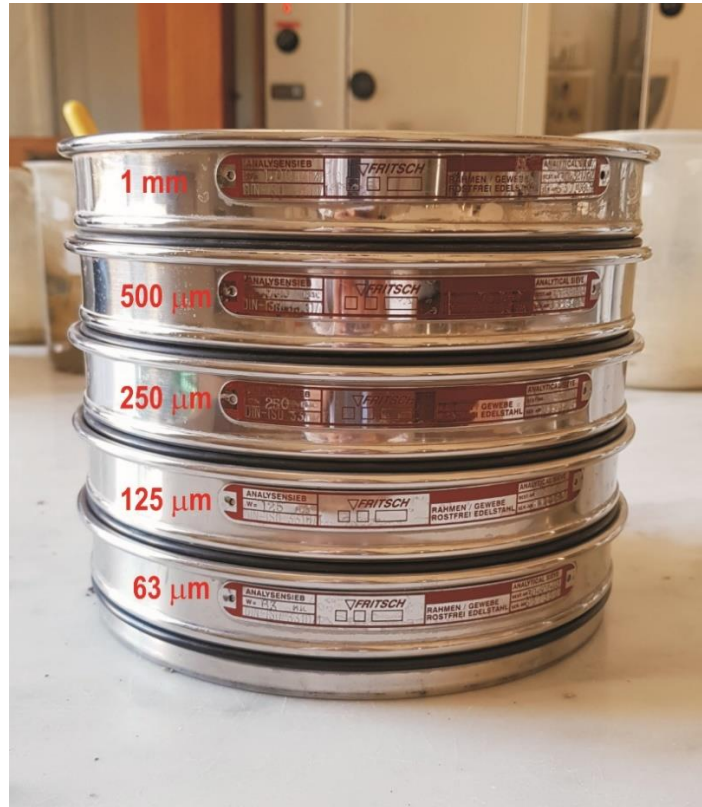


Fig. 2.12: Overlapped sieves used for the granulometric analyses.

2.5.2 Radiometric dating

The most common technique to date recent freshwater and marine sediments up to about 120-150 years ago (Andersen, 2017) and to assess sediment accumulation rates (SAR) is based on the measurements of ^{210}Pb and ^{137}Cs activities along core records (Abril, 2003; Tylmann et al., 2016).

^{210}Pb isotope is a natural radio-nuclide and belong to the ^{238}U decay series (Abril et al., 2014; Incarbona et al., 2016). It is characterized by a half-life of 22.3 years and its daughter isotope is ^{210}Po (Andersen, 2017). ^{210}Pb is commonly present in marine sediments where it accumulates from two different sources: the decay of *in situ* ^{226}Ra that generates the supported ^{210}Pb , and the atmospheric fallout that generates the unsupported ^{210}Pb (Kosnik et al., 2015; Andersen, 2017). In

marine environments, ^{210}Pb of atmospheric origin usually binds with fine-grained particles suspended in the water column, depositing and accumulating at the sediment-water interface as excess ^{210}Pb . This is why the presence of fine-grained sediments along the record is mandatory to correctly apply ^{210}Pb -chronologies. The radiometric dating of marine sediments is based upon the determination of the vertical distribution of unsupported ^{210}Pb , by subtracting supported ^{210}Pb activity from the total activity (Hollins et al., 2011).

^{137}Cs isotope is an artificial radionuclide commonly used as an independent tracer to validate the chronologies provided by ^{210}Pb dating (Smith, 2001). This isotope derives from nuclear fission and its main sources in the atmosphere were two historical events: the nuclear weapons testing (peaked in 1963) that were carried out in the Pacific Ocean by United States and the accident that occurred in 1986 at the nuclear central of Chernobyl.

Both collected cores (Aim 2 as described in Fig. 1.2) were chronologically constrained by measuring activities of ^{210}Pb and ^{137}Cs isotopes along the sedimentary records. The radiometric dating was carried out at the CNR-ISMAR Institute of Bologna, Italy. ^{210}Pb activity was measured via alpha counting of its daughter isotope ^{210}Po , assuming secular equilibrium between them as described in Incarbona et al. (2016) and Rizzo et al. (2009). ^{210}Po was extracted from the sediment using hot HNO_3 and H_2O_2 , and spiked with ^{209}Po (NIST standard SRM 4326, diluted to 0.43 Bq g^{-1}) used as a yield monitor. The solution was evaporated to near dryness and the nitric acid was eliminated using concentrated HCl , the residue was then dissolved in 1.5 N HCl and Iron was reduced using ascorbic acid. Finally, Po isotopes were plated onto a silver disk overnight at room temperature. Based on the decreasing concentration of excess ^{210}Pb , sedimentation rates were calculated following the Constant Flux:Constant Sedimentation model (Robbins, 1978), assuming that both the atmospheric flux of excess ^{210}Pb to the sediment-water interface and the sediment supply remain constant over time (Abril, 2014).

^{137}Cs measurements were carried out placing dried samples in plastic containers and counting emissions by gamma spectrometry. Gamma emissions of ^{137}Cs were counted at 661.7 keV photo-peak for 24-72 h using Ortec germanium detectors (OrtecHPGeGMX-20195P and GEM-20200) calibrated against a sediment spiked with the Amersham reference standard solution QCY48A by using the same counting geometry. The detectors were coupled to a multi-channel analyser and shielded by a 10 cm thick layer of lead.

2.6 Statistical analyses

The softwares Excel and PAST v4.01 (Hammer et al., 2001) were utilized to calculate Pearson's (r) and Spearman's (r_s) coefficients with relative p -value, respectively, and to elaborate scatter-plots in order to verify a statistically significant correlation between the calculated absolute abundances of *Amphistegina lobifera* and *A. lessonii* and environmental parameter (Aim 2, as described in Fig. 2.4).

The software PRIMER v6 (Clarke & Gorley, 2006) with PERMANOVA+ add-on (Anderson et al., 2008) was utilized to perform uni- and multivariate analyses on the total benthic foraminiferal community structure recorded in sediment samples collected from the Maltese and Sicilian coasts, in order to assess the potential impact of *A. lobifera* colonization on native assemblages in three different areas within the Sicily Channel (Aim 3, as described in Fig. 2.4). Two consecutive statistical analyses were performed: a first analysis was conducted taking into account the foraminiferal community structure collected at different depths from the area at most advanced stage of invasion, i.e. the Maltese Islands, while the second analysis also included the communities from areas subject to earlier stages of invasion, i.e. the Southern and Southeastern coasts of Sicily. To verify differences in the impact of *A. lobifera* to native communities at different depths, we used an orthogonal experimental design with two factors: 'Depth' (fixed, 2 levels: 2-5 m and 15-20 m) and 'Site' (random, 3 levels: Balluta Bay, Hondoq ir-Rummien and Crystal lagoon). To verify the potential effects of *A. lobifera* colonization on native assemblages at the same sampling depth but across areas at known different stages of invasion (Fig. 2.4), we used a nested design with two factors: 'Area' (fixed, 3 levels: Maltese Islands - advanced stage, Southern Sicily - medium stage and Southeastern Sicily - early stage) and 'Site' (random, 3 levels, nested in 'Area': Maltese Islands: 1- Balluta Bay, 2- Hondoq ir-Rummien and 3- Crystal lagoon; Southern Sicily: 4- Marzamemi, 5- Isola delle correnti, 6- Porto Palo; Southeastern Sicily: 7- Agnone, 8- Baia Arcile and 9- Brucoli).

In both analyses, we used as response variables the multivariate community structure (the complete species composition), as well as three univariate indices, calculated from the tool 'Diverse' in PRIMER v.6: species richness, Shannon & Weaver (1949) index of diversity (using \log_e) and Pielou (1984) index of evenness. Multivariate tests were run from Bray-Curtis similarity matrices obtained from the square-root abundance data, while resemblance matrices for univariate response variables were created using the Euclidean distance matrices. Subsequently, permutational analyses of variance (PERMANOVAs) were performed using permutation of residuals under a reduced model, and p -values related to the pseudo-F ratios were calculated with 9999 random permutations

(Underwood, 1997). Whenever there were not enough possible permutations to get a reasonable test, the Monte Carlo *p*-values were used instead (Clarke & Warwick, 2001; Clarke & Gorley, 2006). When significant differences among factors or their interactions were observed, post-hoc pairwise tests were performed.

Additionally, non-metric multidimensional scaling (nMDS) analyses were also used to graphically visualise multivariate patterns of variation in two dimensions, showing the detected different responses of the communities across sites and bathymetries (first analysis), or across areas and sites (second analysis). SIMPER tests (Similarity Percentage; Clarke, 1993) were performed after significant PERMANOVA main tests and pairwise tests to assess which species mostly contributed to the similarity within and dissimilarity among groups (with a cut-off level of 50%; only species with a percentage contribution $\geq 2\%$ were reported).

CHAPTER 3

SPREADING OF NON-INDIGENOUS AND CRYPTOGENIC FORAMINIFERA IN THE CENTRAL MEDITERRANEAN SEA

This chapter is largely based on a paper published on *Estuarine, Coastal and Shelf Science*, by **Guastella R.**, Marchini A., Caruso A., Cosentino C., Evans J., Weinmann A. E., Langer M. R. and Mancin N., entitled: “*Hidden invaders*” conquer the Sicily Channel and knock on the door of the Western Mediterranean sea.

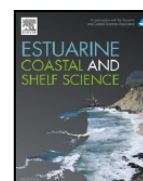
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“Hidden invaders” conquer the Sicily Channel and knock on the door of the Western Mediterranean sea



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3.1 Introduction

One aspect of global change is the dispersal of marine non-indigenous species due to human activities, which is known to be redefining the biogeography of the oceans (Occhipinti-Ambrogi, 2007). However, accurate information on the temporal occurrence, abundance, spatial distribution and effect of NIS on native communities is required in order to understand how they are affecting marine biodiversity (Ojaveer et al., 2014). In particular, small-sized taxa, such as protists, are largely overlooked and often stay undetected for years, sometimes centuries, while their impacts on native communities and habitats are poorly known.

Few non-indigenous species of benthic foraminifera have entered the Mediterranean through the Suez Canal. In the Mediterranean Sea, the most widespread and successful fora-NIS is *Amphistegina lobifera*, that initially colonized the Eastern Mediterranean becoming particularly abundant and forming dense populations there (Langer et al., 2012). More recently, *A. lobifera* was recorded in the Central Mediterranean and in the southern Adriatic Sea, where it has extended its invasion range reaching the Maltese and Pelagian Islands (Yokes et al., 2007; Caruso & Cosentino,

2014) and the southern coasts of Albania (Langer & Mouanga, 2016), respectively. Recent genetic studies (Schmidt et al., 2016; Prazeres et al., 2020) have confirmed the Indo-Pacific origin of the Mediterranean populations of *A. lobifera*. For other foraminiferal species, such as the congeneric *Amphistegina lessonii* and *Sorites orbiculus*, *S. variabilis*, *Amphisorus hemprichii*, *Coscinospira arietina*, *C. hemprichii*, *Cymbaloporella squamosa*, *Heterostegina depressa* and *Pseudolachlanella slitella*, the origin of central Mediterranean populations is still debated; thus they should be considered cryptogenic (Marchini et al., 2015).

The Sicily Channel is centrally located in the Mediterranean Sea, representing a natural biogeographical corridor for non-indigenous species coming from the Red Sea and range-expanding Atlantic taxa entering through the Strait of Gibraltar. It also acts as the oceanographic boundary between the colder western and warmer eastern Mediterranean basins (Azzurro et al., 2014; Di Lorenzo et al., 2017 and references therein). Therefore, the Sicily Channel is the ideal region to assess the ability of thermophilic foraminifera to breach this boundary, especially for the highly invasive *A. lobifera* that is known to be limited by the 14 °C winter isotherm (Hallock, 1999; Langer & Hottinger, 2000; Triantaphyllou et al., 2012).

This study provides an updated overview of the current distribution and establishment status of the non-indigenous species *A. lobifera* and the cryptogenic *A. lessonii* in the Sicily Channel, also furnishing new records of the genus *Amphistegina* and other cryptogenic species of benthic foraminifera. Additionally, the new records were used to create updated species distribution models (SDM) in order to assess the potential distribution of *A. lobifera* in the Central and Western Mediterranean Sea driven by the current and future climate conditions.

3.2 Materials and methods

3.2.1 Study areas and sampling strategy

Sampling was carried out at five different areas, the first four located in the Sicily Channel and the last one located in the Western Mediterranean (Fig. 3.1): Maltese Islands (A), southeastern coast of Sicily (B), Pantelleria Island (C), Favignana Island (D) and northwestern coast of Sicily (E). The first record of *A. lobifera* in the Maltese Islands was in 2006 (Yokes et al., 2007), but it had not been previously reported from the other study areas, although, according to recent species distribution models (Weinmann et al., 2013), all five study areas have medium to high values of habitat suitability for this species under current climate conditions.

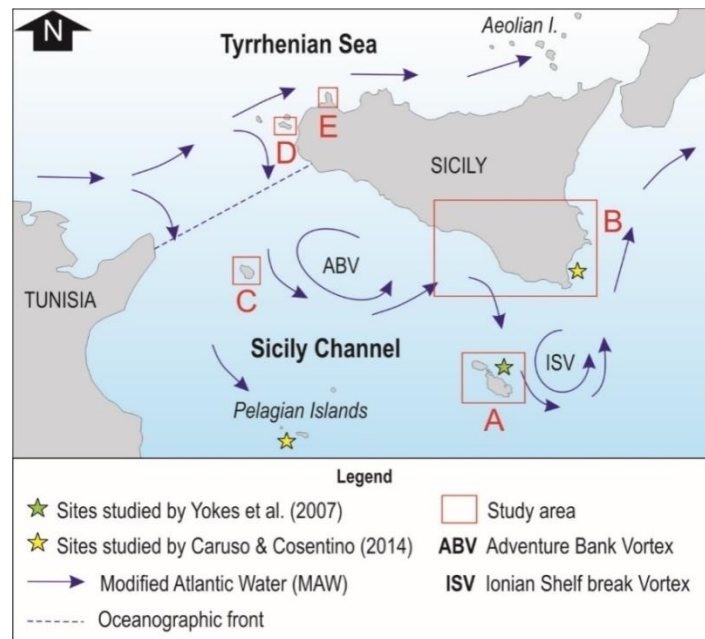


Fig. 3.1: Map of the surface circulation in the Sicily Channel (redrawn after Drago et al., 2010) together with the location of the five sampling areas: A) Maltese Islands, B) Southern Sicily, C) Pantelleria Island, D) Favignana Island, E) Northwestern Sicily. The sites previously studied by Yokes et al. (2007) and Caruso & Cosentino (2014) are indicated with green and yellow stars, respectively.

Sampling strategy followed the standard procedures suggested by the FOBIMO protocol (Schönfeld et al., 2012). A total of 98 sediment and 51 algal samples were collected at different depths from 56 sites, collecting two or three replicate samples from each locality by using a Van Veen grab or a small corer operated by a diver. Further details on sampling methods are reported in **Chapter 2 paragraph 2.2.1** of this thesis and in Appendix 1.

Additionally, unpublished results from surveys conducted by Prof. Caruso A. and Dr. Cosentino C. (University of Palermo) in 2015 and 2016 along the southeastern Sicilian coasts were also included in the present work, allowing assessment of possible changes in relative abundances of *A. lobifera* in the same area over a temporal range of 3 years. In particular, a total of 6 sites were sampled between 2015 and 2016, three sites in summer 2015 (34- Noto, 35- Vendicari, 36- Punta Secca; Appendix 1) and three sites in summer 2016 (37- Capo Passero, 38- Isola delle correnti, 39- Punta delle formiche; Appendix 1), respectively.

3.2.2 Sampling processing and foraminiferal analyses

Samples were prepared for the foraminiferal analyses following the standard procedures suggested by the FOBIMO group (Schönfeld et al., 2012). All the details on samples preparation are reported in **Chapter 2** of this thesis, *paragraphs 2.3.1* and *2.3.2* for sediment and algal samples respectively, while details on the foraminiferal analyses are reported in **Chapter 2, paragraph 2.4.1**.

3.2.3 Species distribution models (SDMs)

In order to obtain the updated SDMs for *Amphistegina lobifera*, the newly obtained records of the species were processed together with previous records published in Langer et al. (2012), Weinmann et al. (2013), Mouanga & Langer (2014) and Langer & Mouanga (2016), complemented with a few additional findings from other locations of the Central Mediterranean (e.g. Caruso & Cosentino, 2014), for a total of 126 records from the Eastern and Central Mediterranean, the Adriatic and Tyrrhenian Seas. The Bio-ORACLE database (<http://www.bio-oracle.org/>) was used to obtain the environmental data, including oceanographic variables derived by remotely sensed and *in situ* measured datasets (Tyberghein et al., 2012; Assis et al., 2018), and to obtain also future datasets, based on Representative Concentration Pathway (RCP) scenarios described within the Fifth Assessment Report of the IPCC (Collins et al., 2013). In particular, the RCP 4.5 scenario was used for the proposed SDMs, which assumes that global emissions will peak in the mid 21st century and translates to a warming range of 0.9-2.0 °C in the period 2046-2065 and of 1.1-2.6 °C in the period 2081-2100 (Collins et al., 2013). Additionally, the annual minimum temperature and the mean primary productivity were applied as limiting constraints, respectively for the temperature limit of *A. lobifera* (14 °C) and for potentially unsuitable areas characterized by high eutrophication conditions. The obtained SDMs provide the potentially suitable areas for modern *A. lobifera* specimens in the Mediterranean Sea and the future distribution ranges projected for 2040-2050 and 2090-2100 scenarios, based on predicted environmental change (Tyberghein et al., 2012; Assis et al., 2018).

The software Maxent 3.4.1 (Philips et al., 2006) was utilized to obtain the SDMs; it uses a grid-based machine-learning algorithm following the principles of maximum entropy (Jaynes, 1957). During the modelling process, the software starts with a uniform distribution, successively fits it to the data (occurrence records of *A. lobifera* and environmental variables), tests the predictive capability and improves the model by iteratively permuting and varying the input variables and features thereof. This is measured in the log likelihood or “model gain” displaying the increasing distance from the uniform distribution (Elith et al., 2011). Maxent automatically selected a total of 10,000 random background points within the range of *A. lobifera* in the Central and Eastern Mediterranean Sea, the Tyrrhenian Sea and the Red Sea, and then predicted the relative suitability of the habitat, interpreted as the potential distribution of the studied taxon. The logistic output format with suitability values ranging from 0 (unsuitable) to 1 (optimal) was used (Philips & Dudík, 2008), setting to 0.5 by default the probability of presence at sites with “typical” conditions (Elith et al., 2011). Additionally, a multivariate environmental similarity surface (MESS) analysis was

carried out, but did not highlight an extrapolation of probability values within the target area, thus suggesting high confidence for model predictions. The modelling process was performed with 50 replicates and the average predictions across all replicates were used for further processing. The software allows for model testing by calculating the Area Under the Curve (AUC), which refers to the Receiver Operation Characteristic (ROC) curve (Philips et al., 2006) and evaluates the ability of the model to distinguish presence from background points. In order to compute AUC scores, the set of occurrence records were randomly split into training (70%) and test subsets (30%), used for model calibration and testing of the predictive performance, respectively. The continuous probability surfaces of the SDMs were subsequently converted into presence/absence maps using the “Equal training sensitivity and specificity logistic threshold” as recommended by Liu et al. (2005).

3.3 Results

A total of ~ 90 native species of benthic foraminifera was recognized, mainly represented in both algal and sediment samples by the shallow-water genera *Peneroplis*, *Elphidium*, *Rosalina*, *Lobatula*, *Cibicides*, *Ammonia*, *Planorbulina* and the miliolids *Quinqueloculina* and *Triloculina*. Epiphytic species, such as *Lobatula lobatula*, *Peneroplis pertusus*, *Rosalina bradyi*, *Planorbulina mediterraneensis* and *Quinqueloculina disparilis*, usually dominated in the assemblages recorded in algal samples and show a higher number of stained specimens. On the contrary, in sediment samples the most common taxa were both epiphytic and epifaunal or shallow infaunal species, such as *Elphidium crispum*, *Elphidium macellum*, *Ammonia inflata*, *Ammonia parkinsoniana*, *Textularia pala* and *Uvigerina mediterranea*. These were characterized by a higher abundance of unstained specimens, often with abraded tests. In sediment samples from the Maltese sites some reworked Miocene fossils of benthic and planktonic foraminifera (i.e. *Anomalinoidea helacinus*, *Heterolepa bellincionii*, *Stilostomella monilis*, *Uvigerina striatissima*, nodosariids and the planktonic genera *Globigerinoides* and *Globigerina*) occurred together with modern benthic foraminifera. These fossil taxa derived from the erosion of Miocene outcrops (from ~ 25 to ~ 6 Ma in age) and in most samples were particularly abundant, well preserved and often difficult to distinguish from modern dead specimens of benthic foraminifera. For this reason, in these samples, the census count was limited to specimens dyed by Rose Bengal or dead specimens of unequivocal modern origin. *Amphistegina lobifera* occurred in most sediment samples from the Maltese Islands and living specimens were also found for the first time in algal and sediment samples from southeastern Sicily, Pantelleria and Favignana Islands. Additionally, some cryptogenic species of benthic foraminifera

were recorded: *Amphistegina lessonii*, *Amphisorus hemprichii*, *Coscinospira arietina*, *Coscinospira hemprichii* and *Sorites orbiculus* (Fig. 3.2, Appendix 2).

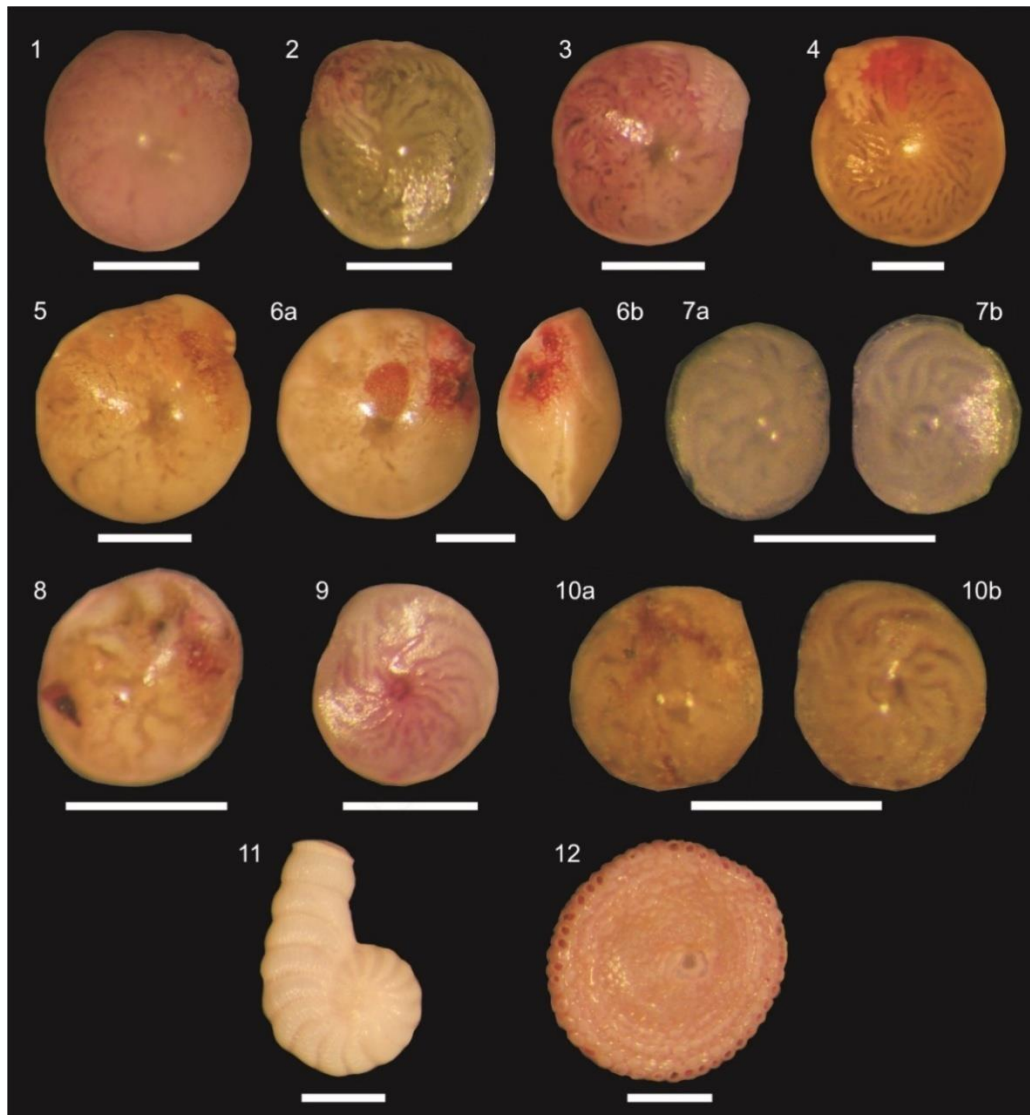


Fig. 3.2: Photomicrographs of stained and unstained non-indigenous and cryptogenic benthic foraminifera recorded in the Sicily Channel. 1- Umbilical view of Rose Bengal stained specimen of *Amphistegina lobifera* (site 12, Malta Island). 2- Dorsal view of Rose Bengal stained specimen of *A. lobifera* showing the typical chamber sutures strongly lobulated (site 11, Malta Island). 3- Umbilical view of Rose Bengal stained specimen of *A. lobifera* showing the typical chamber sutures strongly lobulated (site 36, southern Sicily). 4- Dorsal view of Rose Bengal stained specimen of *A. lobifera* (site 40, southern Sicily). 5- Umbilical view of unstained specimen of *A. lobifera* showing a brownish colour of the last chambers characteristic of the living animal (site 53, Pantelleria Island). 6- Rose Bengal stained specimen of *A. lobifera*: a) umbilical view, b) profile (site 5, Favignana Island). 7- Unstained specimen of *A. lessonii* characterized by a small-sized planoconvex test and typical chamber sutures forming long falciform arcs on the dorsal side: a) umbilical view, b) dorsal view (site 7, Malta Island). 8- Umbilical view of Rose Bengal stained specimen of *A. lessonii* showing a planoconvex test characterized by falciform chamber sutures (site 5, Favignana Island). 9- Dorsal view of Rose Bengal stained specimen of *A. lessonii* showing a planoconvex test characterized by well visible falciform chamber sutures (site 36, southern Sicily). 10- Unstained small-sized specimen of *A. lessonii* showing a brownish colour of the last chambers

characteristic of the live specimens: a) umbilical view, b) dorsal view (site 54, Pantelleria Island). 11- Unstained specimen of *Coscinospira arietina* (site 11, Malta Island). 12- Rose Bengal stained specimen of *Sorites orbiculus* (site 36, southern Sicily). Scale bars are 500 μm . Details of sampling sites are given in Appendix 1.

3.3.1 Maltese Islands

Stained specimens of *A. lobifera* were found in samples from most of the studied localities, except for only three sites of the Maltese Islands (14- Ghadira Bay, 21- Ghajn Tuffieha Bay and 22- Golden Bay) (Fig. 3.3A-B, Appendix 2). Relative abundance of *A. lobifera* ranged from 1% to 98%, reaching the highest values (> 70%) in the northern part of Malta island and between the islands of Comino and Gozo (sites 3, 5, 11, 12, 13, 26, 27, 28 and 31; Fig. 3.3B, Appendix 2), where the sea floor mainly consists of coarse-grained coralline limestone particles, while lowest abundances were recorded in fine-grained substrata. The congeneric *A. lessonii* was usually present but with small-sized specimens and lower abundances than *A. lobifera*, ranging from 3% to 8% relative abundance, mostly in the northern part of Malta island (sites 2, 15, 16 and 19; Fig. 3.3B, Appendix 2). The other cryptogenic species (*A. hemprichii*, *C. arietina*, *C. hemprichii* and *S. orbiculus*) showed low abundances (2%-8%) and a homogeneous distribution (Fig. 3.3B, Appendix 2).

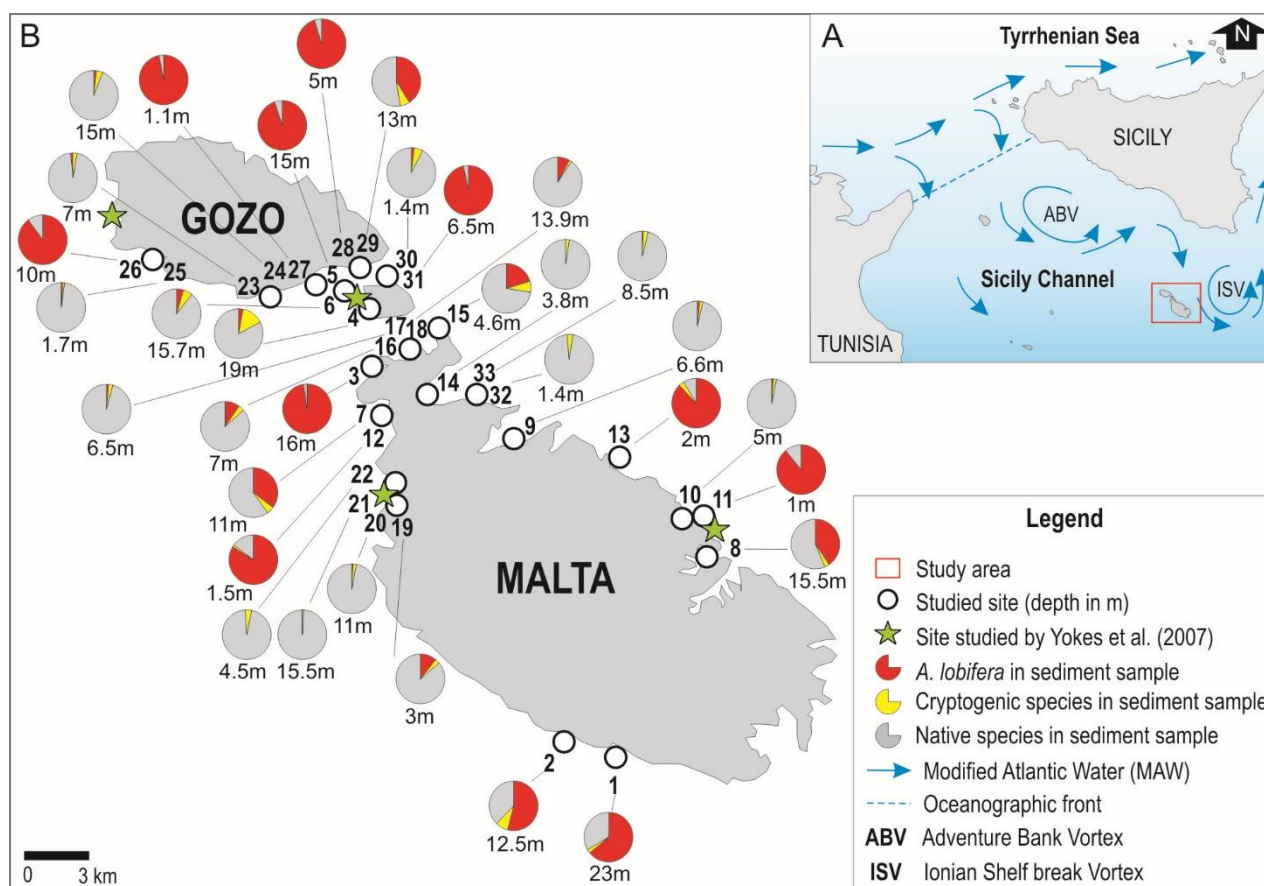


Fig. 3.3: A) The study area located in the Sicily Channel; B) Relative abundances of *Amphistegina lobifera* and cryptogenic foraminifera recorded in sediment samples from the Maltese Islands.

3.3.2 Southern Sicily

Amphistegina lobifera was not found in sediment samples collected in 2015 (Fig. 3.4B) and it was first recorded in 2016 at Isola delle Correnti (site 38, Fig. 3.4C, Appendix 2) with relatively low abundances (10%-12% on algae and < 1% in sediment). One year later, in 2017, both juvenile and adult stained specimens of *A. lobifera* were recorded in algal and sediment sampled from seven study sites (sites 41, 42, 43, 44, 45, 46 and 48; Fig. 3.4D, Appendix 2) with relative abundance ranging from 1% to 50%. The highest values (35%-50%) were recorded in the southernmost portion of the area, around site 38 (Fig. 3.4C), where *A. lobifera* was recorded for the first time in 2016, while lower abundances were recorded at sites located westward and northward of this area. Very rare specimens (< 1%) of the congeneric *A. lessonii* had been documented in 2012 at Vendicari (site 35) (Caruso & Cosentino, 2014). In samples collected in 2017, it often occurred together with *A. lobifera* but exhibiting lower abundances (1%-4%) and smaller test sizes. Additionally, three other symbiont-bearing cryptogenic taxa (*S. orbiculus*, *A. hemprichii* and *C. arietina*) were recorded, but only at a few localities and usually with very low abundances (< 1%; Fig. 3.4D, Appendix 2).

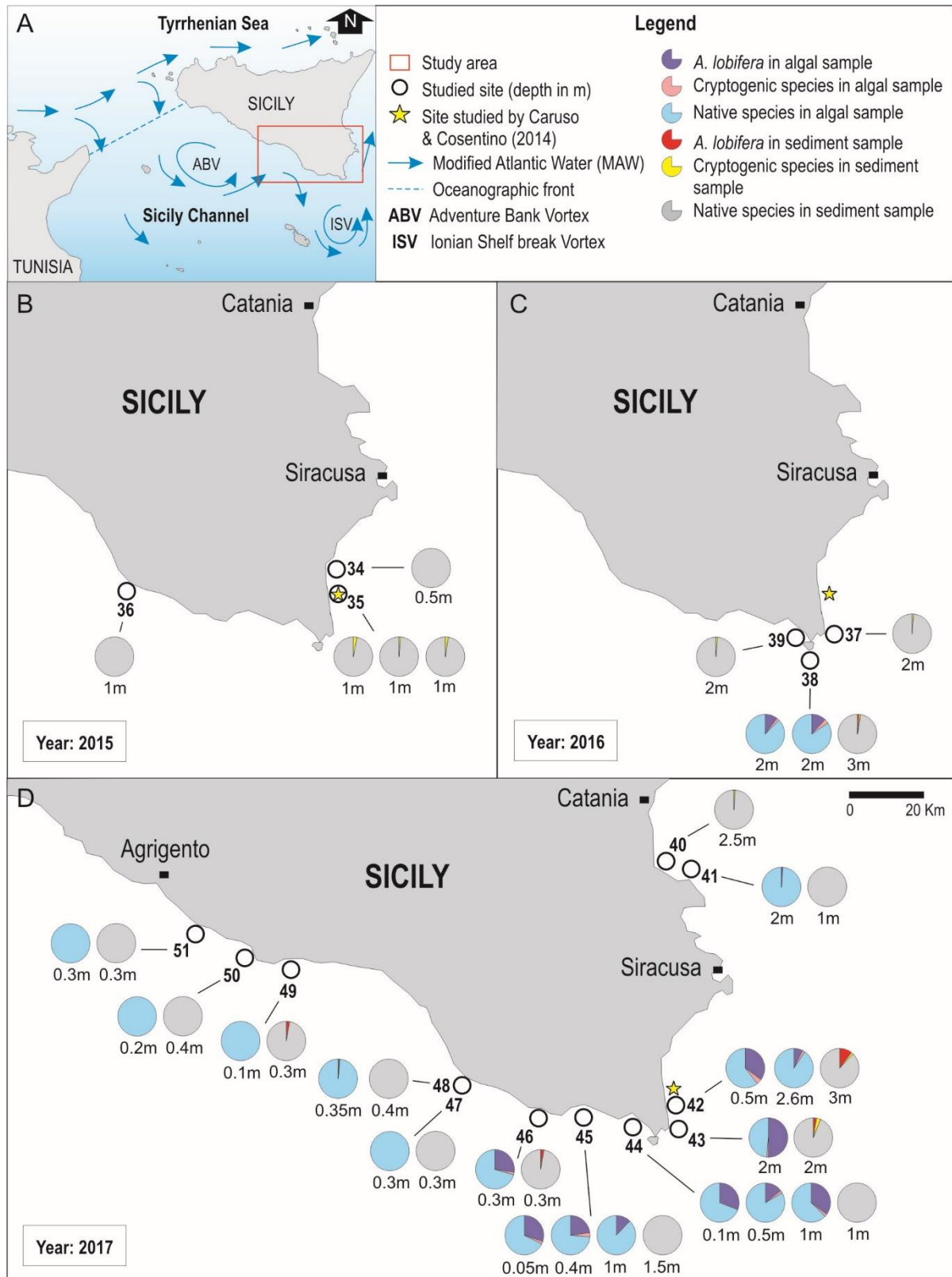


Fig. 3.4: A) The study area located in the Sicily Channel; Relative abundances of *Amphistegina lobifera* and cryptogenic foraminifera recorded in sediment and algal samples from Southern Sicily: B) Quantitative data from the sampling surveys conducted in 2015; C) Quantitative data from the sampling surveys conducted in 2016; D) Quantitative data from the sampling surveys conducted in 2017. A marked increase in the relative abundance and an extension of the geographic distribution of *A. lobifera* emerge from the comparison of the 2016 and 2017 results.

3.3.3 Pantelleria and Favignana islands and northern Sicily

In Pantelleria Island, *A. lobifera* was recorded at all study sites, with relative abundances ranging from 2% to 82% and recording the highest values (39%-82%) in the southern and eastern portion of the island (sites 53 and 54; Fig. 3.5B, Appendix 2). Although the Rose Bengal treatment was not applied to the samples collected from this site, the characteristic greenish to brownish colour of the cytoplasm and the extrusion of pseudopods (Fig. 3.2, image 5) enabled easily recognizing live specimens. The congeneric *A. lessonii* co-occurred with *A. lobifera* in this area, in both algal and sediment samples, with small-sized tests and low abundances not exceeding 7% (Fig. 3.5B, Appendix 2). Additionally, *C. arietina* and *S. orbiculus* were present with relative abundance of ~ 1% (Fig. 3.5B, Appendix 2), whereas *A. hemprichii* and *C. hemprichii* were not found.

In Favignana Island, stained specimens of *A. lobifera* were found for the first time in summer 2018 with low abundances (2%-3%) on algal samples collected at very shallow depths (1-4 m) from Lido Burrone and Cala del Passo (sites 58 and 59; Fig. 3.5C, Appendix 2), but the species was absent in sediment samples. The congeneric *A. lessonii* and *S. orbiculus* only occurred at site 59 with very low abundances (< 1% and ~ 2% respectively; Fig. 3.5C, Appendix 2).

In Northern Sicily, no amphisteginids were found, whereas *A. hemprichii* and *S. orbiculus* occurred at the two study sites (60- Santa Margherita and 61- San Vito) with abundances of ~ 15% (Fig. 3.5D, Appendix 2).

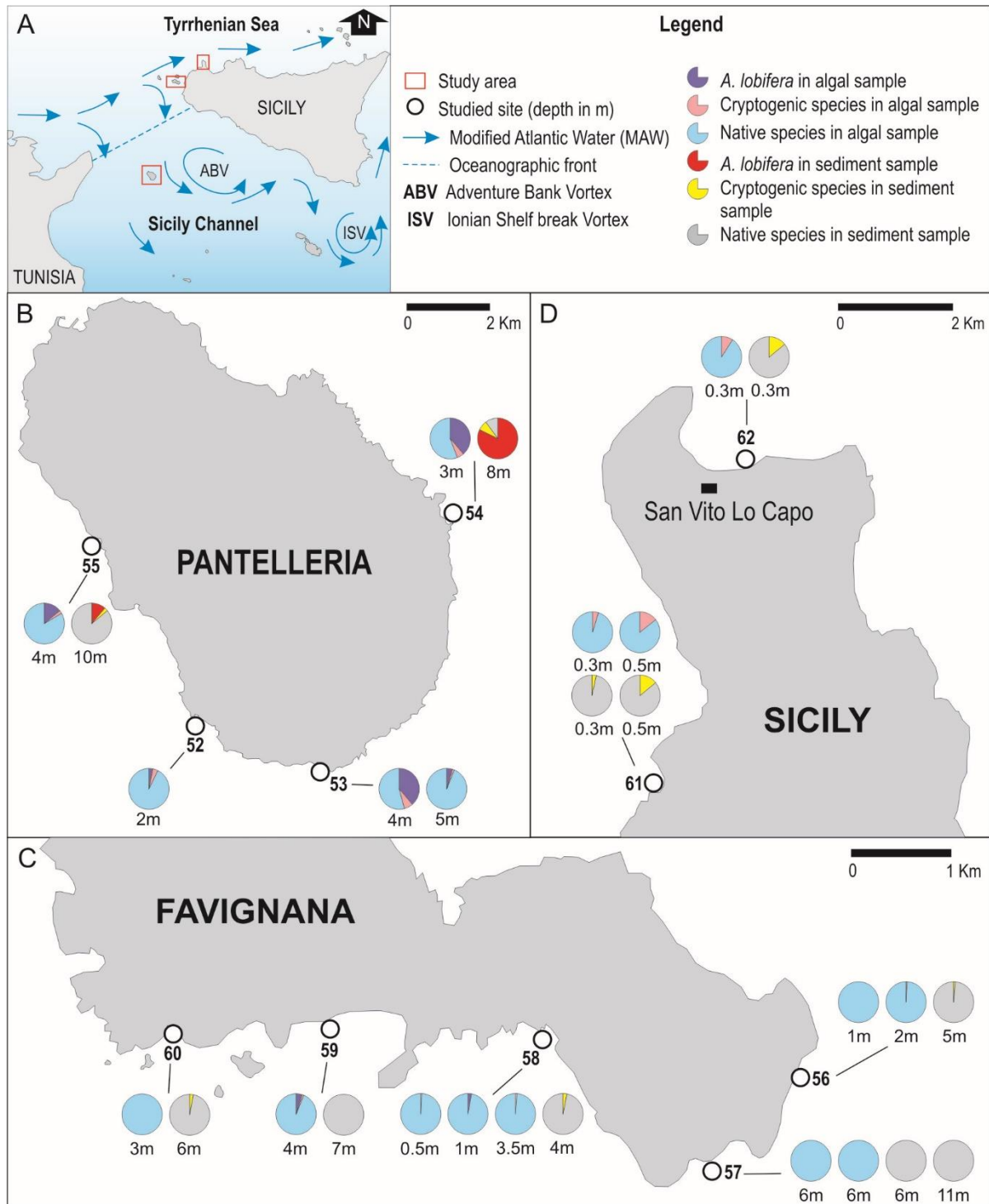


Fig. 3.5: A) The study areas located in the Sicily Channel and Tyrrhenian Sea; Relative abundances of *Amphistegina lobifera* and cryptogenic foraminifera recorded in sediment and algal samples from Pantelleria Island (B), Favignana Island (C) and Northern Sicily (D).

3.3.4 New distribution models

On the basis of the previously known occurrence and the new records obtained through the present study (Appendix 2), a new species distribution model for *A. lobifera* in the Mediterranean Sea was computed, obtaining good AUC values (AUC_{training}: 0.7978 and AUC_{test}: 0.7825 based on 88

training and 37 test records). Annual minimum temperature contributed with 64.1% to the model performance.

The model obtained under current climate conditions (Fig. 3.6) shows the highest values of habitat suitability (colours from orange to yellow) along the coasts of the Levantine Sea and the Aegean Sea. Habitat suitability gradually decreases along the western coast of Greece, Albania and northern Tunisia. In the Sicily Channel, habitat suitability is lower (usually less than 0.4) and further decreases westward, while suitability values > 0.5 are present along the coasts of southern Croatia, western Italy (Lazio and Campania), southern France, eastern and southern Spain, Morocco and Algeria.

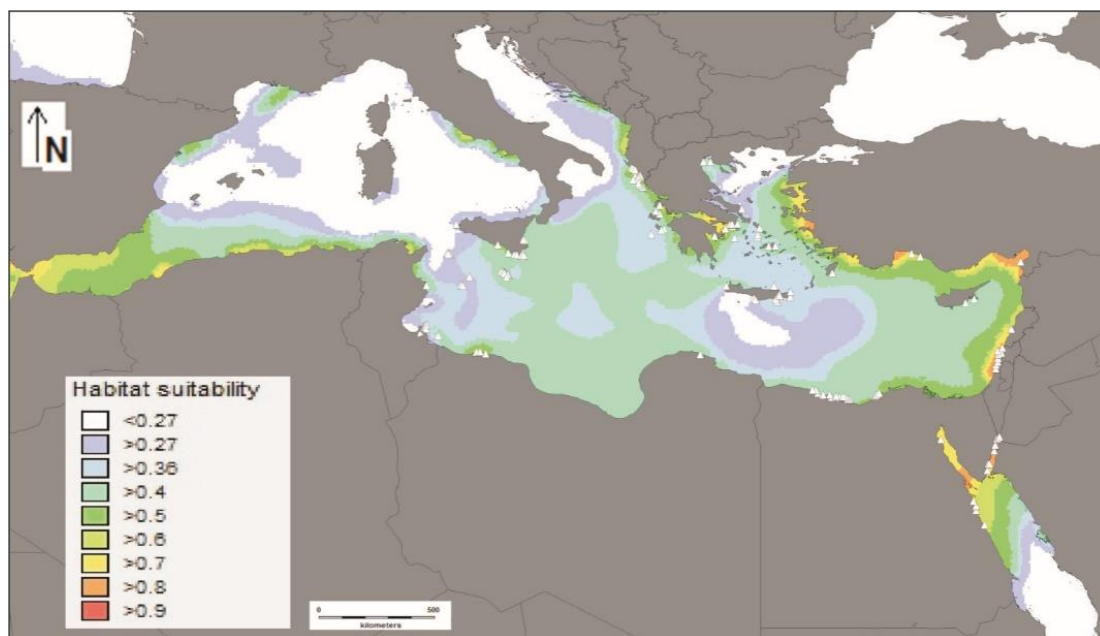


Fig. 3.6: Species distribution model for *A. lobifera* under current climate as projected by Maxent. White triangles represent occurrence records for the computation of the species models. Coloured areas highlight habitat suitability, increasing from unsuitable (white) to moderate (green) and highly suitable (orange).

Based on the obtained projection onto the RCP4.5 scenario, the overall habitat suitability for *A. lobifera* in the Mediterranean Sea will increase in 2040-2050 (Fig. 3.7), leading to potential range expansions into the Western Mediterranean. The projection suggests that *A. lobifera* will follow a continuous north-westward spreading, reaching the Adriatic and Tyrrhenian Sea in the Central Mediterranean and the Alboran and Balearic Sea in the Western Mediterranean.

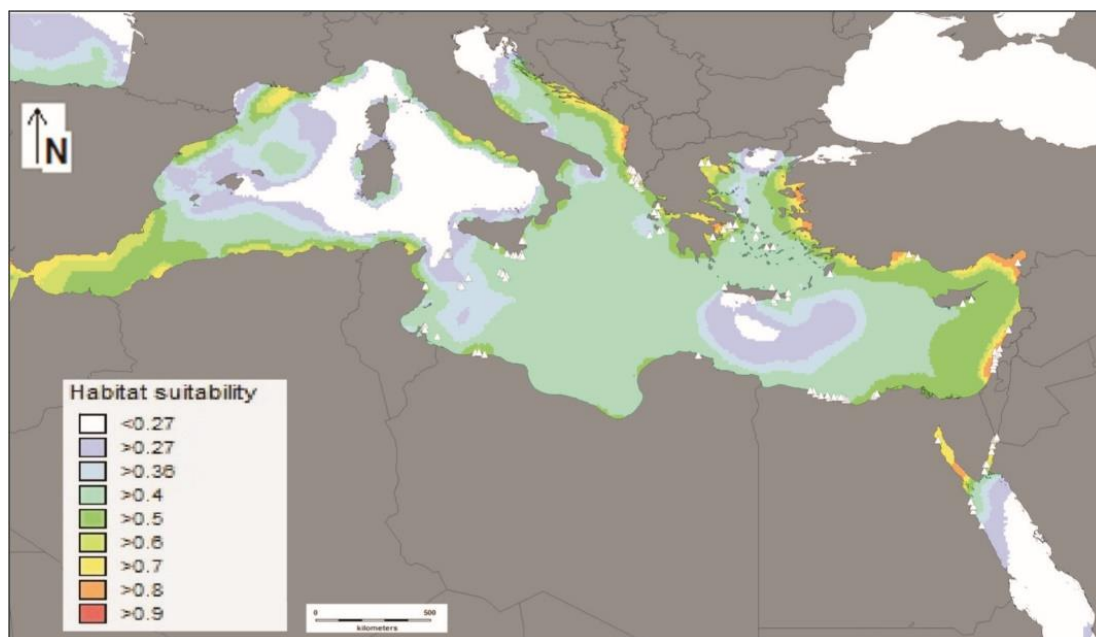


Fig. 3.7: Species distribution model for *A. lobifera* under future climate (2040-2050) as projected by Maxent. White triangles represent occurrence records for the computation of the species models. Coloured areas highlight habitat suitability, increasing from unsuitable (white) to moderate (green) and highly suitable (orange).

In the years 2090-2100, habitat suitability is expected to furtherly increase westward (Fig. 3.8), reaching values > 0.6 into the Alboran Sea and along the coasts of Italy in both Tyrrhenian and the Adriatic Sea. The coasts of northern Albania, Montenegro and Croatia are also expected to show favourable conditions.

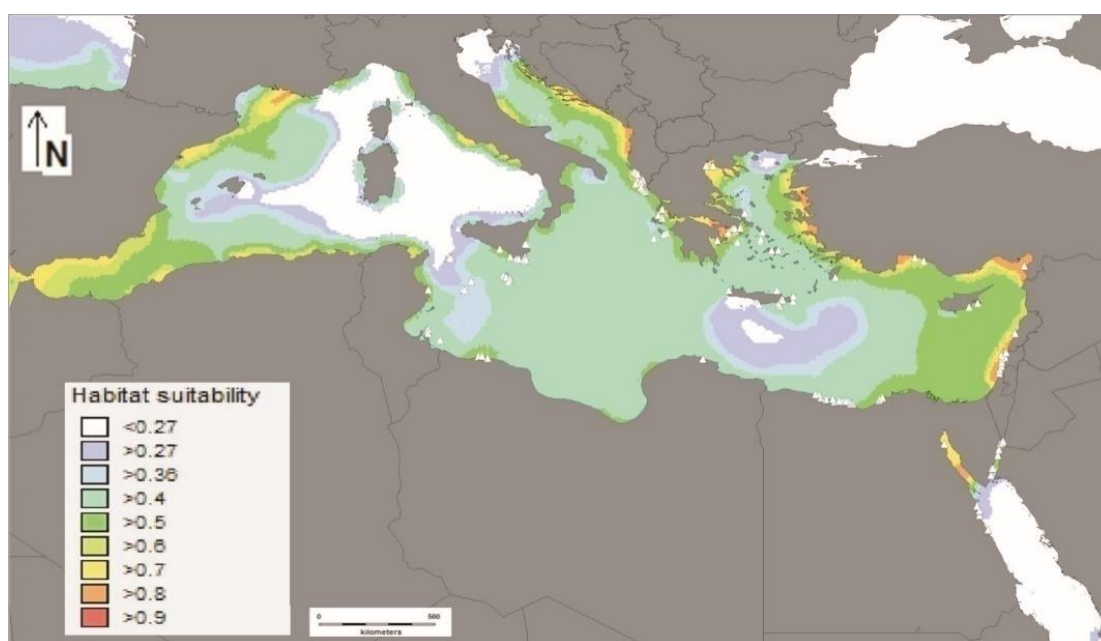


Fig. 3.8: Species distribution model for *A. lobifera* under future climate (2090-2100) as projected by Maxent. White triangles represent occurrence records for the computation of the species models. Coloured areas highlight habitat suitability, increasing from unsuitable (white) to moderate (green) and highly suitable (orange).

3.4 Discussion

3.4.1 Spatial and temporal patterns and possible spreading vectors

The non-indigenous species *Amphistegina lobifera* was first recorded in the Sicily Channel with the following temporal succession: in southern Tunisia in 1979 (Blanc-Vernet et al., 1979), in the Pelagian Islands in 2005 (Caruso & Cosentino, 2014), in the Maltese Islands in 2006 (Yokes et al., 2007) and finally around Djerba (Tunisia) in 2014 (El Kateb et al., 2018). In the present study, *A. lobifera* was documented in three new areas of the Sicily Channel (Fig. 3.4D and Fig. 3.5B-C): in south-eastern Sicily (along the coast between Catania and Ragusa) and in Pantelleria and Favignana islands. At present, these last two records represent the westernmost limit of the distribution range of *A. lobifera* in the Mediterranean basin. Within the Sicily Channel, the species *A. lessonii* was first recorded in south-eastern Sicily in 2012 (Caruso & Cosentino, 2014) and the occurrences from the Maltese Islands and Pantelleria and Favignana islands resulting from this study represent three new records for this cryptogenic taxon. In all the studied sites, this species co-occurs with the congeneric *A. lobifera* but always with smaller-size specimens and with lower abundances, probably due to the shallow sampling depth used in this study (generally < 10 m) given that *A. lessonii* is known to prefer deeper habitats (Mateu-Vicens et al., 2009).

The colonization process by *A. lobifera* is particularly evident along the coasts of south-eastern Sicily, where this foraminiferal assemblage has markedly increased its relative abundance, passing from 12% of the total benthic foraminiferal assemblage in 2016 to ~ 50% in 2017, colonizing a coastal area of over 150 km (Fig. 3.4). In Pantelleria Island, the very high abundance recorded indicates that *A. lobifera* had reached the area much earlier than the first record (2017) resulting from this study. On the contrary, the lower abundance recorded in Favignana Island suggests that the species arrived there more recently. Additionally, this last new record, located at the boundary with the Tyrrhenian Sea, indicates an average expansion rate of ~ 13.2 km per year (taking into account that the Suez Canal was opened in 1869). Although this value is slightly higher than previous estimations for Mediterranean amphisteginids (Langer et al., 2013), it agrees with expansion rates of other Lessepsian species (Hiddink et al., 2012). Our results also suggest that the range expansion rates have probably increased over the last two decades (~ 20 km year⁻¹). Since a particularly steep warming trend has been recorded in the Mediterranean Sea over the same period (Pastor et al., 2017), this evidence suggests that the north-western spreading of thermophilic amphisteginids is following the current climate change.

In situ observations, meta-analyses and epigenetic studies across habitats and taxa have suggested that the invasion success is related to a species' ability to adapt to several environmental conditions (i.e., phenotypic plasticity; Daehler, 2003; Karatayev et al., 2009; Davidson et al., 2011; Ardura et al., 2017; Cardeccia et al., 2018). Previous studies have documented that, in the Indo-Pacific region, *A. lobifera* thrives at tropical and subtropical latitudes, at depths < 20 m with mid to high light conditions (Langer & Hottinger, 2000; Triantaphyllou et al., 2012 and references therein). Additionally, *in situ* observations carried out at the Maldives have reported bleaching of *Amphistegina* specimens when temperature exceeds 30 °C and solar irradiation is too intense (Spezzaferri et al., 2018). This is why *A. lobifera* seeks protection beneath algal thalli or reef rubble when light is too strong (Beavington-Penney & Racey, 2004). Despite these evidences, in some sites of this work several living specimens of *A. lobifera* were found attached to algae at very shallow depths (from 50 cm to 5 cm) (Fig. 3.4, sites 42, 44, 45 and 46; Appendix 2), where radiation can be particularly intense and water temperature exceeds 30 °C. Additionally, a high thermal tolerance has also been observed in *A. lobifera* specimens found in the Eastern Mediterranean and introduced through the Suez Canal (Schmidt et al., 2016), suggesting that the Mediterranean populations of *A. lobifera* are well adapted to high temperature and solar irradiation (Prazeres et al., 2017; 2020; Weinmann & Langer, 2017).

Based on our results, three different stages of colonization for *A. lobifera* can be hypothesized within the Sicily Channel (Fig. 3.9): an 'early stage', where *A. lobifera* sporadically occurred at the study sites with low abundances (< 20%); a 'medium stage', where the species occurred in different sites of the study area with higher abundances (varying between 20% and 50%); and an 'advanced stage', where the species was found at most of the study sites with very high abundances (> 50%). In most of the localities around the Maltese Islands and Pantelleria, *A. lobifera* presents very high abundances often exceeding 80% and, as a result, strongly dominates benthic foraminiferal assemblages, with a consequent reduction in relative abundance of native species (Appendix 2). Although *A. lobifera* was already considered as established in the Maltese Islands by Yokes et al. (2007), the present study shows that *A. lobifera* occurs along virtually all the coasts of the archipelago and also at Pantelleria, reaching very high abundances.

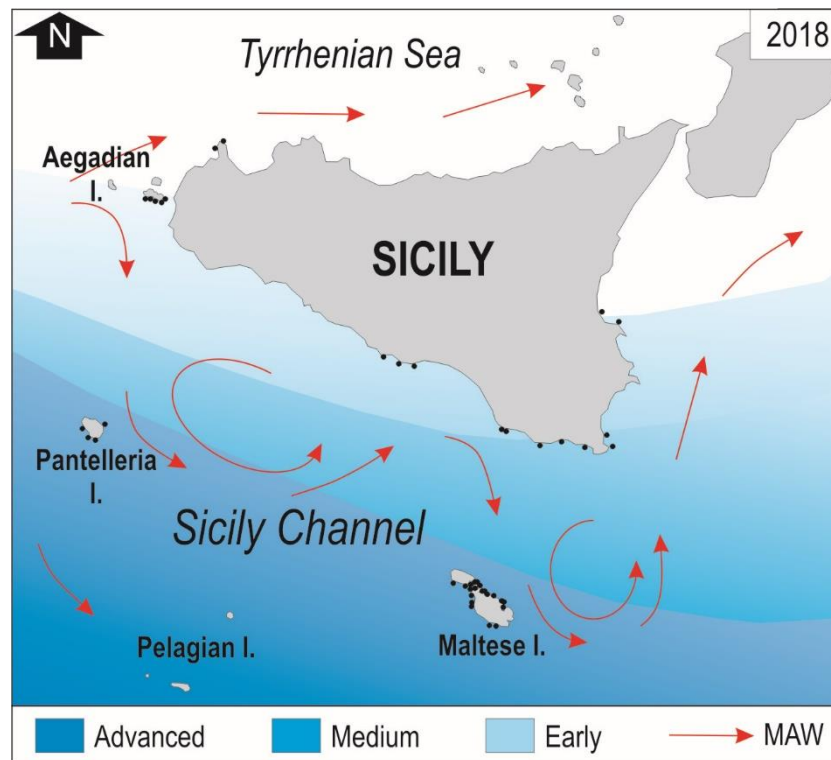


Fig. 3.9: Current geographic distribution and invasion success of *Amphistegina lobifera* in the Sicily Channel. The studied sites are indicated with black points. The colonization process seems to proceed northwards, probably favoured by the surface circulation of the MAW.

The present distribution and abundance of *A. lobifera* within the Sicily Channel also suggest that the colonization process is proceeding northward, probably driven by the surface currents that move from the Pelagian and Maltese Islands towards southern Sicily (Ionian Shelf break Vortex; Fig. 3.1). Additionally, the findings of living specimens attached on algal thalli or seagrass leaves at very shallow depths (5 cm) suggest that *A. lobifera* could be passively transported via floating algae, floating rafts or marine litter (e.g. Finger, 2018); all these vectors may strongly facilitate its spread over large distances (Katsanevakis & Crocetta, 2014). Moreover, since living specimens of *A. lobifera* have been recently documented in faecal pellets of the herbivorous rabbitfish *Siganus luridus*, other potential vectors, such as ichthyochory, may further be involved in its dispersal process (Guy-Haim et al., 2017).

Finally, the cryptogenic foraminifera (*A. lessonii*, *A. hemprichii*, *C. arietina*, *C. hemprichii* and *S. orbiculus*) are also widespread in the Sicily Channel, where they occur in most of the study sites but always with very low abundances (< 5%). Although these observations are preliminary and further studies are needed, the fact that they show a lower invasion success suggests that they may have a lower adaptive ability in the colonization of new areas when compared to *A. lobifera*.

3.4.2 Present and future ranges: implications from SDM for *Amphistegina* in the Mediterranean Sea

The distribution model computed for present-day *A. lobifera* (Fig. 3.6) traces occurrence records very well, especially in the Eastern and Central Mediterranean, including the Nile Delta, the Levantine Basin (Egypt, Gaza, Israel, Lebanon, Syria and Cyprus) and the Aegean Sea (Turkey and Greece). In fact, *A. lobifera* has been reported from Egypt, Libya and Tunisia (El Kateb et al., 2018), the Maltese Islands (Yokes et al., 2007), Corfu (Zenetos et al., 2010), the Pelagian Islands (Caruso & Cosentino, 2014) and the Adriatic Sea (Langer & Mouanga, 2016). These records show that amphisteginids have been rapidly spreading northwestward towards the Tyrrhenian Sea and into the Adriatic Sea, strongly supporting range extensions previously predicted by species distribution models (Langer et al., 2012; Weinmann et al., 2013). In particular, the new records of *A. lobifera* from the Sicily Channel provided by this study, combined with recent records from Tunisia (El Kateb et al., 2018) and from the southern Adriatic Sea (Langer & Mouanga, 2016), allowed to refine the lower temperature tolerances of this foraminiferal species, which survives and thrives close to its current distribution limit. Given that the winter SST has previously been considered one of the main agents controlling the latitudinal distribution of amphisteginids in the Mediterranean basin (Zmiri et al., 1974; Betzler et al., 1997; Langer & Hottinger, 2000), the rapidly rising sea surface temperatures and the extension of climate belts may alter the range of several species (Tittensor et al., 2010). The Mediterranean Sea is one of the most strongly affected areas in the world by rising temperatures: in the Western Mediterranean, shallow-water temperature has increased between 0.8 °C and 4.0 °C over the last 30 years (Prieur, 2002; Vargas-Yanez et al., 2008; Coma et al., 2009), while, in the Eastern Mediterranean, surface waters have warmed by 1.0 °C over the last 20 years (Theocharis, 2008). Therefore, the observed range extension of thermophilic amphisteginids suggests that the northwestern spread is following the current SST increase. The decreasing habitat suitability along the Strait of Sicily (Fig. 3.6) supports the observations provided by this study (Fig. 3.9), showing decreasing abundances of *A. lobifera* if proceeding northwestward from the Maltese Islands to Favignana Island. Suitable habitats are also predicted for the present-day along the coast of Algeria, Morocco and the Alboran Sea, suggesting that other environmental factors (e.g. dispersal capacity, eutrophication) restrict the current range of *A. lobifera* besides the winter SST. Based on the RCP4.5 scenario, *A. lobifera* is expected to have a high potential to colonize wide areas in the Adriatic Sea, Central and Western Mediterranean. Thus, according to new SDMs provided by this study (Fig. 3.7-8), several areas may potentially be subject to rapid invasions by amphisteginids in the future favoured by ongoing sea warming.

3.5 Final remarks

The Mediterranean Sea is strongly influenced by global warming, since SSTs have increased faster compared to the global oceans. According to this study, the current range extension of *Amphistegina lobifera* is following the SST increasing trend and isotherm shifts recorded in the Mediterranean Sea (Coll et al., 2010; Lejeusne et al., 2010; Bianchi et al., 2013). Future scenarios obtained from new species distribution models predict increases in habitat suitability and a range expansion towards the Western Mediterranean and the northern Adriatic Sea. Up to a few years ago, the colonization of the Western Mediterranean by thermophilic species coming from the Red Sea was considered unlikely due to winter temperatures acting as an oceanographic barrier. On the other hand, the new records and current distribution patterns of *A. lobifera* within the Sicily Channel provided by this work demonstrate a rapid spreading towards the Western Mediterranean.

CHAPTER 4

INVASION HYSTORY OF *AMPHISTEGINA* SPP. IN MALTA ASSESSED FROM SEDIMENT CORES

This chapter is based on an article submitted to *Scientific Reports*, by **Guastella R.**, Marchini A., Caruso A., Evans J., Cobianchi M., Cosentino C., Langone L., Lecci R. and Mancin N., entitled: *Reconstructing bioinvasion dynamics through micropaleontological analysis highlights the role of temperature change as a driver of alien foraminifera invasion.*

4.1 Introduction

Large gaps of knowledge remain in respect to several aspects of the marine bioinvasion phenomenon, such as vectors of introduction, environmental and socio-economic impacts, and spatial and temporal patterns of invasions (Katsanevakis et al., 2014a; Essl et al., 2015; Seebens et al., 2017; Ojaveer et al., 2018). Sporadic monitoring and surveillance, weak taxonomic knowledge, or cryptic behaviour of some species have left several marine invasions undetected for years, decades or even centuries (Carlton 2009; Griffiths et al., 2010; Zenetos et al., 2019). Several approaches have been attempted to compensate for this gap and to reconstruct the timing of first introduction events and the subsequent stages of invasion, such as: re-examination of old museum or herbarium collections (e.g. Hughes & Lowry, 2010; Ahnelt, 2016; Steen et al., 2017), analysis of published descriptions (e.g. Zullo, 1992), interviews to local fishermen or shellfish farmers (e.g. Longo et al., 2007; Bariche et al., 2014; Azzurro et al., 2019), molecular tools (e.g. Panova et al., 2011; Ordóñez et al., 2016; Scorrano et al., 2017, Deldicq et al. 2019) and radiometric dating (e.g. Petersen et al., 1992; Albano et al., 2018). Despite all the different approaches utilized, the gap still persists because long and continuous time-series are difficult to obtain, due to the often sporadic and intermittent availability of marine biodiversity data. Larger organisms such as fishes or bivalve molluscs are pretty well known, because they are regularly caught or searched for by fishermen and shell collectors, respectively (Crocetta et al., 2018; Azzurro et al., 2019). Benthic invertebrates are relatively known too, being the object of routine monitoring (Borja et al., 2009). Conversely, microscopic taxa such as foraminifera or other unicellular eukaryotes can remain unnoticed for several years after their introduction until their populations reach outstanding densities (Gómez, 2019; Guastella et al., 2019 and references therein).

In the present study, a micropaleontological approach was applied in order to investigate, along two sediment cores chronologically constrained through radiometric dating, the first arrival and temporal trends of the foraminiferal non-indigenous species *Amphistegina lobifera* and the

cryptogenic *A. lessonii* in Malta Island (Central Mediterranean Sea). Additionally, the colonization dynamics of the two populations were related with the trends of sea surface temperature (SST) measured in the area, in order to assess whether sea warming has driven their invasion patterns. In the last decade, both species have been reported from the Central Mediterranean, with a northern distribution limit in Albania (Adriatic Sea, Langer & Mouanga, 2016) and a western distribution limit in Favignana Island (Egadian archipelago) at the western tip of the Sicily Channel (Guastella et al., 2019), indicating that the colonization is sharply proceeding favoured by global change (Weinmann et al., 2013; Guastella et al., 2019). Furthermore, profound ecological changes have been documented where *Amphistegina* spp. have become the dominant species (e.g. Langer et al., 2012 and reference therein); consequently, the highly invasive species *A. lobifera* has been included in the list of marine invasive alien species (IAS) as one of the species with high potential impact (Tsiamis et al., 2019). In this respect, combining present observations, future climate projections and literature data from ancient sedimentary successions, hypotheses on the future outcomes of this invasion process in the Mediterranean Sea were also elaborated in the present study.

4.2 Materials and methods

4.2.1 Study area and sample collection

Sampling was carried out in Sliema Creek within Marsamxett Harbour (35°54'16.7"N; 14°30'27.5"E; Fig. 4.1A-C; Appendix 1), a natural bay near station '4' of Yokes et al. (2007). This site was selected after multiple and careful inspections, because it displays the required condition of sediment grain size, shelter, depth and absence of human activities that could have altered sedimentation on the sea-floor. Furthermore, in 2018, it was characterized by a well-established population of *Amphistegina* spp., with relative abundances of *A. lobifera* of ~ 40% (Fig. 4.1B). The rocky outcrops around the site are exclusively composed by rocks belonging to the *Globigerina* Limestone Formation (Aquitania-early Langhian), formed in outer shelf to upper slope environment (e.g. Baldassini & Di Stefano, 2017 and references therein). Thus, no fossil amphisteginids were recorded and the relative abundance of other reworked fossil foraminifera (mainly deep-water taxa such as *Cibicides pseudoungerianus*, *Heterolepa bellincionii*, *Neoeponides schreibersii*, *Reussella spinulosa* and *Spiroplectammina carinata* and the planktonic genera *Globigerina*, *Globigerinoides* and *Globoquadrina*) is very low (~ 3% , Fig. 4.1B; Appendix 2).

For this study, two sediment cores were collected from the selected site: the first one (CORE18) was sampled at 16 m depth in May 2018, and the second one (CORE19) was sampled at 17 m depth on September 2019, respectively (Fig. 4.1C). All the criteria used for the identification of the

sampling site and the methodologies applied for the collection and preparation of the cores are reported in **Chapter 2**, *paragraph 2.2.2* of this thesis.

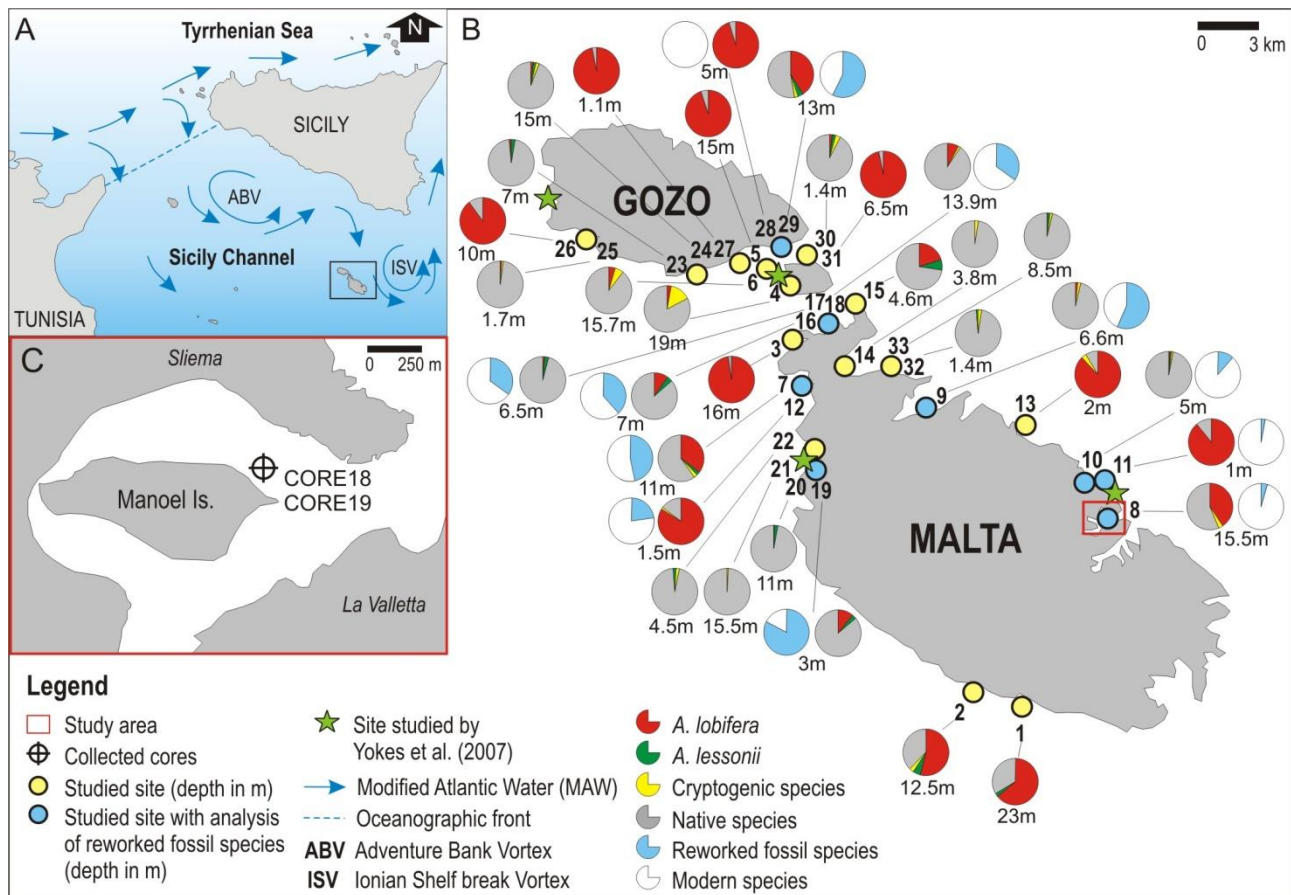


Fig. 4.1: A) Study area (Sicily Channel, Central Mediterranean Sea); B) Current distribution and relative abundance of *A. lobifera* (in red) and *A. lessonii* (in green) (modified from Guastella et al., 2019) and calculated relative abundance of reworked fossil foraminifera (in light-blue); C) Sampled site within the Marsamxett Harbour of Malta Island; the two cores were collected respectively in May 2018 and September 2019.

4.2.2 Grain-size, radiometric and micropaleontological analyses

Both cores were chronologically constrained by measuring activities of ^{210}Pb and ^{137}Cs isotopes along the sedimentary record. In order to do so, two conditions have to be satisfied: sediments have to be fine-grained, since ^{210}Pb usually binds with fine-grained particles suspended in the water column before settling at the sea floor, and cores have to be continuous (without *hiatuses*) and undisturbed (not mixed vertically). Grain-size analysis was therefore carried out for both cores in order to quantify the content of fine-grained sediments along the records and to check for possible erosional unconformities. All the details on the applied techniques for both grain-size and radiometric analyses are reported in **Chapter 2**, *paragraphs 2.5.1* and *2.5.2* of this thesis.

For micropaleontological analyses, core samples (a total of 91, sectioned at 1-cm intervals) were prepared as washed residues and analysed in their foraminiferal content under a stereomicroscope. The absolute abundances of *A. lobifera* and the congeneric *A. lessonii* were calculated along the cores as number of individuals recorded per gram of dry sediment (N g^{-1}). Additionally, the absolute abundance of calcareous nannoplankton was calculated on smear slides as total number of individuals recorded per mm^2 and then used as proxy for hydrodynamism. Since nannoplankton accumulation occurs only in low-energy systems, this independent proxy was used to estimate if the energy at the sampling site was low enough to permit settling of fine-grained particles (size $< 30 \mu\text{m}$) and, consequently, of radionuclides used for the radiometric dating. The applied methodologies are extensively described in **Chapter 2**, paragraphs 2.3.1 and 2.4.1.

4.2.3 Sea Surface Temperature (SST) dataset

The target species *A. lobifera* and *A. lessonii* are indicative of warm tropical and subtropical waters (Langer & Hottinger, 2000) and their range expansion seems to be driven by the ongoing climate change (Langer et al., 2013). Given that, in the semi-enclosed Mediterranean basin, sea warming can be more preponderant than in the open ocean, the Sea Surface Temperature (SST) trend of the Sicily Channel during the last 60 years was taken into account in order to verify its relation with the abundance of *Amphistegina* spp. recorded in the two collected cores. SST was extracted from 3D temperature simulated by the reanalyses of Mediterranean Sea [ref. MEDSEA_REANALYSIS_PHY_006_009, horizontal spatial resolution $\sim 6 \text{ km}$], available as monthly averages for the whole period 1955-2015 years. SST was analysed at 16 m depth (the same bathymetry of the core collection) in the grid point of the model closest to the sampling site of sediment cores [Lat. $35^{\circ}56'15''\text{N}$; Long. $14^{\circ}30'0''\text{E}$]. Starting from the available temperature data (Appendix 3), two different curves were elaborated: curve of annual average SST, obtained by calculating the average SST for each year from 1955 to 2015, and curve of annual wintry average SST, obtained by calculating the average SST during the winter season represented by the months of January, February and March. Additionally, the SST annual and wintry anomalies were elaborated starting from the SST mean during the period 1955-2015 and during the winter seasons, respectively. The first one was obtained by subtracting the SST averaged over the whole period from each annual mean, while the second one was obtained by subtracting to each wintry mean the SST averaged over all winter seasons (from January to March) during the last 60 years.

4.3 Results

4.3.1 Granulometric curves and calcareous nannoplankton content down core

Along both cores, the fine-grained fractions (very fine sand and mud) were continuously present with fairly high abundances varying between 50% and 15% (Fig. 4.2), thus satisfying the first point required to apply ^{210}Pb -dating. High-energy episodes (e.g. storm waves), which could have altered the normal deposition or caused erosional surfaces, can be reasonably excluded due to the absence of abrupt variations in grain-size down core and the continuous occurrence of nannoplankton (Fig. 4.2). This allowed to satisfy also the second point required and to confirm that the collected sediment cores were suitable for radiometric dating through ^{210}Pb .

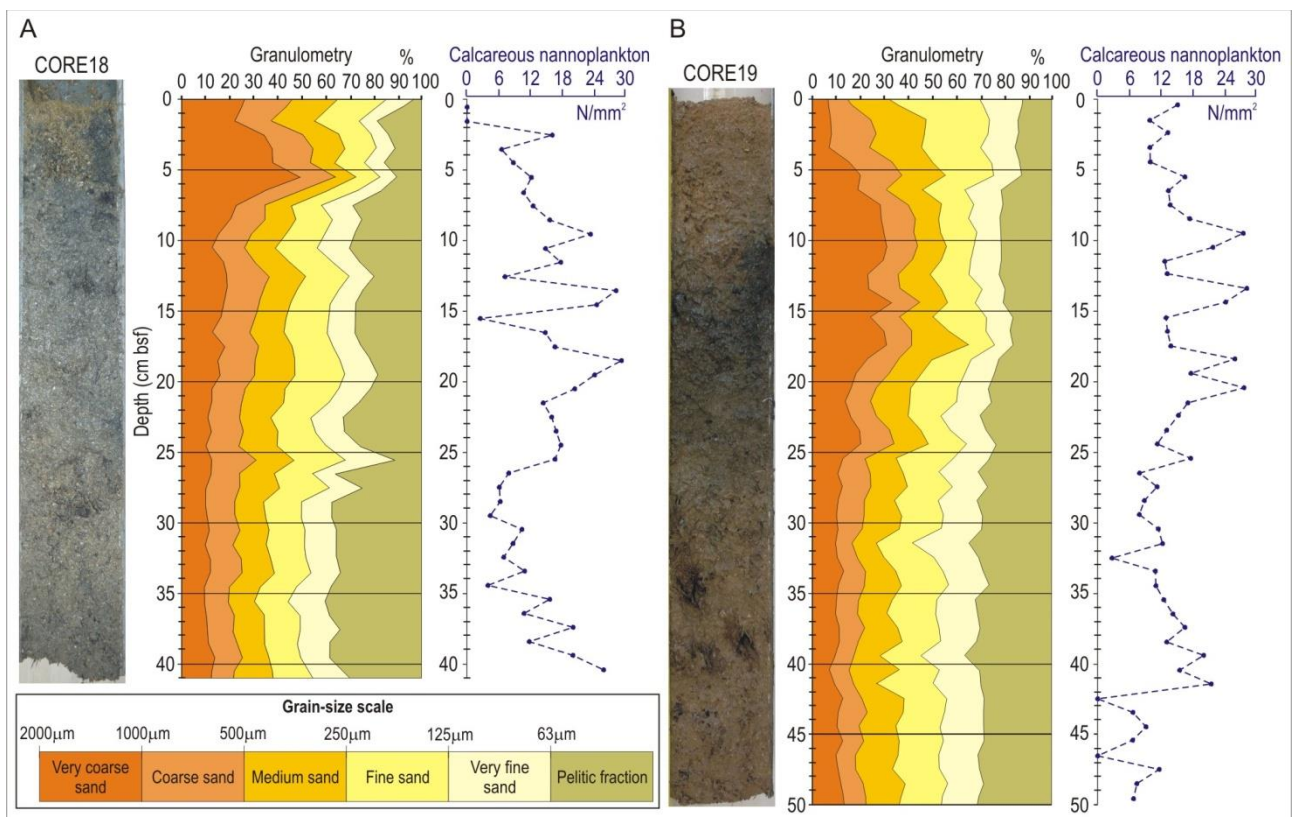


Fig. 4.2: From left to right: photo of CORE18 (A) and CORE19 (B), granulometric curves according to the grain-size subdivisions proposed by Blott & Pye (2012), and curves of absolute abundance of calcareous nannoplankton down core.

4.3.2 Radiometric dating

Along both cores, curves of ^{210}Pb activity were very similar indicating a good replicability of the collected data. Both curves showed the typical activity-depth profile, with higher values at the core top that rapidly decrease down core (Fig. 4.3; Appendix 4). In CORE18, ^{210}Pb isotope rapidly halved within the first 20 cm below sea floor (bsf); this interval was used to calculate a constant

Sediment Accumulation Rate (SAR) of about 0.2 cm yr^{-1} (Fig. 4.3, scatter plot A). The derived age model provided an estimated time interval of about 5 years for each centimetre of sediment. In CORE19, ^{210}Pb activity halved within the first 35 cm bsf furnishing a higher number of measurements that were used to calculate a more refined SAR of 0.22 cm yr^{-1} (Fig. 4.3, scatter plot B) and consequently a second more constrained age model, where each centimetre of sediment cores corresponded to a time interval of about 4.5 years.

Unfortunately, no ^{137}Cs was recorded in either core, thus an independent validation through this method was not possible. While the absence of the Chernobyl peak of ^{137}Cs is common in sediments collected in the southern part of the Mediterranean Sea due to the dispersion pattern of ^{137}Cs fallout that followed the accident, it is surprising to have no signal of nuclear bomb experiments. Nevertheless, the absence of ^{137}Cs in both cores supports the finding that in this area the ^{137}Cs supply is negligible.

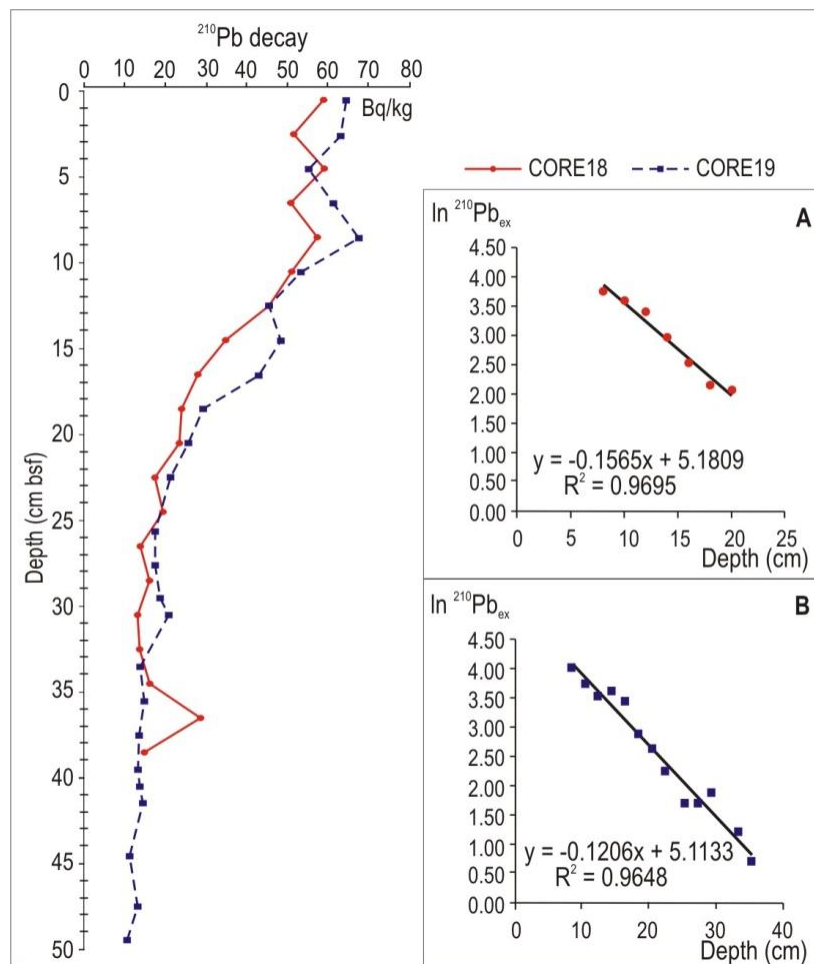


Fig. 4.3: ^{210}Pb decay showing the typical activity-depth profile that decrease down core. Insets on the right report a constant SAR, respectively of 0.20 cm yr^{-1} for CORE18 (A) and 0.22 cm yr^{-1} for CORE19 (B); this last value was utilised in the applied age model, which leads to an estimated time interval of about 4.5 years for each cm of sediment.

4.3.3 *Amphistegina spp. content down core*

Along both records, the absolute abundance curves of *A. lobifera* and *A. lessonii* were characterized by the same distribution pattern down core with a typical decreasing trend (Fig. 4.4; Appendix 2), indicating that the two records were well replicated and have not suffered high energy episodes of vertical mixing. In CORE18 *A. lobifera* was continuously present from cm 0-1 to cm 14-15 bsf, while in CORE19 its lowest occurrence was slightly deeper, corresponding to cm 16-17 bsf. The highest abundances were recorded in the upper portion of both cores, between cm 0-1 and cm 6-7 bsf, from 3.96 N g⁻¹ to 1.11 N g⁻¹ in CORE18 and from 7.06 N g⁻¹ to 1.02 N g⁻¹ in CORE19, respectively. Moreover, in both records the absolute abundances of *A. lobifera* decreased abruptly starting from cm 6-7 bsf down to the lowest occurrences, where the minimum values were recorded (0.28 N g⁻¹ in CORE18; 0.13 N g⁻¹ in CORE19).

The congeneric *A. lessonii* showed the same distribution pattern down core with the highest abundances in the upper portion of both records, respectively from 3.13 N g⁻¹ to 0.85 N g⁻¹ in CORE18 and from 3.06 N g⁻¹ to 1.04 N g⁻¹ in CORE19, then rapidly decreasing from cm 6-7 bsf down to the lowest occurrences, which corresponded to cm 14-15 bsf in CORE18 and to cm 17-18 bsf in CORE19, where the minimum values were recorded (0.28 N g⁻¹ in CORE18; 0.13 N g⁻¹ in CORE19).

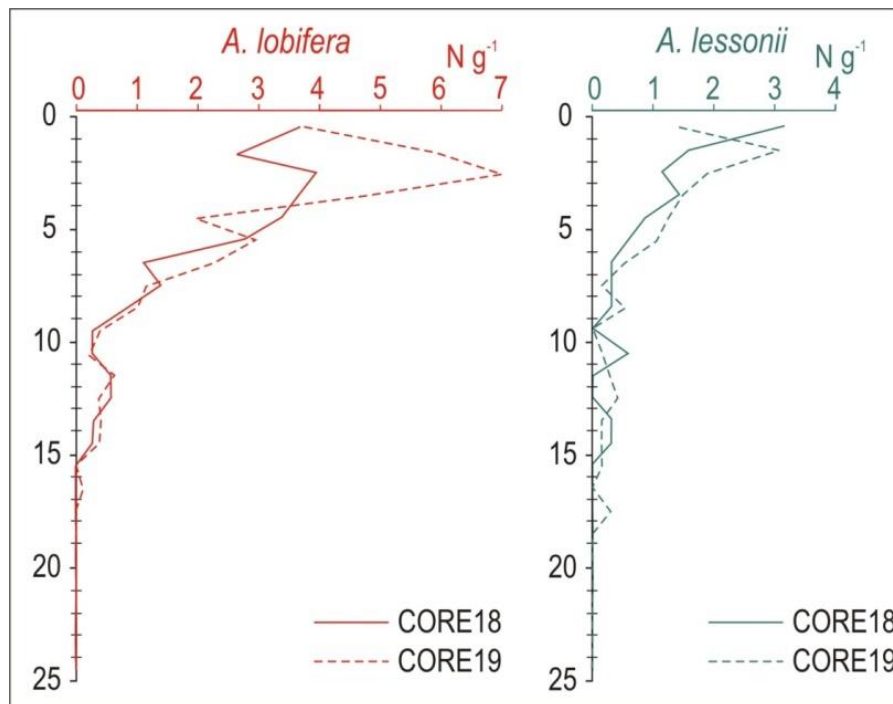


Fig. 4.4: Curves reporting absolute abundances of *A. lobifera* (in red) and *A. lessonii* (in green) with a characteristic decreasing trend moving from core top to bottom.

4.3.4 Sequential stages of invasion and comparison with SST trends

Based on the second age model (1 cm of sediment corresponds to a time interval of 4.5 years), the first occurrence of *A. lobifera* in Marsamxett Harbour can be dated to the mid-1940s (Fig. 4.5). During the first decades of its colonization, up to the end of the 1980s, *A. lobifera* was present but with very low abundances, probably in response to environmental conditions not favourable for the growth of dense populations. Starting from the 1990s, *A. lobifera* increased more rapidly, reaching the maximum peak of abundance between 2005 and 2010, when it was recorded in the Maltese Islands for the first time by Yokes et al. (2007). Thus, the first occurrence of *A. lobifera* was reported in 2006 with a lag time of about 60 years from its true first arrival; additionally, the establishment of dense populations and their spread along the Maltese coasts were reported by Guastella et al. (2019) with a lag time of about 30 years (Fig. 4.5).

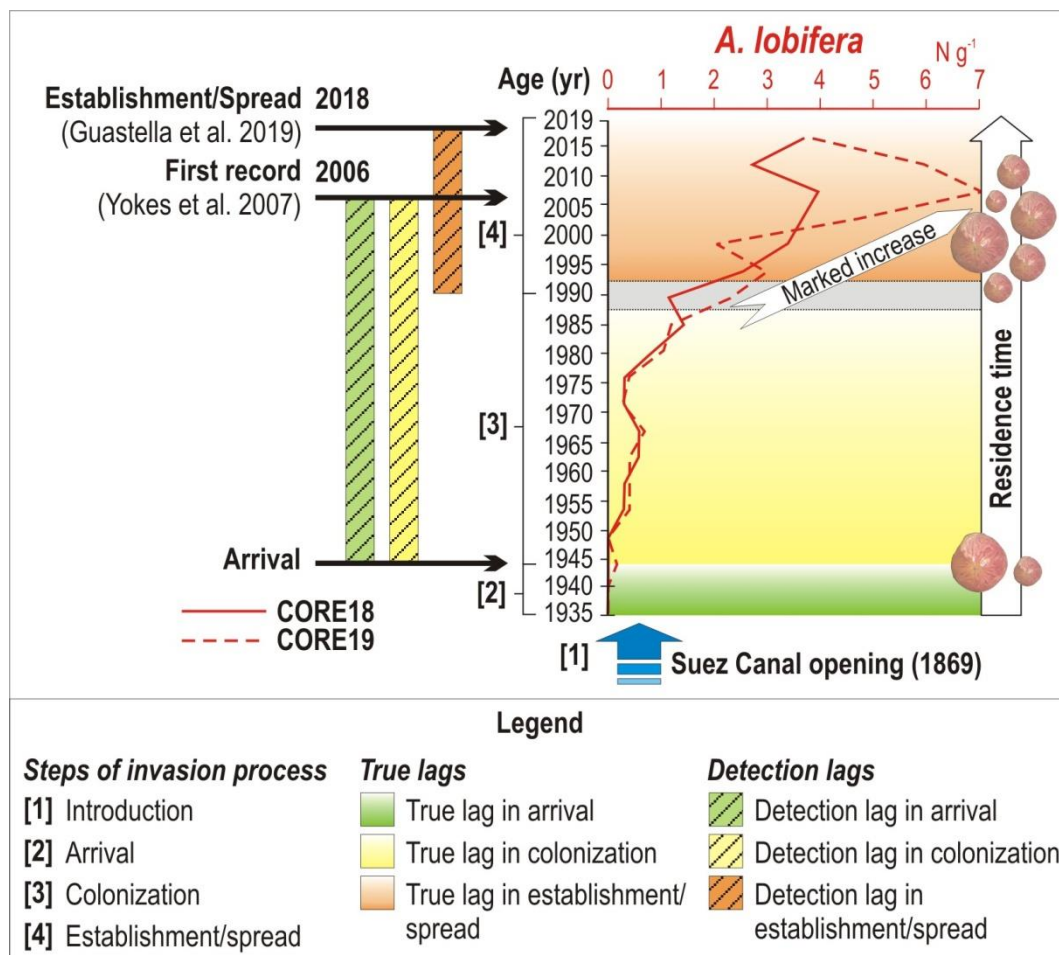


Fig. 4.5: Curves of absolute abundance of *A. lobifera* with the different steps of invasion process (on the left): [1] introduction of the species after the opening of Suez Canal in 1869; [2] first arrival occurred in the mid-1940s; [3] subsequent gradual colonization and [4] establishment and population increase, started in the 1990s. The first record of *A. lobifera* by Yokes et al. (2007) occurred with a lag time of about 60 years, while the establishment/spread unravelled by Guastella et al. (2019) occurred with a lag time of about 30 years.

Similarly, the first occurrence of the congeneric *A. lessonii* in Marsamxett Harbour can be dated at the end of 1930s (Fig. 4.6). Although with lower abundances than *A. lobifera*, *A. lessonii* displayed a similar invasion dynamic: its population was restricted to a few specimens during the first decades, then rapidly increased in abundance starting from the 1990s, up to the first record made in 2018 (Guastella et al., 2019). Thus, the first record of *A. lessonii* in Malta was reported with a lag time of about 80 years, while the establishment of permanent populations with a lag time of about 30 years (Fig. 4.6).

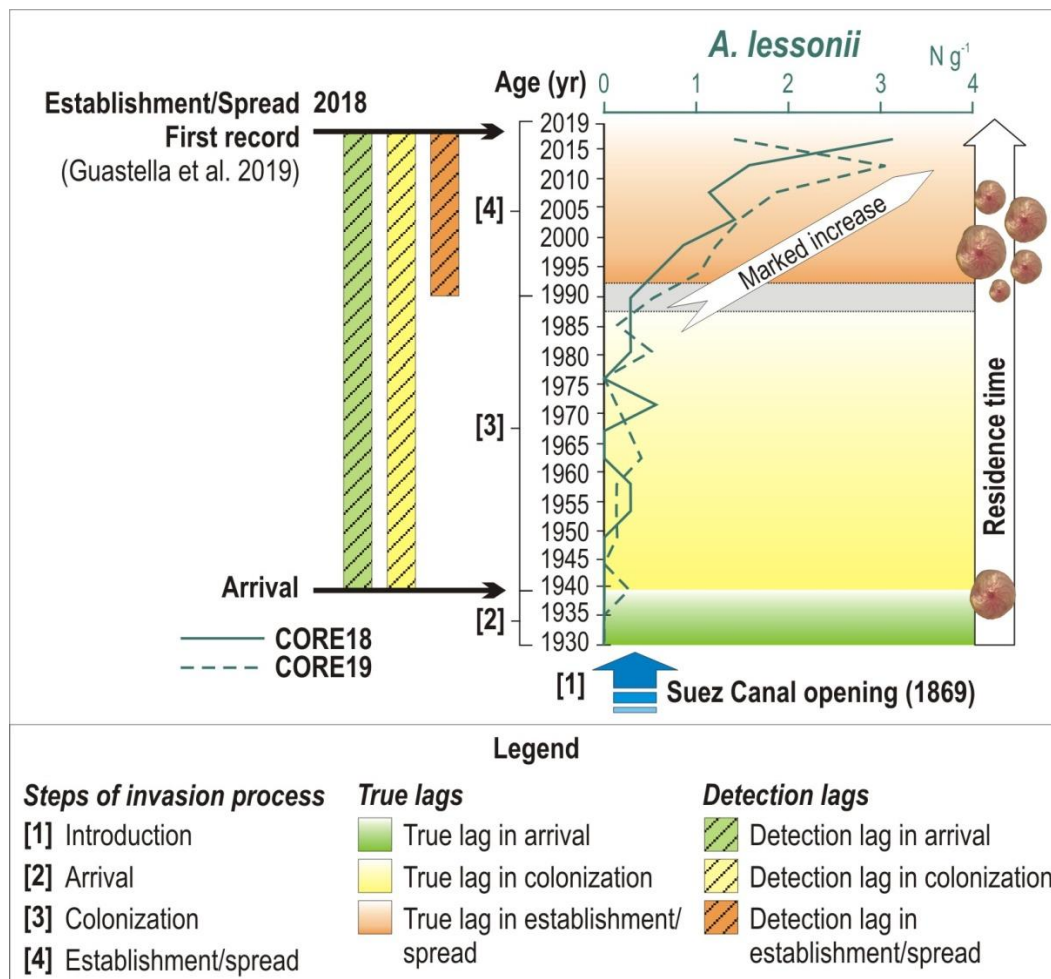


Fig. 4.6: Curves of absolute abundance of *A. lessonii* with the different steps of invasion process (on the left): [1] introduction of the species after the opening of Suez Canal in 1869; [2] first arrival occurred at the end of 1930s; [3] subsequent gradual colonization and [4] establishment and population increase, started in the 1990s. The first record of *A. lessonii* by Guastella et al. (2019) occurred with a lag time of about 80 years, while the establishment/spread occurred with a lag time of about 30 years.

By comparing the increasing abundances of *A. lobifera* and *A. lessonii* recorded along the sediment cores with the annual average SST measured during the last 60 years, a strong similarity emerged (Figs. 4.7 and 4.8). Between the 1980s and the 1990s, a progressive rise of the annual average SST was recorded, with an average overall increase of $\sim 1^\circ\text{C}$; in the same timeframe, *A. lobifera* and *A.*

lessonii abundances started to markedly increase as well. The high Pearson's and Spearman's coefficients (r and r_s) and a p -value of less than 0.05 showed a statistically significant correlation between the increasing annual average SST and the absolute abundances of *A. lobifera* (CORE18: $r = 0.71$, $r_s = 0.75$ and $p < 0.01$; CORE19: $r = 0.79$, $r_s = 0.85$ and $p < 0.001$) and *A. lessonii* (CORE18: $r = 0.71$, $r_s = 0.68$ and $p < 0.01$; $r = 0.74$, $r_s = 0.75$ and $p < 0.01$). A stronger correlation resulted from the comparison with the curve of annual average wintry SST (Figs. 4.7 and 4.8). Since the 1980s, the average SST increased rapidly during the winter, exceeding definitively 15 °C at the beginning of the 1990s and leading today to an average overall rise of ~ 0.7 °C. This correlation was statistically significant, as supported again by the high r and r_s and relative p -value of less than 0.05, for both *A. lobifera* (CORE18: $r = 0.79$, $r_s = 0.78$ and $p < 0.001$; CORE19: $r = 0.78$, $r_s = 0.81$ and $p < 0.001$) and *A. lessonii* (CORE18: $r = 0.61$, $r_s = 0.58$ and $p < 0.05$; CORE19: $r = 0.65$, $r_s = 0.74$ and $p < 0.05$).

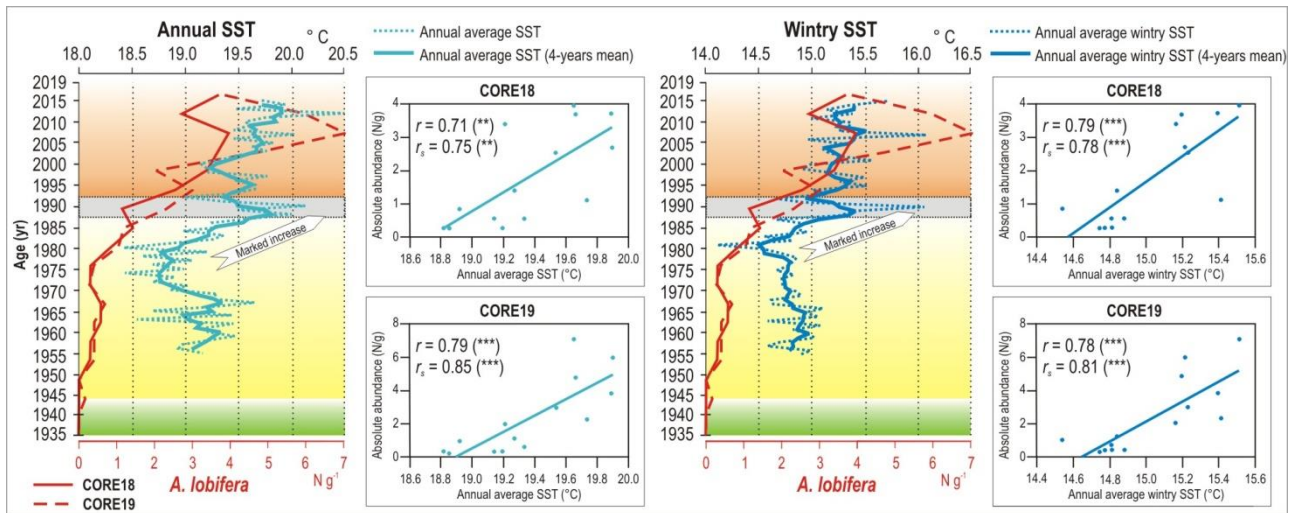


Fig. 4.7: Timeline of absolute abundance of *A. lobifera* plotted against the annual SST (left) and wintry SST (right) simulated since 1955 to 2015, accompanied by scatter-plots of *A. lobifera* abundances recorded in CORE18 and CORE19 compared to the annual average and wintry SST. Pearson's and Spearman's correlation coefficients (r and r_s) are also reported and p -values are expressed with following symbols: $p > 0.05$ (NS), $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

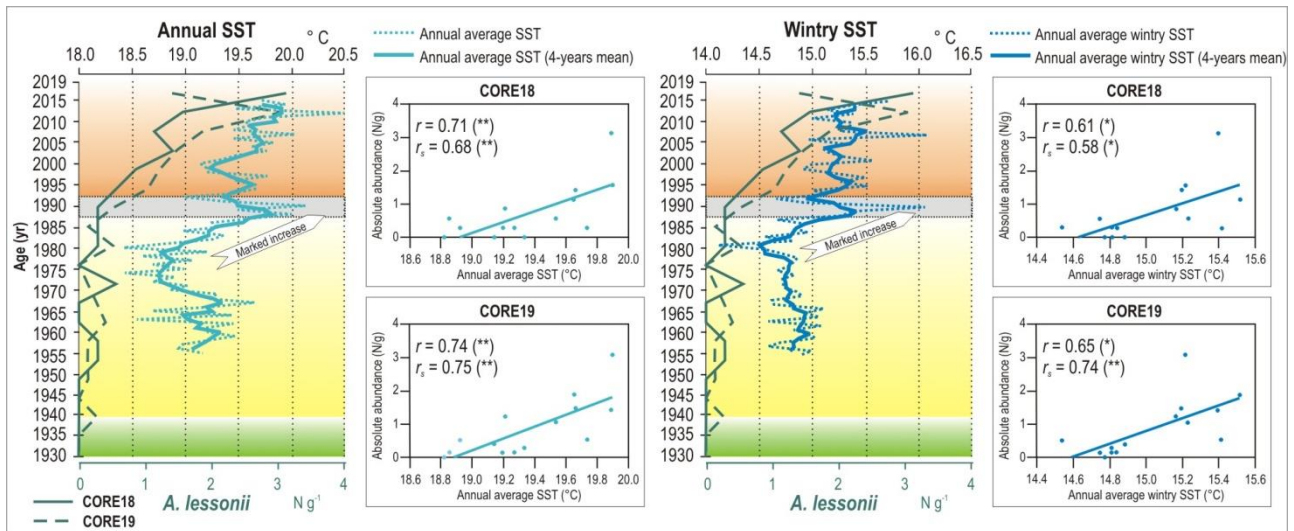


Fig. 4.8: Timeline of absolute abundance of *A. lessonii* plotted against the annual SST (left) and wintry SST (right) simulated since 1955 to 2015, accompanied by scatter-plots of *A. lessonii* abundances recorded in CORE18 and CORE19 compared to the annual average and wintry SST. Pearson's and Spearman's correlation coefficients (r and r_s) are also reported and p -values are expressed with following symbols: $p > 0.05$ (NS), $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***)

The aforementioned relation was further supported by the time series of annual SST anomalies evaluated against the 1955-2015 average, at 16 m depth in the grid point of the model closest to the sampling site of sediment cores in Malta (Figs. 4.9 and 4.10). Figures 4.9 and 4.10 show that both *A. lobifera* and *A. lessonii* increased their abundance only when the annual SST anomaly curve exhibited positive values, corresponding to the warmer phase (with temperature higher than 1955-2015 average) starting from 1985 and still in progress (Marullo et al., 2011). This result was also confirmed by the statistically significant correlation between *Amphistegina* spp. abundances and annual SST anomaly, as evidenced by the high r and r_s and relative p -value of less than 0.05, respectively for *A. lobifera* (CORE18: $r = 0.71$, $r_s = 0.75$ and $p < 0.01$; CORE19: $r = 0.80$, $r_s = 0.86$ and $p < 0.001$) and for *A. lessonii* (CORE18: $r = 0.68$, $r_s = 0.66$ and $p < 0.01$; CORE19: $r = 0.74$, $r_s = 0.76$ and $p < 0.01$). A statistically significant correlation emerged also when comparing the increasing abundances of *Amphistegina* spp. with the annual wintry SST anomaly, as supported again by r , r_s and p -value for both *A. lobifera* (CORE18: $r = 0.79$, $r_s = 0.78$ and $p < 0.001$; CORE19: $r = 0.80$, $r_s = 0.83$ and $p < 0.001$) and *A. lessonii* (CORE18: $r = 0.61$, $r_s = 0.57$ and $p < 0.05$; CORE19: $r = 0.66$, $r_s = 0.72$ and $p < 0.05$) (Fig. 4.8-9).

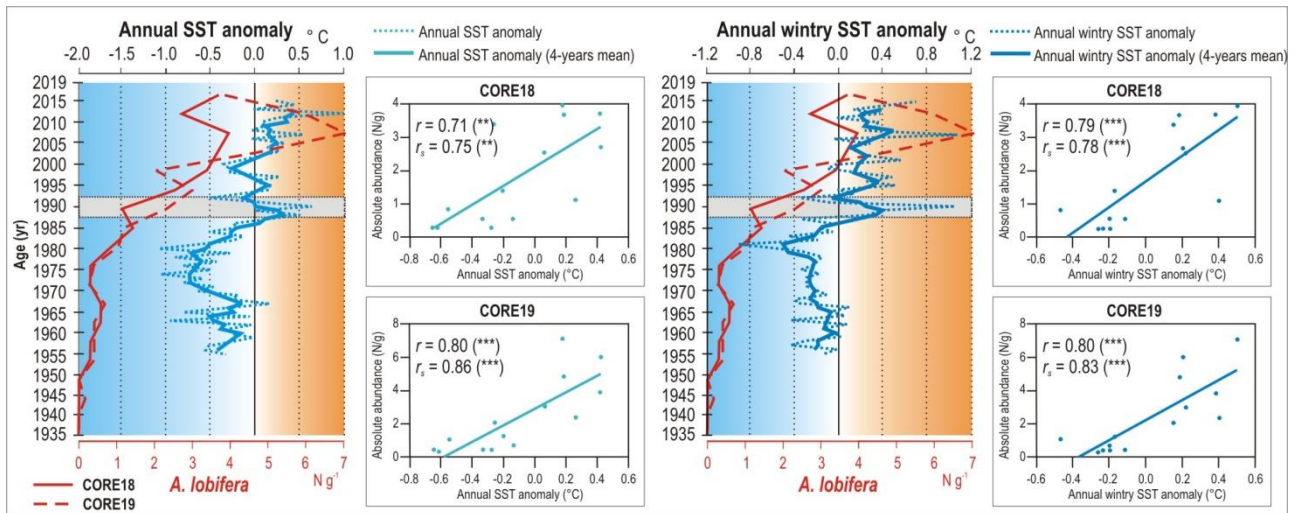


Fig. 4.9: Timeline of absolute abundance of *A. lobifera* plotted against the annual SST anomaly (left) and wintry SST anomaly (right) evaluated over the period 1955-2015, accompanied by scatter-plots of *A. lobifera* abundances recorded in CORE18 and CORE19 compared to the annual and wintry SST anomalies. Pearson's and Spearman's correlation coefficients (r and r_s) are also reported and p -values are expressed with following symbols: $p > 0.05$ (NS), $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

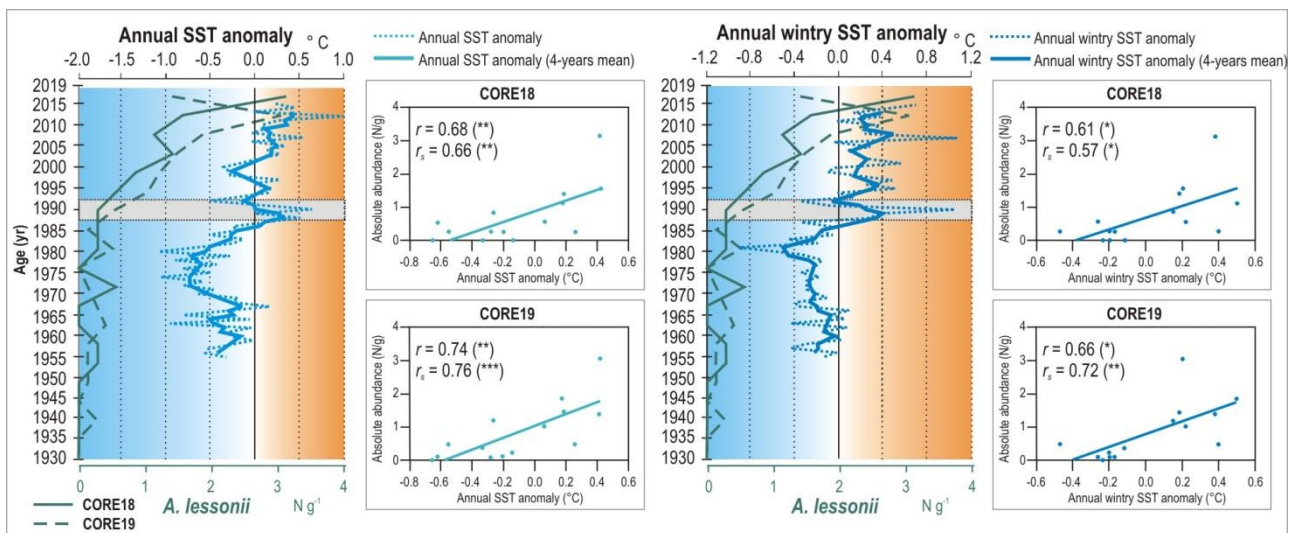


Fig. 4.10: Timeline of absolute abundance of *A. lessonii* plotted against the annual SST anomaly (left) and wintry SST anomaly (right) evaluated over the period 1955-2015, accompanied by scatter-plots of *A. lessonii* abundances recorded in CORE18 and CORE19 compared to the annual and wintry SST anomalies. Pearson's and Spearman's correlation coefficients (r and r_s) are also reported and p -values are expressed with following symbols: $p > 0.05$ (NS), $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

4.4 Discussion

4.4.1 The response of Amphistegina spp. to warming trends

Satellite measurements coupled to complex ocean models and literature data over the last decades have largely documented and predicted sea surface warming in the semi-enclosed Mediterranean

basin (e.g. Shaltout & Omstedt, 2014; Pastor et al., 2017; Sakalli, 2017; Macias et al., 2018). A consistent increase in temperature was documented by the analysis of decadal SST trends starting from 1980-1983, which could be part of a 70-year variation linked to the Atlantic Multi-decadal Oscillation (AMO, Marullo et al., 2011; Pastor et al., 2017), followed by a notable acceleration of the Mediterranean surface warming trend during the last two decades (Pastor et al., 2017; Sakalli, 2017). The redistribution of marine biodiversity and the spread of thermophilic invaders are known to be promoted by global increase in sea temperatures (e.g. Occhipinti-Ambrogi, 2007; Walther et al., 2009; Marras et al., 2015; Molinos et al., 2015; Walsh et al., 2016); therefore, an accelerated warming rate should be taken into serious account when studying relationships between invasion processes and climate change, especially in the semi-enclosed Mediterranean Sea where the climatic signals can be amplified (Pastor et al., 2017). In fact, a progressive shifting of the horizontal and vertical distribution of Mediterranean marine species and an increase of tropical alien species to the detriment of temperate native species due to the sea warming have already been documented (Lejeusne et al., 2010; Raitsos et al., 2010; Marbà et al., 2015; Azzurro et al., 2019; Bianchi et al., 2019).

The present work, based on the analysis of historical records preserved in radiometrically-dated sediment cores, reconstructs the invasion history of cryptic species and unravels the 70 year-long population dynamics of the Indo-Pacific species *A. lobifera* and the cryptogenic *A. lessonii* in the Central Mediterranean, where they have been exposed to a progressive heating. According to data derived from the present study, these two tropical species reached Malta at the beginning of the 1940s, several decades earlier than their first records by Yokes et al. (2007) and Guastella et al. (2019), but they did not develop dense populations for at least 50 years. A reasonable explanation of the protracted lag time between initial introduction and the population explosion can be found in the altered seasonal patterns of sea temperature triggered by global climate change. In fact, the exponential growth of both populations began only after 1990 and accelerated after 2003, when the wintry SST repeatedly exceeded 15 °C (Fig. 4.6) and the warmer phase abruptly increased its intensity and frequency along the timeline (Fig. 4.7). Indeed, as demonstrated by laboratory experiments and direct observations, 15 °C is known to represent a thermal threshold for both *A. lobifera* and *A. lessonii*, which are unable to calcify the tests at surface temperatures below that value (Titelboim et al., 2019). In particular, *A. lessonii* displays higher sensitivity than the congeneric *A. lobifera*, which has so far achieved a higher invasion success in the Mediterranean basin probably thanks to this fact (Titelboim et al., 2019). Conversely, *A. lessonii* demonstrated a better tolerance for higher temperatures (> 32 °C) than *A. lobifera* in laboratory conditions (Schmidt et al., 2016; Titelboim et al., 2019), leading to hypothesize that in the future, according to the

predicted Mediterranean warming (e.g. Sakalli, 2017; Macias et al., 2018), *A. lessonii* could possibly overcome *A. lobifera*.

The geological history of the Mediterranean Sea teaches us that this has already happened in the past. During the Late Pliocene (3.1-2.51 Ma), SST in the southern portion of the Sicily Channel was 6-7 °C warmer than modern average temperatures, which vary between 19 °C and 21 °C (Drago et al., 2010; Herbert et al., 2015; Plancq et al., 2015; Tzanova & Herbert, 2015). In these conditions, *A. lessonii* strongly dominated Mediterranean amphisteginid populations, also characterized by minor abundances of the congeneric species *A. targionii* and *A. gibbosa* (Di Bella et al., 2005). *Amphistegina lessonii* probably developed its acclimation ability earlier, thanks to a longer colonization history during the warmer Pliocene Mediterranean, when the species recolonized the basin after the abrupt extinction that occurred during the Messinian salinity crisis in the Late Miocene (e.g. Hayward et al., 2009 and reference therein). Exactly as in the past, this ability could advantage *A. lessonii* even in the future, considering that the warming trend is going to continue.

This hypothesis, however, might be considered realistic only if invaders spread and acclimate to new environments fast enough to keep pace with climate change (Hiddink et al., 2012; Marras et al., 2015; Molinos et al., 2015). Based on a literature data and meta-analyses, Sorte et al. (2010) estimated that the poleward shift of marine species in response to climate change happens at an average rate of $\sim 19 \text{ km yr}^{-1}$. In the Mediterranean Sea, *A. lobifera* was estimated to have spread at a rate of 13.2 km yr^{-1} (Guastella et al., 2019). This estimate, however, was based on the known sequence of first records of *A. lobifera* along the eastern and central Mediterranean coasts, but the latter has been significantly backdated by the results achieved in the present study. In fact, a 60 years-long delay emerged in the assessment of the ‘first arrival’ of *A. lobifera* in Malta. A similar bias exists for all the Mediterranean records, since the present findings down core precede the first record of *A. lobifera* in the entire Mediterranean Sea. Additionally, according to data provided by this work, the species arrived in the Central Mediterranean at least in the mid-1940s, that is only 70 years after the opening of the Suez Canal occurred in 1869. As calculated along the North-African coasts, Malta is about 3200 km far from Port Said at the mouth of the Suez Canal, a distance that *A. lobifera* (and possibly also *A. lessonii*) have covered in about 70 years with a spreading rate of $\sim 45 \text{ km yr}^{-1}$. This value is consistent with the spreading rates estimated for other Erythraean invaders that move in response to climate change (referred to inter-quartile range of 25-75%: $17.0\text{-}49.8 \text{ km yr}^{-1}$ for continental shelf dispersal and $12.6\text{-}35.1 \text{ km yr}^{-1}$ for straight-line dispersal; Hiddink et al., 2012). Therefore, according to the present results, both species seem to possess the spreading ability for successfully responding to the ongoing climate change.

4.4.2 The micropaleontological approach as a reliable procedure in studies of invasion dynamics

This study is the first one to apply a ‘micropaleontological approach’ for investigating the marine bioinvasion by a small-sized non-indigenous species of benthic foraminifera. In monitoring activities, several advantages can be derived by applying analyses and methods commonly used in Micropaleontology, such as: (1) possibility of analysing long historical records (up to about one century) on a continuous base; (2) possibility of analysing early stages of invasions (e.g. see Walsh et al., 2016), which are often unknown because several alien species tend to remain overlooked for a long time after their first arrival (Carlton, 2009); (3) possibility of directly exploring the response of the target species to environmental parameters (e.g. sea surface temperature). In this work, all the three points mentioned above are treated with a quantitative and replicable approach, differently from several other studies, which compensate for the lack of continuous and direct measurements by reconstructing bioinvasion dynamics from qualitative observations (e.g. Delaney et al., 2008; Azzurro et al., 2019) or from modelling approaches (e.g. Salihoglu et al., 2011; Kanary et al., 2014; Mellin et al., 2016).

In this work, we demonstrated that the micropaleontological approach is a very useful tool in studying of marine bioinvasions, especially for the smallest species that are more likely to be overlooked until they become invasive and for which we need more accurate information on invasion histories. However, using the analysis of sediment core samples to reconstruct the invasion dynamics of non-indigenous marine organisms has some limitations. First of all, this method applies only to taxa with mineralised remains that can persist in tanatocoenoses of sediments, such as protists (e.g. foraminifera, diatoms, radiolarians, dinoflagellates, calcareous nannoplankton), calcifying cnidarians, echinoids, shelled molluscs, etc. (Albano et al., 2018). Then, the core sediment containing the target species should mirror both its habitat (in terms of substratum, grain-size, depth, etc) and its real distribution through time, but at the same should be devoid of vertical mixing, free from bioturbation and free from reworked fossil specimens, which could misrepresent the original first occurrence of their modern counterparts along the core record. Finally, faunal variations along the core record strongly depend on the sediment accumulation rate (SAR); in fact, given that a single centimetre of sediment can correspond to several years, reconstructing annual or seasonal time series and recognizing single peculiar events is only possible with very high SAR.

Nonetheless, the micropaleontological approach deserves to be included in the toolkit for studying of invasion dynamics by the smallest marine organisms with mineralised remains.

4.5 Final remarks

Accurate data on the arrival and temporal dynamics of alien taxa are essential to understand invasion patterns and their drivers, and hence to design and implement effective management measures. Such data are often lacking, especially for small-sized taxa that often remain undetected or are only sporadically recorded until their populations reach outstanding densities.

In the present work, micropaleontological analysis of radiometrically-dated sediment cores was used for the first time to reconstruct invasion histories of two non-indigenous species of benthic foraminifera, the Lessepsian *A. lobifera* and the congeneric cryptogenic *A. lessonii*. Both species have a mineralised shell that persists in sediments after death, thus it is possible to accurately determine along a historical record the real date of introduction and to link the subsequent temporal changes in abundance to variations in environmental parameters.

Using this approach, a considerable lag-time between the first records of the target species and their true first occurrences in the studied area emerged. Additionally, by relating the temporal dynamics of the two populations with trends in sea surface temperature measured in the area, a strong correlation was also demonstrated. This indicates that Mediterranean SST increase has the potential to trigger population outbreaks of thermophilic alien species coming from the Red Sea. Therefore, the micropaleontological approach adopted in the present work can be an important addition to the toolkit for studying bioinvasions, serving to elucidate invasion histories, identify the main drivers of invasion success, and ultimately better inform management decisions.

CHAPTER 5

OCCURRENCE OF *A. LOBIFERA* IN A TSUNAMITE: A CASE OF “FAILED INVASION”

5.1 Introduction

5.1.1 *The problem of the ‘early failed invasions’*

During the last decades, biological invasions have been largely investigated because they represent a serious global environmental problem leading to significant ecological and economic consequences (Zhang et al., 2020). Consequently, invasion ecologists have mainly focused on the study of species that have successfully invaded new areas causing significant damages to ecosystem functioning and biodiversity loss (Lockwood et al., 2013; Jeschke & Heger, 2018), and little attention has been given to failed invasions and to the causes that determine invasion failure (Marchetti et al., 2004; Ings et al., 2010; Zenni & Nuñez, 2013).

Invasion failure can occur during any stage of the process (Blackburn et al., 2011): at early stages, if alien species are not able to survive, reproduce or maintain a sustainable population in the new recipient environment, or at later stages, if they do not spread after naturalization, thus failing to successfully invade the new area (Zenni & Nuñez, 2013). Many factors have been suggested to drive the success or failure of biological invasions (Lockwood et al., 2013; Zenni & Nuñez, 2013; Jeschke & Heger, 2018). Primarily, the propagule pressure could be insufficient to survive demographic stochasticity and sustain the settlement of a new population (Simberloff, 2009). Secondly, habitat match between source and recipient regions could be inadequate and, consequently, the introduced organisms could find unfavourable environmental conditions (e.g. substrate, temperature, salinity, nutrients, light etc.) for their establishment and reproduction – a concept known as ‘abiotic resistance’ (Fenoglio et al., 2016; Zhang et al., 2020). Interspecific relations may also play a role in this: the introduced organisms may have to face novel microbial, herbivore or predator communities, distinct competitive regimes or novel prey species, or may simply be unable to find available ecological niches – a concept known as ‘biotic resistance’ (Levine, 2000; 2004; Colautti & Lau, 2015). Opportunistic traits that increase survival and reproduction in the novel environments are known to promote establishment and proliferation of alien species (Garcia-Ramos & Rodriguez, 2002; Miller et al., 2007). As a consequence, if the newcomers are not able to adjust to new environmental conditions, they have scarce probability to carry out a successful invasion. Genetic bottleneck effects may play a role in this process (Zenni & Nuñez, 2013). Although unreported in the literature, we can not exclude that in rare instances

invasions can fail due to stochastic events, such as forest fires, ice storms, hurricanes and typhoons, that can abruptly change environmental conditions, triggering extinction of newcomers.

In most cases, early failed invasions remain unknown, thus they are very difficult to detect and study, especially when unintentional introductions are concerned (Phillips et al., 2010). However, the identification of failed invasions, as well as the causes that determine failure of an invasion, is fundamental to advance the general understanding of invasion ecology and to support management actions (Carlton, 1996b; Marchetti et al., 2004; Zenni & Nuñez, 2013). Similarly, early stages of invasions are often very poorly known, because species attract researchers' attention only after having successfully established in a new area. This hinders understanding the timing of first introduction events and the subsequent early stages of invasion. A variety of approaches have been used to compensate for this gap and try to reconstruct the colonization history of alien species: re-examination of old museum or herbarium collections (e.g. Hughes & Lowry, 2010; Ahnelt, 2016; Steen et al., 2017), analysis of published descriptions (e.g. Zullo, 1992; Galil et al., 2018), interviews to local fishermen or shellfish farmers (e.g. Longo et al., 2007; Bariche et al., 2014; Azzurro et al., 2019), molecular tools (e.g. Panova et al., 2011; Bock et al., 2015; Cristescu, 2015; Rius et al., 2015; Ordóñez et al., 2016; Scorrano et al., 2017, Deldicq et al. 2019) and radiometric dating (e.g. Petersen et al., 1992; Albano et al., 2018). In particular, the analysis of historical records preserved in sediment cores allows to reconstruct the colonization history of alien species and to identify the first introduction events, as well as possible early failed invasions.

In this study, we report the early failed invasion of the foraminiferal non-indigenous species *Amphistegina lobifera* and the cryptogenic congeneric *A. lessonii* in Malta (Central Mediterranean Sea). This result, which had not been originally expected, was achieved through the analysis of two sediment cores, by using techniques of micropaleontology and radiometry. Additionally, we identify the cause for the invasion failure, that is one of the most catastrophic geological event occurred in the Mediterranean Sea: the 1908 Messina earthquake.

5.1.2 The 1908 Messina tsunami and the effects in the Maltese Islands

One of the most catastrophic natural events on Earth is tsunami, an anomalous wave mostly generated by strong earthquakes or submarine landslides, that is known to be harmless in the open sea but that becomes dangerous when it reaches the coast, because it amplifies its destructive force, flooding wide areas and damaging lands and buildings, causing loss of human life (e.g. Gusiakov et al., 2019 and references therein).

The central Mediterranean Sea has a high tsunami risk, due to its intense seismicity and the presence of several active volcanoes (Papadopoulos et al., 2014). In the Mediterranean Sea, one of the most destructive examples associated with an intense earthquake was the Messina tsunami of 1908, which caused over 80,000 victims and is considered the worst disaster in the modern history of the Mediterranean area (Guidoboni et al., 2007). On 28th December 1908, a severe earthquake (Mw 7.1) happened in the Messina Strait, destroying major towns in the eastern Sicily and southern Calabria, including Messina and Reggio Calabria, and causing a violent tsunami characterized by at least three major waves over 10 m high, which propagated in all directions reaching the coasts of the Maltese Islands, Albania, Montenegro and the Greek Ionian Islands (Guidoboni et al., 2007; Pino et al., 2009). Although more than 100 years have passed after this catastrophic event, the cause of the 1908 Messina tsunami remains unresolved (e.g. Billi et al., 2008); nonetheless, recent studies have proposed a dual earthquake/submarine landslide mechanism (Schambach et al., 2020 and references therein). The tsunami waves arrived in the Maltese Islands, ~ 250 km south of the epicentre (Fig. 5.1A), about one hour after the earthquake, and caused damages mostly along the eastern coasts of the archipelago, as documented by historical chronicles, local newspapers and field evidences (e.g. Borg et al., 2016; Mottershead et al., 2014; 2017). The peculiar topography of the eastern coast, characterized by shore platforms, amplified the impact of tsunami waves that flooded the shores causing massive landslides and the formation of several boulders (Mottershead et al., 2014; 2017; Biolchi et al., 2016). In the Grand Harbour (La Valletta), boats broke their moorings and the house at Msida, Sliema and Pieta were inundated (Borg et al., 2016 and references therein). On the contrary, Marsamxett Harbour, located into a lateral creek, was sheltered from the direct impact of tsunami waves, thus no significant damages to buildings and boats have been reported (Borg et al., 2016).

5.2 Materials and methods

5.2.1 Sampling site and core collection

Two sediment cores were collected at 16 m and 17 m depth, respectively on May 2018 and September 2019, in the natural bay of Marsamxett Harbour (35°54'16.7''N; 14°30'27.5''E; Fig. 5.1A-C; Appendix 1) in Malta Island. All the criteria used for the identification of the sampling site and the methodologies applied for the collection and preparation of the cores are the same as described in **Chapter 4, paragraph 4.2.1** of this thesis. Thanks to its peculiar location within a narrow creek (Fig. 5.1C), the sampling site results partly sheltered by the probable direction of the tsunami waves impacting on the north-eastern coast of the island of Malta.

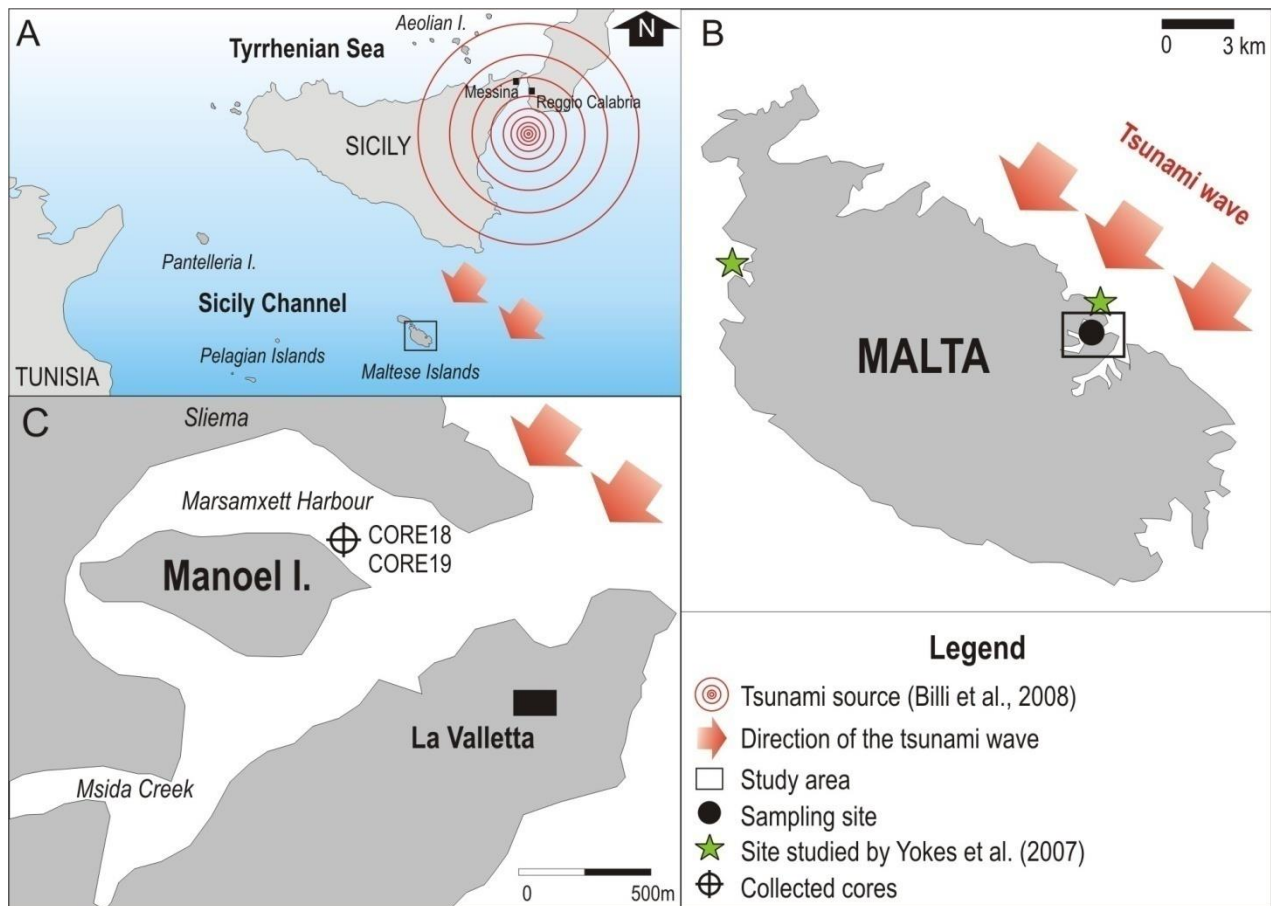


Fig. 5.1: A) Study area located in the Maltese Islands (Sicily Channel, Central Mediterranean Sea) and the epicentre of the tsunami generated by the Messina earthquake occurred in 1908; B-C) Sampled site in Marsamxett Harbour (Malta island).

5.2.2 Sedimentological, radiometric and micropaleontological analyses

The grain-size and radiometric analyses were carried out for both sediment cores according to the methodologies described in **Chapter 2, paragraphs 2.5.1 and 2.5.2** of this thesis. Additionally, the porosity curve was calculated for both sediment cores. Sediment samples were weighed, oven-dried at 55 °C, and then re-weighed to determine water content. Porosity was calculated from the loss of water between wet and dry sediments according to equations suggested by Berner (1971), assuming a sediment density of 2.6 g cm⁻³ and a water density of 1.034 g cm⁻³.

The foraminiferal content was quantified in 91 washed residues prepared according to the procedures described in **Chapter 2, paragraph 2.3.1** of this thesis. The absolute abundance of benthic foraminifera (including the target species *A. lobifera* and *A. lessonii*) was calculated along the cores as number of individuals recorded per gram of dry sediment (N g⁻¹). Epyphitic foraminifera were classified into four groups following Langer (1993): A- permanently attached, B- temporarily attached, C- suspension feeding and D- permanently motile. Taxonomic attributions at

the species level (when necessary) followed Cimerman & Langer (1991) and Hottinger et al. (1993). Quantitative data are reported in Appendix 2. The calcareous nannoplankton content was analysed on standard smear slides using a polarizing light microscope at 1250X magnification, in order to calculate absolute abundance of calcareous nannoplankton as total number of individuals recorded per mm².

5.3 Results

5.3.1 Sedimentological signatures

The first collected core (CORE18) was 41 cm long, while the second one (CORE19) was 50 cm long. Once sectioned, the two cores showed similar sedimentological signatures, thus indicating a good replicability of the collected records (Fig. 5.2). Both cores were gradually getting coarser upwards, which permitted to subdivide the studied records into two distinct portions: lower portion and upper portion. Sediments from the lower portion, starting from the bottom up to about cm 24-25 below sea floor (bsf), mainly consisted of fine and very fine sands and mud (60-70%) and were characterised by a grey colour, probably due to anoxic conditions of the sediments (Fig. 5.2A-B). This was suggested by both the smell of hydrogen sulphide present during the core sectioning and the very high abundance of *Posidonia oceanica* remains, mainly rhizomes well visible to the naked eyes (Fig. 5.2D). Sediments from this core portion were homogeneous, without any appreciable lamination, and contained also abundant mollusc shells (often fragmented) and large benthic foraminifera.

Contrarily, sediments from the upper portion, starting from about cm 24-25 bsf up to top, were mainly formed by very coarse sands and, in equal proportion, by coarse and medium sands. The coarse-grained fractions gradually increased in percentage going up to top, going from 25-30% to 60-70% of the whole sediment, while fine and very fine sands and mud were subordinated; particularly the mud fraction had percentages between 5% and 18% (Fig. 5.2A-B). In this interval, *P. oceanica* remains rapidly disappeared and mollusc shells became rarer. Additionally, the upper portion of sediment cores was characterised by abundant iron Fe-oxides, as well shown by both the rust-coloured tube of CORE19 and the light brown to yellow colour of sediments towards the top of both cores (Fig. 5.2E).

Compared to the grain-size curves, porosity curves showed an opposite trend, with the highest values (0.6-0.8) in the lower fine-grained portion of both cores, then progressively decreasing upwards, along with the increase of sediment grain-size (Fig. 5.2C).

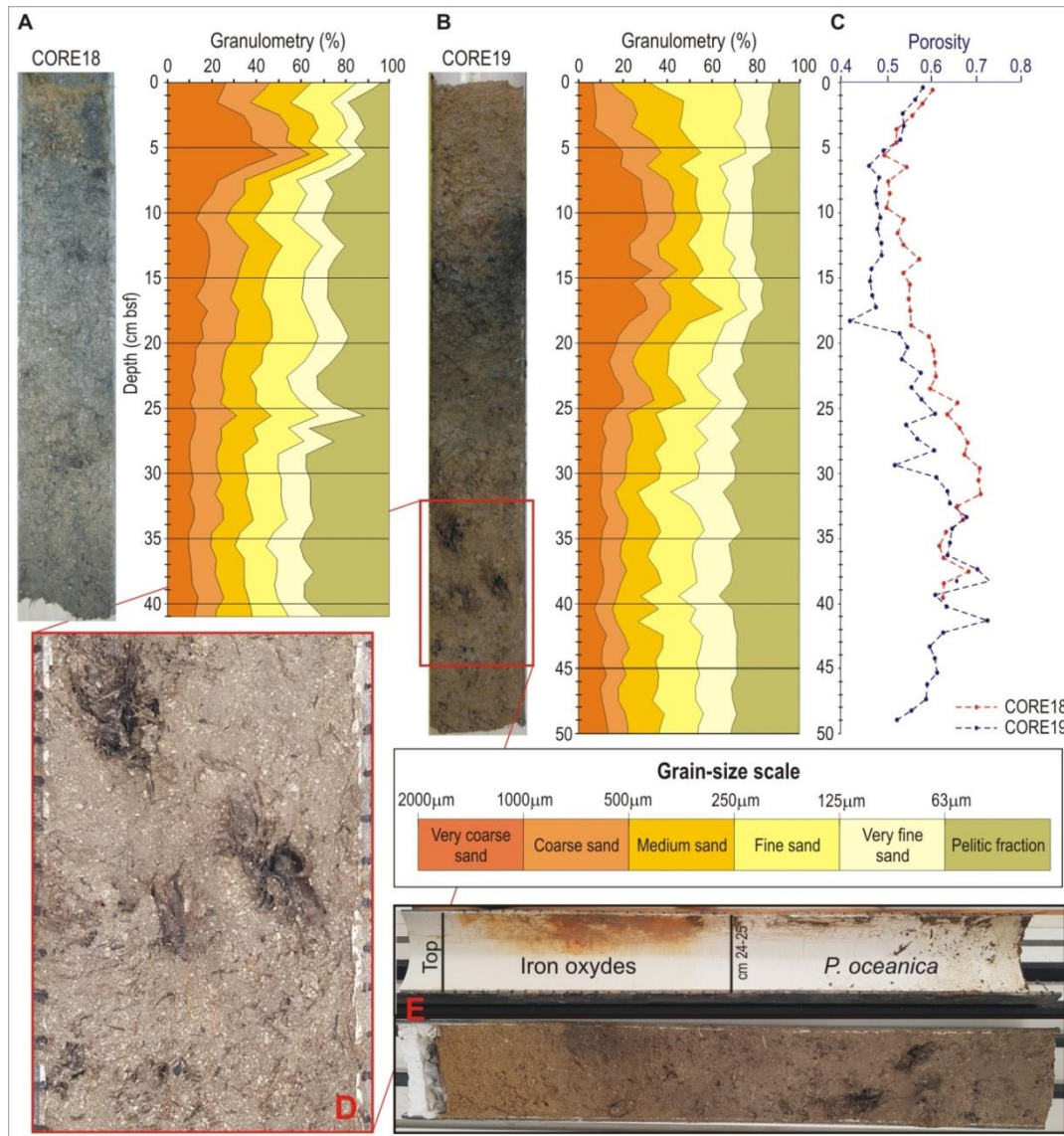


Fig. 5.2: Images of sectioned cores and sediment grain-size curves of CORE18 (A) and CORE19 (B), respectively; C) porosity curves of the studied records; D) Detail of CORE19 showing abundant rhizomes of *P. oceanica* and macroscopic mollusc shells often fragmented; E) Inner side of the tube containing CORE19 (top) and sectioned CORE19 (bottom); note the rust-coloured portion due to high oxidation from cm 24-25 bsf up to the top.

5.3.2 ^{210}Pb and ^{137}Cs chronologies

Both cores were radiometrically dated by measuring activities of ^{210}Pb and ^{137}Cs isotopes along the sedimentary record. As previously described in **Chapter 4, paragraph 4.3.2** of this thesis, the results of this analysis indicate that each centimetre of sediment core corresponds to a time interval of about 4.5 years (Fig. 4.3).

5.3.3 Foraminiferal content down core

In both cores, the same distribution pattern was recorded for both *A. lobifera* and *A. lessonii*, thus indicating that the collected data are well replicated (Fig. 5.3; Appendix 2). In the lower portion of

the records, both species were sporadically and discontinuously present with very low abundances ($< 1 \text{ N g}^{-1}$): the lowest occurrences were recorded at cm 39-40 bsf and at cm 37-38 bsf for *A. lobifera* and *A. lessonii*, respectively, while the highest occurrences were recorded at cm 28-29 bsf and at cm 30-31 bsf, respectively (Fig. 5.3). After an interval of over 10 cm where both species were missing, *A. lobifera* re-appeared in the studied records, namely at cm 16-17 bsf in CORE19 and at cm 14-15 bsf in CORE18, while *A. lessonii* re-appeared at cm 17-18 bsf in CORE19 and at cm 14-15 bsf in CORE18. In the upper portion of both cores, *A. lobifera* and *A. lessonii* were continuously present with an increasing trend of abundance upwards, which became more accentuated from cm 6-7 bsf up to top, where the highest abundances were recorded (Fig. 5.3).

Along both records, benthic foraminiferal assemblage was abundant and well diversified (Appendix 2). The highest total abundance was recorded in the lower portion of both cores up to cm 24-25 bsf (average values of $\sim 1000\text{-}1200 \text{ N g}^{-1}$ in both cores, with an abundance peak of $\sim 2000 \text{ N g}^{-1}$ at cm 47-48 bsf in CORE19), then abundance gradually decreased (respectively up to cm 7-8 bsf in CORE19 and 5-6 bsf in CORE18, with an average value of $\sim 400 \text{ N g}^{-1}$). Finally, in the uppermost centimetres of both records, total abundance rapidly increased again ($\sim 1700 \text{ N g}^{-1}$ at the top of CORE18) (Fig. 5.3; Appendix 2).

Benthic foraminiferal assemblage from both records was dominated by epiphytic taxa, which were continuously and abundantly present along the entire record (Fig. 5.3). Epiphytic foraminifera were mainly represented by permanently motile miliolids (Group D, according to Langer, 1993), particularly the genera *Peneroplis* and *Quinqueloculina*, with total abundances varying between 250 and 1000 N g^{-1} in CORE18 and 200 and 800 N g^{-1} in CORE19. The other epiphytic groups (A-C) presented lower abundances, usually not exceeding 400 N g^{-1} . Group A and B (taxa permanently and temporarily attached, according to Langer, 1993) were characterised by a decreasing trend of abundance upwards, starting from cm 24-25 bsf up to top, that was more accentuated for the epiphytic foraminifera of Group A. In particular, among the taxa belonging to this last group, the species *Miniacina miniacea* disappeared in the upper portion of both records, while the species *Planorbulina mediterranensis* markedly reduced its abundance, going from 440 N g^{-1} at cm 48-47 bsf to $\sim 7 \text{ N g}^{-1}$ at cm 17-16 in CORE19. Finally, the species *Sorites orbiculus* had a decreasing trend of abundance in the lower portion of both records up to cm 24-25 bsf, then, after an interval with a constant low abundance (average values $\sim 10 \text{ N g}^{-1}$), it markedly increased its abundance ($30\text{-}40 \text{ N g}^{-1}$) starting from cm 6-7 bsf up to top. Other benthic foraminifera, such as the epifaunal genus *Ammonia* and the infaunal genus *Textularia*, were always subordinated (Fig. 5.3).

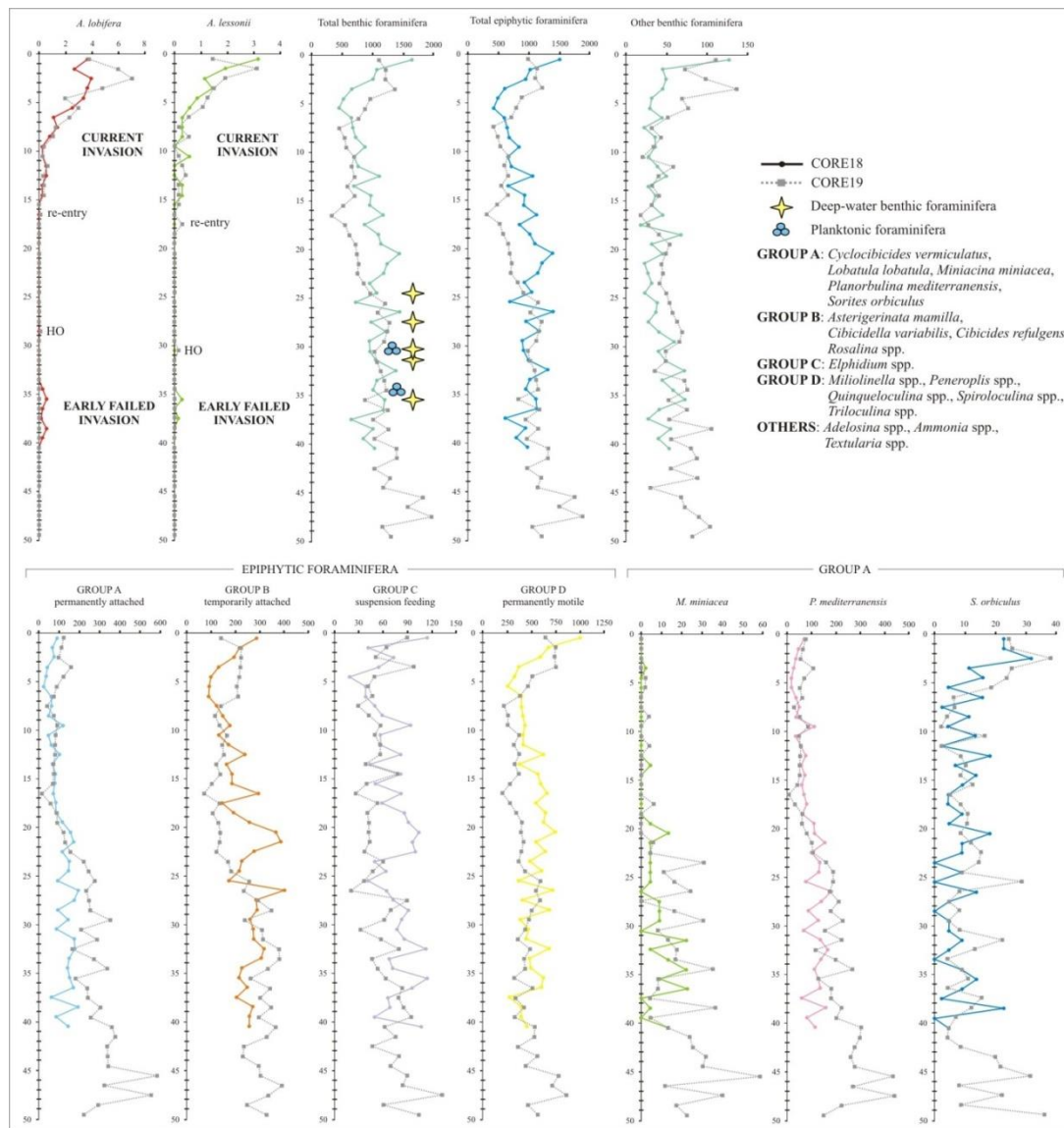


Fig. 5.3: Absolute abundance (as number of specimens per gram of dry sediment; $N\ g^{-1}$) of benthic foraminiferal assemblage along the studied records (from the left): *A. lobifera*, *A. lessonii*, total benthic foraminifera, total epiphytic foraminifera and other benthic foraminifera. Below, absolute abundance of epiphytic foraminifera subdivided into four groups (A-D) according to Langer (1993) (from the left): Groups A to D and some species belonging to Group A.

Along both records, planktonic foraminifera were missing and only very few specimens were found in some samples from the lower portion (Fig. 5.3), occurring together with very rare benthic foraminifera, such as *Bolivina variabilis*, *Buccella granulata*, *Bulimina marginata*, *Reussella spinulosa* and *Uvigerina peregrina*. These infaunal species usually characterise deep sediments in the Mediterranean Sea (Murray, 2006; Milker et al., 2009) and their presence is quite unusual in the littoral zone (Fig. 5.4). Reworked fossil foraminifera, which were very rare and sporadically present along both records, consisted exclusively of few Miocene planktonic specimens belonging to the genera *Globigerinoides* (*G. immaturus* and *G. trilobus*) and *Globigerina*, both of them derived from the Globigerina Limestone Formation that crops out all around the studied site.



Fig. 5.4: Deep-sea benthic foraminifera recorded in the lower portion of sediment cores. 1- *Siphonina reticulata*, cm 31-32 bsf. 2- *Angulogerina angulosa*, cm 31-32 bsf. 3- *Uvigerina peregrina*; a) specimen from cm 31-32 bsf, b) specimen from cm 29-30 bsf. 4- *Siphouvigerina* sp., cm 27-28 bsf. 5- *Reussella spinulosa*, cm 24-25 bsf. 6- *Bulimina aculeata*, cm 30-31 bsf. 7- *Bolivina variabilis*, cm 31-32 bsf. 8- *Brizalina* sp., cm 27-28 bsf. 9- *Fissurina* sp., cm 27-28 bsf. 10a,b- *Buccella granulata*, cm 31-32 bsf. 11- *Lenticulina cultrata*, cm 24-25 bsf. Scale bars are 100 µm.

Finally, very abundant remains of *Posidonia oceanica*, sometimes with epiphytic foraminiferal specimens still attached to the larger fragments, were found in the lower portion of both cores, together with mollusc shells, mainly represented by gastropods and subordinately bivalves, and echinoid plates and spines. Most of mollusc shells were still perfectly coloured, and some of them were broken with sharp edge fractures (Fig. 5.5, images 7-10). On the contrary, the mollusc shells

found in the upper portion of sediment cores were not so well preserved, showing an opaque external surface, sometimes abraded and often bio-perforated by sponges or microscopic algae (Fig. 5.5 image 14).

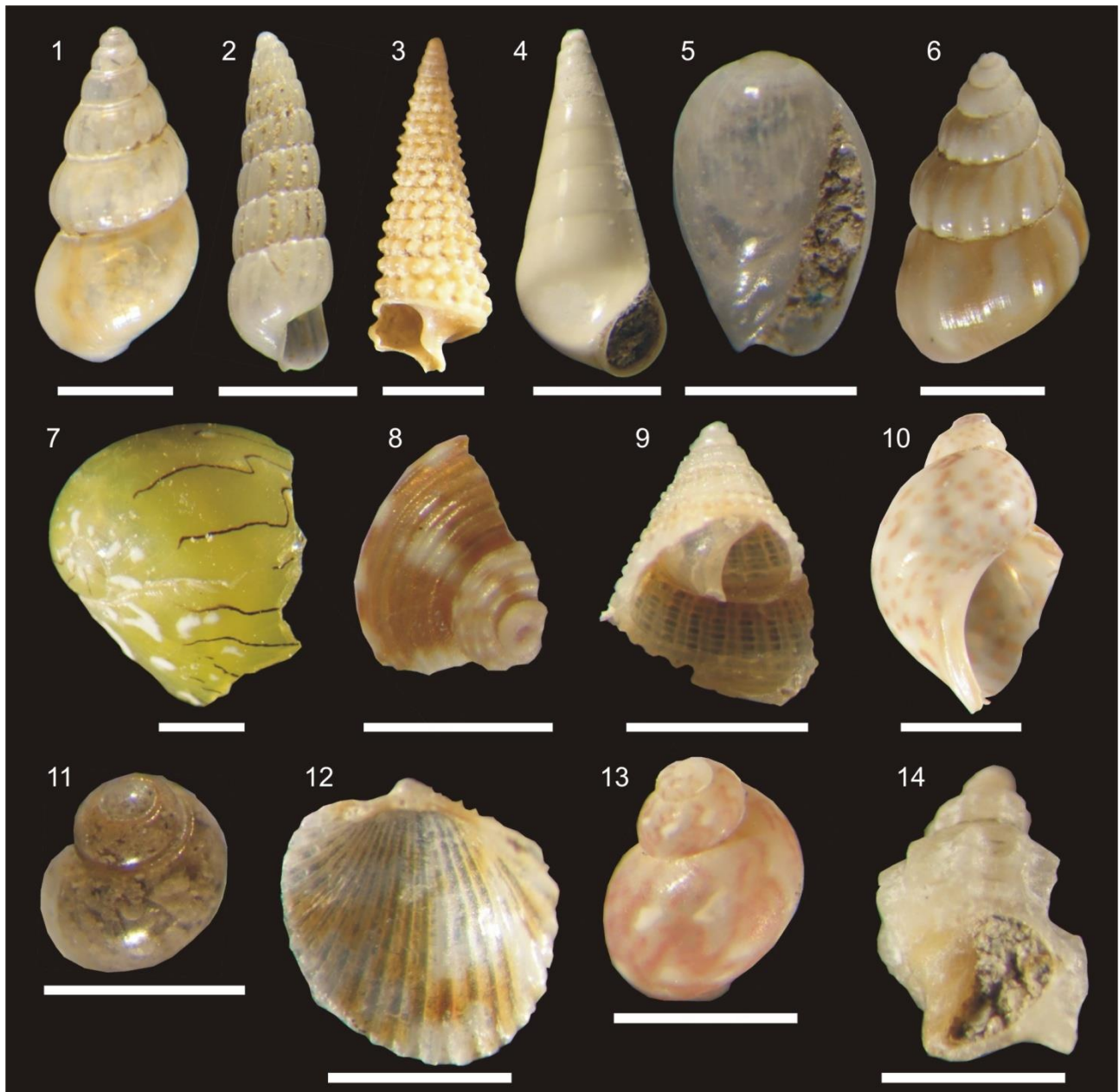


Fig. 5.5: Gastropod and bivalve remains from the lower portion of both sediment cores. One gastropod specimen from the upper core portion (14) is also reported, for comparison purposes. 1- *Rissoa* sp. (Rissoidae), cm 33-34 bsf. 2- *Turbonilla* sp. (Pyramidellidae), cm 32-33 bsf. 3- Triphorid specimen not identified, cm 32-33 bsf. 4- *Parvioris ibizenca* (Eulimidae), cm 29-30 bsf. 5- *Gibberula* sp. (Cysticidae), cm 35-36 bsf. 6- *Pusillina radiata* (Rissoidae), cm 32-33 bsf. 7- Fragmented shell of *Smaragdia viridis* (Neritidae), cm 32-33 bsf. 8- Fragmented shell of *Gibbula ardens* (Trochidae), cm 30-31 bsf. 9- Fragmented shell of unidentified mollusc, cm 31-32 bsf. 10- Fragmented shell of *Tricolia pullus* (Phasianellidae), cm 31-32 bsf. 11- *Rissoella* sp. (Rissoellidae), cm 29-30 bsf. 12- *Parvicardium* sp. (Cardiidae), cm 27-28 bsf. 13- Perfectly preserved shell of *Tricolia pullus* (Phasianellidae), cm 35-36 bsf. 14- Opaque and partly abraded shell of unidentified mollusc from the upper core portion. Scale bars are 1 mm.

5.3.4 Calcareous nannoplankton content down core

Since in shallow waters the wave motions can prevent the deposition of small-sized sediment particles, such as nannoplankton remains ($< 20 \mu\text{m}$ in size), the analysis of calcareous nannoplankton content was used to investigate the hydrodynamic conditions at the sea-floor of the sampling site. The continuous occurrence of nannoplankton down core in both records (Fig. 5.6; Appendix 2), collected at 16 m and 17 m depth respectively, indicated that the energy conditions were suitable for the nannoplankton deposition.

In the lower portion of CORE19, total abundance of nannoplankton had an average value of 8 N mm^2 , starting from the bottom up to cm 41-42 bsf, where a peak of $\sim 20 \text{ N mm}^2$ was recorded. Total abundance was characterised in both cores by a decreasing trend up to cm 26-27 bsf (minimum values of $4\text{-}7 \text{ N mm}^2$) and followed by a marked increase from cm 25-26 bsf upwards (Fig. 5.6). Nannoplankton assemblages were dominated by reworked fossil taxa, late Oligocene to early Miocene in age, supplied from the active erosion of rocks belonging to the *Globigerina* Limestone Formation cropping out all around the Marsamxett Harbour. Modern species were always rare to very rare (abundances varying from 0 to 10 N mm^2) and mainly consisted of the long-ranging *Coccolithus pelagicus*, *Gephyrocapsa oceanica* and *Emiliana huxleyi*. The strategist species sporadically and discontinuously occurred along both records with abundances $< 1 \text{ N mm}^2$. Noteworthy is the sporadic occurrence of *Florisphaera profunda*, a taxon living in the deep photic zone (100-200 m depth) in the open sea, recorded at cm 35-36 bsf and cm 30-31 bsf together with deep-sea benthic foraminifera (Fig. 5.3; Appendix 2).

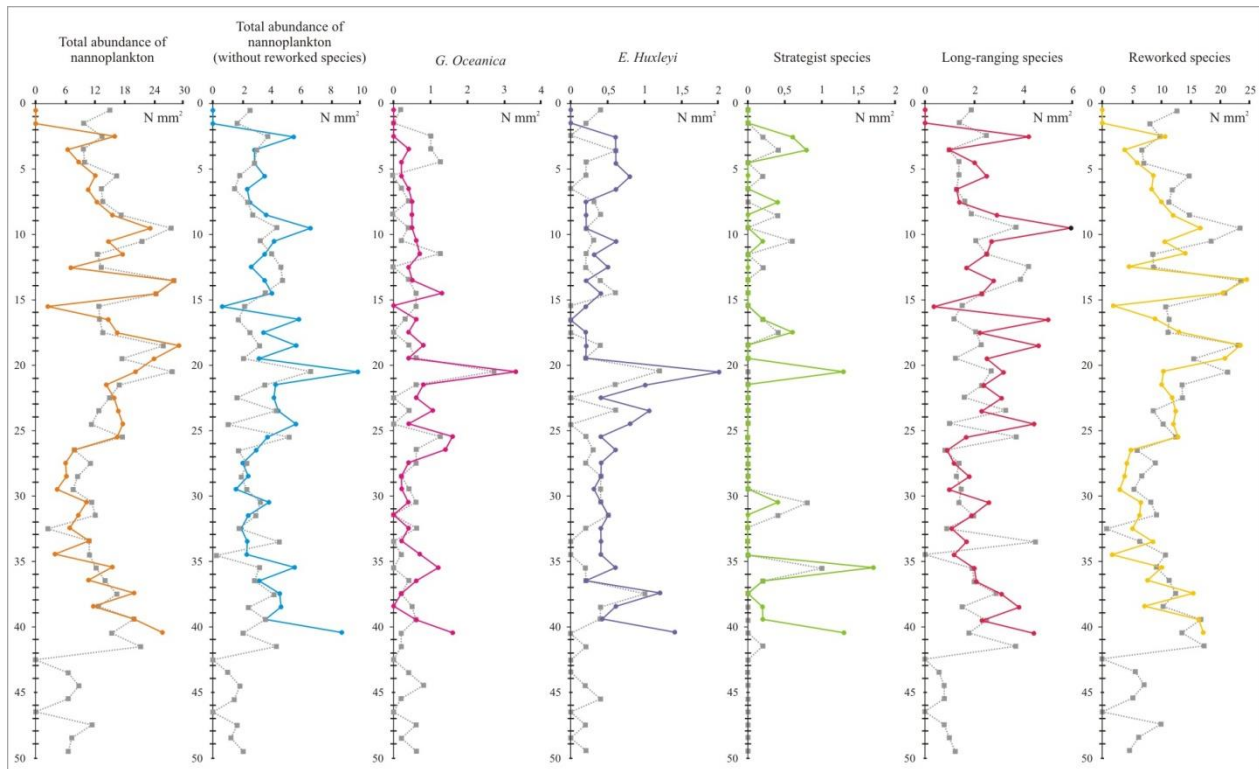


Fig. 5.6: Curves reporting the absolute abundance (N mm^{-2}) of nanoplankton taxa recorded in both cores (from the left): total nanoplankton, total modern nanoplankton (excluding reworked species), *G. oceanica*, *E. huxleyi*, strategist species, long-ranging species and reworked species.

5.3.5 Cumulative evidences of a sudden high energy depositional event

Our samples showed that *A. lobifera* and *A. lessonii* had arrived very early in time in Marsamxett Harbour, Malta (Fig. 5.3). However, their population disappeared soon later and it took a few decades before the two species re-appeared in the records (see **Chapter 4** of this thesis). If the age model derived from radiometric analyses (~ 4.5 years for each centimetre of sediment; Fig. 4.3) is regularly applied along the whole cores, we should conclude that the onset of the early failed invasion recorded at cm 39-40 bsf occurred in the early thirty years of the nineteenth century. However, this is clearly an unrealistic conclusion because the opening of the Suez Canal, where the Mediterranean populations of *A. lobifera* come from (Schmidt et al., 2016; Prazeres et al., 2020), occurred in 1869. Therefore, we formulated two alternative interpretations of the age model to explain the presence of *A. lobifera* in the bottom portion of the cores: I) altered patterns of ^{210}Pb decay, but this seems improbable since ^{210}Pb activity curves recorded in both sediment cores show otherwise; and II) occurrence of a rapid depositional event in the lower portion of both cores, which could have altered the sedimentation rate and caused the sudden accumulation of a thick layer of sediments.

This second hypothesis is supported by the collected data showing several evidences for a sudden high energy depositional event. Both cores can be subdivided into two distinct portions (lower and upper), since significant changes in sediment grain-size, porosity and micropaleontological content occurred at around cm 24-25 bsf. The lower portion of both cores was mainly composed by grey mud and very fine sands with a high porosity and a high content of undecomposed organic matter with symptoms of anoxic conditions, which are compatible with a sudden burial of live organisms. Sediments also contained abundant mollusc shells perfectly preserved, still coloured and, sometimes, broken with sharp edge fractures (Fig. 5.5), definitely different from the depigmented, eroded and bio-perforated shells of the upper layers, which have likely stayed for a long time on the sea-floor before burial. Moreover, very abundant remains of *P. oceanica* with epiphytic foraminifera (e.g. *M. miniacea*, *P. mediterraneensis* and *S. orbiculus*), sometimes still attached to the largest leaves (Fig. 5.2) were present within the mud. Finally, the decreasing trend of the calcareous nannoplankton content indicates a probable increase of the depositional energy at the sea-floor that kept the finest particles suspended. All these evidences suggest a very rapid burial of molluscs, foraminiferal shells and *P. oceanica* remains, probably caused by a high energy depositional event that, at the same time, uprooted *P. oceanica* from the sea-floor, broke mollusc shells and prevented the consistent deposition of nannoplankton. The marked and sudden increase of nannoplankton abundance in the upper portion of both cores, starting from cm 25-26 bsf up to top, suggests a consequent rapid decrease of the depositional energy that allowed the accumulation of the finest sediment particles, as nannoplankton remains. Additionally, the upper portion of both cores was characterised by an increase in sediment grain-size and a consequent decrease of porosity, which probably favoured the circulation of interstitial waters within the sediments and the strong oxidation of iron minerals.

5.4 Discussion

5.4.1 The historical tsunami generated by the 1908 Messina earthquake

Based on the proposed age model of 4.5 years per each centimetre of sediment, the upper portion of both cores, from the top down to cm 24-25 bsf, corresponds to a time interval spanning from 2019-2018 to 1905. In particular, sediments from cm 24-25 bsf have accumulated from 1905 to 1910 thus they probably recorded also 1908, the year of the catastrophic earthquake occurred off the coast of Messina (Sicily). This abrupt event generated a violent tsunami, which reached the Maltese Islands about one hour later impacting on the eastern coast and causing significant damages (Borg et al., 2016; Mottershead et al., 2014; 2017). We argue that the lower muddy portion of both cores could

represent the tsunamiite accumulated by the tsunami waves entering the natural bay of Marsamxett Harbour.

In sediment cores collected from deep-water settings (e.g. off the Augusta Bay, eastern Sicily; Smedile et al., 2011; 2012) tsunamiites are easily recognizable because of their peculiar sedimentary signatures, such as coarse-grained and gradated layers, the evident erosional base and a high content of both epiphytic foraminifera, remains of *P. oceanica* and beach debris displaced from the shore by the backwash wave. On the contrary, in sediment cores collected from coastal areas, tsunamiites are more difficult to detect because, first of all, these transitional environments are continuously subject to reworking by waves and littoral currents and to anthropic activities, and also because tsunami waves generally leave little or no sedimentary record (Dawson & Stewart, 2007; Noda et al., 2007; Judd et al., 2017). Additionally, tsunami deposits may be similar to traces of other high energy depositional events including storm waves, thus leading to mistake frequently tsunamiites with tempestite layers (Nanayama et al., 2000).

In shallow-water contexts, tsunami deposits usually do not exceed a few tens of centimetres in thickness and do not display peculiar sedimentary signatures, except for: I) a high content of mud, sometimes characterised by rare laminae; II) an abrupt erosional surface at the base, which is cut when seabed sediments are suspended, depending on the coastal topography and on the hydrodynamic conditions during transport and sedimentation (Dawson & Stewart, 2007; Papadopoulos et al., 2014); III) abundant biogenic remains, such as mollusc shells, marine plants, ostracods, diatoms and foraminifera, which commonly float within the mud and can show broken shells due to the turbulent water transport (Dawson & Stewart, 2007 and references therein) or sometimes derived from deeper environments and transported landwards by the tsunami waves (Nanayama & Shigeno, 2006; Sugawara et al., 2009; Uchida et al., 2010).

Typical depositions triggered by tsunami waves in coastal settings agree with sedimentological and micropaleontological evidences resulted by this work and described above. According to historical chronicals and local newspapers, in the naturally sheltered bay of Marsamxett Harbour, the 1908 tsunami waves caused only a going and coming of the sea during the day (Borg et al., 2016 and references therein). The oscillatory wave movement probably remobilised the sea-bottom sediments, keeping the mud fraction suspended, but without any significant transport landwards nor offshore. Tsunamiite recorded in the studied cores is ~ 25 cm thick and is mostly composed by homogeneous mud and silt containing abundant remains of *P. oceanica* (Fig. 5.2), mollusc shells (Fig. 5.5) and large epiphytic foraminifera, including *A. lobifera* and *A. lessonii* (Fig. 5.3). The occurrence of mollusc shells, often broken but with perfectly preserved and still coloured

periostracum, suggest a sudden burial probably caused by a turbulent water flux (Fig. 5.5). Deep-water benthic foraminifera (Fig. 5.4), very rarely recorded in both cores, were probably been transported landwards by the tsunami waves, together with a few planktonic foraminiferal specimens and open-sea calcareous nannoplankton, such as the species *Florisphaera profunda* (Fig. 5.6). Unfortunately, both cores failed in recording the tsunamiite base, since the basal erosional surface and the abrupt change in sediment grain-size are missing. Therefore, it is impossible to determine when the two *Amphistegina* species really arrived in Marsamxett Harbour. Yet, we can document the presence of their tests within sediments deposited before 1908 and, then, remobilised by the tsunami waves, which is a surprising and significant backdating of their first occurrence in the central Mediterranean Sea.

5.4.2 Triggering mechanisms for the early failed invasion of *Amphistegina* spp.

The 1908 tsunami may hence have caused the failure of the early invasion of *Amphistegina* spp. in Marsamxett Harbour through a cascade of events. First, the deposition of the tsunamiite abruptly buried the seagrass meadow under over 25 cm of muddy sediments, damaging and causing a drastic change of habitat for most benthic foraminiferal species, including the target taxa. In fact, the *P. oceanica* meadow originally existing in the harbour was probably unable to recover and develop new patches, as documented by the absence in both cores of *P. oceanica* remains above the tsunamiite. Since this plant has very low growing rates (Marbà et al., 2002; Cabaço et al., 2008; Pace et al., 2017; Noè et al., 2020), the recovery of *P. oceanica* meadows is considered irreversible in the human time-scale. Therefore, the sea bottom of Marsamxett Harbour seems to have irremediably changed after the 1908 tsunami.

All the benthic community was directly influenced by this sudden depositional event, as supported by the decreasing trend in abundance of permanently and temporarily attached epiphytic foraminifera (Fig. 5.3), which suffered a rapid decline and never fully recovered in the subsequent years, as documented by the disappearance of *M. miniacea*. The two *Amphistegina* species, which abruptly disappeared in Marsamxett Harbour, may have experienced a similar fate. It was only after 30 years, in the early 1940s, that *Amphistegina* spp. made a new appearance in the Marsamxett bay, starting their current, more successful, invasion process (see **Chapter 4** of this thesis).

5.5 Final remarks

This study exceptionally documents two natural events that are usually very difficult to detect both in Geology and Marine Ecology: the impact of a tsunami generated by a historical earthquake and

an early failed invasion by a minute alien species, which are here related by a cause-and-effect relationship.

Recent genetic studies have irrefutably demonstrated the Indo-Pacific origin of *A. lobifera*, which entered the eastern Mediterranean Sea via the Suez Canal (Prazeres et al., 2020). This study documents that *Amphistegina* spp. reached Malta very early, within 40 years of the inauguration of the Suez Canal in 1869. This makes *A. lobifera* one of the most precocious Lessepsian invaders in the central Mediterranean Sea, and proves that this species has spreading capabilities much higher than previously believed, even exceeding the already remarkable results obtained in **Chapter 4, paragraph 4.4.1** of this thesis. However, this early colonization attempt in Malta failed due to a sudden and unpredictable geological event such as the tsunami wave generated by the 1908 Messina earthquake. Only 30 years later, *Amphistegina* spp. re-appeared in Marsamxett Harbour and successfully started its second and more successful invasion process.

Unfortunately, these results are very limited in space, being referred to a small enclosure along the coast of Malta Island, and nothing conclusive can be said about the real invasion history of *A. lobifera* along the whole Maltese Islands, its survival after the 1908 tsunami elsewhere in the region, and its re-appearance in the 1940s (as documented in **Chapter 4** of this thesis). In particular, the re-appearance of *Amphistegina* spp. populations may be due to the arrival of novel propagules from the Eastern Mediterranean, or from other local populations in Malta having survived the 1908 tsunami. Despite repeated attempts to collect additional core samples from other sites along the Maltese Islands or along the southern Sicilian coasts, we failed to identify a site with all the suitable features (listed in **Chapter 2, paragraph 2.2.2** of this thesis) to carry out a similar analysis.

Nevertheless, we can assume that *Amphistegina* spp. remained very rare in Malta Island for several decades, probably due to the environmental conditions still not favourable for the development of dense populations (see **Chapter 4** of this thesis), causing *Amphistegina* spp. to remain overlooked by marine biologists until the beginning of the twenty-first century, when it was recorded for the first time in 2006 by Yokes et al. (2007). By comparing the early failed invasion of *Amphistegina* spp. in Malta with the more recent records of the current colonization process from the eastern and central Mediterranean Sea, it becomes evident that significant mistakes can be made about the first records of alien species, and that very little is known about this phenomenon.

Interestingly, this study reports several evidences of the 1908 Messina tsunami from two sediment cores collected in the shallow water of Marsamxett Harbour (Malta Island), located about 250 km away from the earthquake epicentre. This sudden geological event, in fact, not only resulted in over

80,000 victims and damaged lands and buildings in southern Italy (Guidoboni et al., 2007), but also triggered massive landslides along the eastern coasts of the Maltese Islands (Mottershead et al., 2014; 2017; Biolchi et al., 2016) and, in the Marsamxett bay, dramatically changed the marine habitat. Therefore, this type of stochastic events represents a serious risk not only to human life, buildings and activities (e.g. Galea, 2007) but also for the preservation of marine environments, especially along coastal areas.

In conclusion, the analysis of historical records preserved in sediment cores radiometrically dated allows not only to reconstruct the invasion dynamics of alien species and to detect possible early failed invasions, leading to a better understanding the biological invasion mechanisms, but also to relate the invasion failure to natural events, such as tsunami waves, that could abruptly interrupt or alter the colonization process by alien species.

CHAPTER 6

THE POTENTIAL IMPACT OF *A. LOBIFERA* ON NATIVE FORAMINIFERAL ASSEMBLAGES OF THE SICILY CHANNEL

This chapter describes and discusses preliminary data addressed to satisfy Aim 3. Unfortunately, it was not possible to obtain the complete data set within the end of my doctorate and, consequently, to carry out all the statistical analyses as foreseen in the designed experimental plan because of two main reasons: I) the unexpected results of Aim 2 (see **Chapters 4 and 5**), obtained by the analysis of a first pilot sediment core, required more study through the sampling and analysis of a second core. As a consequence, the work conducted for the whole Aim 2 took longer than originally planned; II) due to the COVID-19 pandemic and the consequent lockdown, the access to the Micropaleontology laboratory of the Department of Earth and Environmental Sciences (University of Pavia) was forbidden from March to June 2020 to both PhD and master degree students, consequently, part of micropaleontological and sedimentological analyses originally planned for spring 2020 were not performed.

6.1 Introduction

Alien marine species may become invasive with severe impacts on biodiversity and ecosystem services (Katsanevakis et al., 2016 and references therein). Although the majority of introductions have only minor effects to the ecosystem, some of them can lead to numerous problems, especially when they have distressing impacts on native biota, causing decline or even extinctions of native species (Thomas et al., 2016). Ecological impacts of invasive alien species are known to span from single-species interactions to entire population declines, from change in community composition to local extinctions, with consequent effects on ecosystem functioning (Blackburn et al., 2014; Katsanevakis et al., 2014b). Therefore, understanding and quantifying the impacts of invasive alien species on native communities are fundamental to assess risk, in order to inform environmental management and set operational objectives to develop mitigation measures (Ojaveer et al., 2015).

In the recent decades, several studies on ecosystems of specific locations have reported the ecological impacts of single or groups of marine alien species, mainly represented by crustaceans, molluscs, macroalgae and fishes (e.g. Katsanevakis et al., 2014b). However, most impacts of bioinvasions are difficult to detect and measure in the marine environment, and standardised frameworks to evaluate impacts are difficult to develop (Ojaveer et al., 2015). In particular, very little is known about the impacts of inconspicuous, small-sized and cryptic marine alien taxa, such as foraminifera. This allows ‘hidden invaders’ to colonize wide marine areas without being noticed

and, in some severe cases, to be recorded only after having already caused significant damage to native communities and, consequently, to local ecosystems.

This is hypothesised to be the case of *Amphistegina lobifera*, the most widespread and successful invader of benthic foraminifera in the Mediterranean Sea, which has been notably rapidly expanding its invasion range. Within its early introduction range, the Levantine basin, consequences of its establishment on ecosystem functioning, species diversity, carbonate production and diversity of native biota have been observed (Langer et al., 2012; 2013; Mouanga & Langer, 2014). In fact, in the Eastern Mediterranean, *A. lobifera* has very successfully established and reproduced, becoming dominant in local benthic assemblages and altering community structure. Worse, due to the mass deposition of its carbonatic remains forming biogenic sands, it has been able to modify rocky bottom habitats of Israel, Turkey and Greece (Hyams et al., 2002; Meriç et al., 2008; Triantaphyllou et al., 2009; Çinar et al., 2011; Langer et al., 2012; Schmidt et al., 2015). That is why amphisteginids were included among the 100 worst invasive species in the Mediterranean Sea (Streftaris & Zenetos, 2006). Although *A. lobifera* has been considered as one of the benthic foraminiferal NIS with the highest potential impact (Tsiamis et al., 2019), the real effects of its colonization on native assemblages and, consequently, on local ecosystem, are poorly known, especially in the Central Mediterranean where *A. lobifera* was only recently recorded (Yokes et al., 2007; Caruso & Cosentino, 2014; Guastella et al., 2019).

The present study aims to measure the potential impact of the highly invasive *A. lobifera* on native benthic foraminiferal assemblages in the Sicily Channel (Central Mediterranean), by comparing the effects on community structure among different stages of invasion: advanced, medium and early. To achieve this, a preliminary analysis was carried out, based on data available from Aim 1 (Appendix 2), in order to establish a suitable experimental design. First, we examined the community structure of native foraminiferal assemblages from samples in our study area (from both Malta and Sicily) that contained very few or no individuals of *A. lobifera*, and which were therefore unaffected or only marginally influenced by this species, in order to evaluate their comparability. Secondly, based again on results of Aim 1 (**Chapter 3** of this thesis), we identified areas within the Sicily Channel that could represent the status of ‘advanced’, ‘medium’ or ‘early’ stage of invasion, obtained from the relative abundances of *A. lobifera* in the foraminiferal assemblages. Third, we designed and carried out an experimental plan aimed at estimating the impact of *A. lobifera* across three stages of invasion on community structure variables: species composition and richness, diversity and evenness.

6.2 Materials and methods

6.2.1 Preparatory analyses and experimental design

Firstly, using the dataset obtained from Aim 1 of this thesis (Fig. 1.2), available as Appendix 2, the composition of native foraminiferal communities was analysed in six sediment samples collected at 1-3 m depth in three sites of the Maltese Islands (19. Gnejna – inner; 25. Xlendi – inner; 30. Santa Maria – inner) and Sicilian coast (40. Agnone; 43. Capo Passero; 45. Pozzallo) respectively, where *A. lobifera* was very rare to absent. In each sample, species richness (S), diversity (H') and evenness (J') were calculated and qualitatively compared across sites, in order to verify if native foraminiferal assemblages in the Sicily Channel had a common structure, or exhibited high variability of community indices, which could possibly mask the impact of the introduced *A. lobifera*. Afterwards, the results of Aim 1 reported in **Chapter 3**, paragraphs 3.3.1, 3.3.2 and 3.4.1 (Figs. 3.3, 3.4 and 3.9) of this thesis, were carefully analysed to identify areas within the Sicily Channel that exhibited characteristics of ‘advanced’, ‘medium’ and ‘early’ stage of invasion. Based on the relative abundance of *A. lobifera*, these areas were identified as follows: Maltese Islands – advanced stage; Southern Sicily – medium stage; and Southeastern Sicily – early stage. The experimental plan of Fig. 2.4 was then designed and applied. The original sampling plan involved the subdivision of each area into two sub-areas, and the collection of samples from two sites in each sub-area: this would have implied collection of samples in 12 sites: 3 areas x 2 sub-areas x 2 sites. However, it was not possible to identify many suitable sites for benthic foraminifera colonization in all the three areas, either because the sea-bottom was rocky at the selected depths, or because the sediment grain-size was too coarse. Therefore, the selection of random sites for sampling was constrained by habitat availability, and the original experimental design was simplified into the following. For each study area, three different sites were sampled (total: 9 sites) at two different bathymetries (2-5 m and 15-20 m depth). Four replicates were collected at each site, for a total number of 72 collected sediment samples. Following the FOBIMO recommendations (Schönfeld et al., 2012), at each site and depth, samples (of approximately 30 g) were collected from the uppermost part of the sea-bottom using a small corer operated by a SCUBA diver (Fig. 6.1). Further details on sampling sites are reported in Appendix 1.

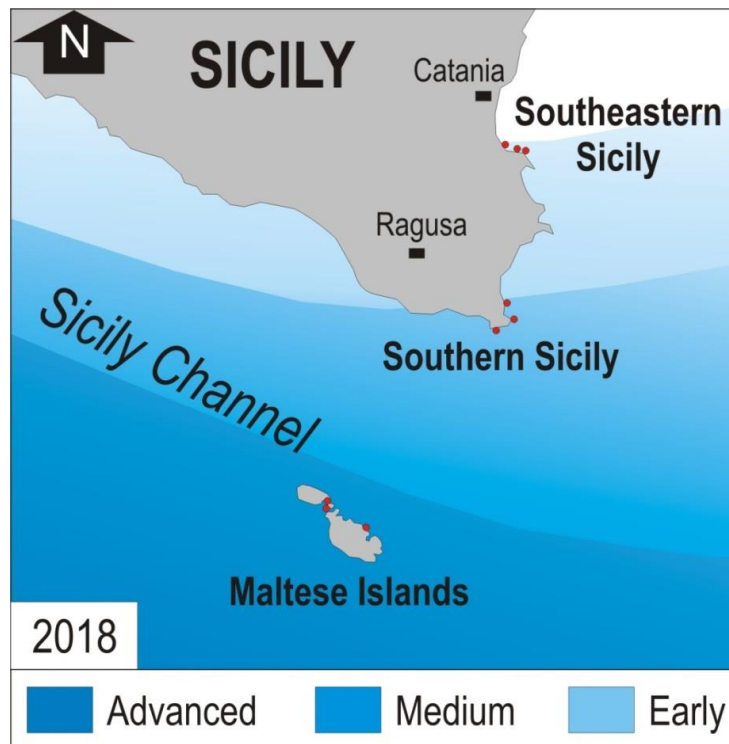


Fig. 6.1: Sampling sites (red points) in the three study areas at different stages of invasion: Maltese Islands (advanced stage), Southern Sicily (medium stage) and Southeastern Sicily (early stage). The subdivision of the Sicily Channel according to the colonization process of *A. lobifera* is based on data collected in 2018 (see **Chapter 3** of this thesis).

6.2.2 Sampling processing and foraminiferal analysis

For each sampling site, three replicates were prepared as washed residues for the foraminiferal analysis. Samples were oven-dried at 40 °C for one day, weighed, washed over a 63 μm sieve and then oven-dried again. When necessary, the obtained washed residues were separated into discrete aliquots using a precision micro-splitter. Modern foraminiferal assemblages were analysed under a stereomicroscope, counting about 300 specimens into a discrete aliquot; when the obtained washed residue was quantitatively scarce, the whole number of specimens within the sample was counted. Foraminiferal census counts were used to calculate the absolute abundances of all species identified, as number of specimens recorded per gram of dry sediment (N g^{-1}).

6.2.3 Statistical analyses

In order to assess the potential impact of *A. lobifera* colonization on native assemblages of the three study areas at known different stages of invasion, two series of statistical analyses were performed. The first analysis considered samples collected from the Maltese Islands (advanced stage of invasion) at the two different depths. This took into account the absolute abundances of modern benthic foraminifera (including *A. lobifera*, cryptogenic and native species) preserved in sediment samples. This first analysis allowed us to verify which habitat (2-5 m or 15-20 m depth) exhibits the

more pronounced effects of *A. lobifera* on native communities (null hypothesis: no difference in foraminifera assemblages between depths). This analysis used an orthogonal design with two factors: ‘Depth’ (fixed: 2 levels) and ‘Site’ (random: 3 levels). Once this was determined, the second analysis estimated the impact of *A. lobifera* on native assemblages among the three areas at different stages of invasion. This was performed taking into account the absolute abundances of modern benthic foraminifera preserved in sediment samples collected at only one bathymetry from all areas (null hypothesis: no difference in foraminifera assemblages across areas). This analysis used a nested design with two factors: ‘Area’, corresponding to the invasion stage (fixed: 3 levels) and ‘Site’ (random: 3 levels, nested in ‘Area’). Both analyses considered the following response variables: multivariate species composition (compared with the Bray-Curtis index of similarity), as well as species richness, Shannon’s diversity and Pielou’s evenness (compared with the Euclidean distance). More details on the univariate and multivariate analyses are reported in **Chapter 2, paragraph 2.6** of this thesis.

6.3 Results

6.3.1 The native assemblages of the Sicily Channel

In the sites of the Maltese Islands and Southern Sicily where the non-indigenous species *A. lobifera* was absent or very rare (sites 19, 25, 30, 40, 43 and 45 of Appendix 1), the structure of native foraminiferal communities exhibited similar values of community structure indices (Tab. 6.1). Although it was observed that species composition is highly variable across sites, probably depending on local conditions (e.g. type of substratum, presence of vegetation, depth, currents, light), the overall structure of native foraminiferal communities was consistently characterised by high species richness (18 to 35 species), diversity (Shannon index 2.13 to 3.10) and evenness (Pielou index 0.74 to 0.89). Therefore, indices of community structure were used as response variables to assess changes in the native community structure caused by *A. lobifera* at different stages of invasion process.

Tab. 6.1: Species richness (*S*), Shannon’s diversity (*H'*) and Pielou’s evenness (*J'*) resulted by relative abundances of native benthic foraminiferal communities from the Maltese Islands and Sicily. The dataset utilised for the analyses is available in Appendix 2.

	Maltese Islands			Sicily		
Index	19. Gnejna	25. Xlendi	30. Santa Maria	40. Agnone	43. Capo Passero	45. Pozzallo
<i>S</i>	27	34	35	18	35	27
<i>H'</i>	2.7614	3.1018	2.9012	2.1257	2.8842	2.9547
<i>J'</i>	0.83786	0.87962	0.816	0.73544	0.81122	0.8965

6.3.2 Comparison between depths in the Maltese Islands

Modern benthic foraminifera communities, preserved in bottom sediment samples from three sites of the Maltese Islands (Balluta Bay, Hondoq ir-Rummien and Crystal lagoon), were composed of a total of ~ 40 species at 2-5 m depth and of ~ 60 species at 15-20 m depth, respectively, including one NIS (*A. lobifera*) and 5 cryptogenic species (*A. lessonii*, *Amphisorus hemprichii*, *Coscinospira arietina*, *C. hemprichii* and *Sorites orbiculus*) (Appendix 2). The most abundant species in samples collected at 2-5 m depth was *A. lobifera*, which mostly contributed to the similarity within replicates of the same site (34% in Balluta Bay; 71% in Hondoq ir-Rummien; 81% in Crystal lagoon; SIMPER test, Tab. 6.3A-C). On the contrary, in samples collected at 15-20 m depth, the species that mostly contributed to the community similarity were native epiphytic taxa, such as *Peneroplis pertusus* (17%), *Quinqueloculina disparilis* (10%) and *Q. bradyana* (4%), *Elphidium macellum* (9%) and *E. crispum* (4%), *Lobatula lobatula* (5%), *Rosalina bradyi* (5%) and *Planorbulina mediterraneensis* (5%), while the non-indigenous *A. lobifera* was less abundant and poorly contributing to the similarity of the community (3% in Balluta Bay; 9% in Hondoq ir-Rummien; 2% in Crystal lagoon; SIMPER test, Tab. 6.3D-F). Cryptogenic species were very rare, especially in the shallower samples where *A. lessonii* and *S. orbiculus* were not found, while in the deeper sediment samples the highest contribution to the community similarity of *A. lessonii* was of 5% and of *A. hemprichii* and *S. orbiculus* was of 3% (SIMPER test, Tab. 6.3A-F).

The PERMANOVA based on Bray-Curtis similarities among foraminifera assemblages detected a significant interaction between the factors ‘Site’ and ‘Depth’ [PERMANOVA: P(permanova) = 0.0001, P(MC) = 0.0001; Tab. 6.2], which appears also evident from the nMDS graph (Fig. 6.2). In fact, each site and each depth exhibit peculiar species composition, probably resulting from aggregate distributions of foraminifera populations. The pairwise analysis confirmed significant differences for the factor ‘Depth’ in all the single sites [P(MC) = 0.0024 (Balluta Bay), P(MC) = 0.0014 (Hondoq ir-Rummien), P(MC) = 0.0005 (Crystal lagoon); Tab. 6.2], also confirmed by high average dissimilarities between depths resulting from the SIMPER test (75% in Balluta Bay, 71% in Hondoq ir-Rummien and 88% in Crystal lagoon; Tab. 6.3G-I).

Tab. 6.2: Results of PERMANOVA tests performed on benthic foraminiferal community structures preserved in sediments at 2-5 m depth and 15-20 m depth: main test (A) and pairwise tests (B), based on a Bray-Curtis similarity matrix.

(A) Main test					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Site	2	9.3946	0.0001	9910	0.0001
Depth	1	3.8004	0.1314	38	0.08
SitexDepth	2	13.475	0.0001	9930	0.0001

(B) Pair-wise test				
Factor 'Site', Level 'Balluta Bay'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	3.8619	0.0974	10	0.0024
Factor 'Site', Level 'Hondoq ir-Rummien'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	5.0931	0.1038	10	0.0014
Factor 'Site', Level 'Crystal lagoon'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	7.2439	0.1008	10	0.0005

Tab. 6.3: Results of SIMPER test performed on benthic foraminiferal community structures preserved in sediments from Balluta Bay, Hondoq ir-Rummien and Crystal lagoon: average similarity at 2-5 m depth (A-C) and at 15-20 m depth (D-F), and average dissimilarity between 2-5 m depth and 15-20 m depth (G-I). Cut-off level corresponds to 50%.

SIMPER test					
A) Average similarity at 2-5 m depth in Balluta Bay = 64.98 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	6.13	22.43	9.83	34.52	34.52
<i>Peneroplis pertusus</i>	1.42	5.20	9.81	8.00	42.52
<i>Elphidium macellum</i>	1.15	3.84	8.18	5.91	48.43
<i>Quinqueloculina bradyana</i>	1.15	3.43	4.15	5.28	53.71
B) Average similarity at 2-5 m depth in Hondoq ir-Rummien = 74.29 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	17.69	52.47	6.40	70.63	70.63
C) Average similarity at 2-5 m depth in Crystal lagoon = 81.81 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	9.32	66.50	13.98	81.29	81.29

SIMPER test					
D) Average similarity at 15-20 m depth in Balluta Bay = 73.15 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Peneroplis pertusus</i>	9.12	5.22	9.00	7.14	7.14
<i>Quinqueloculina disparilis</i>	6.78	4.28	7.70	5.85	12.99
<i>Lobatula lobatula</i>	5.95	3.80	7.27	5.19	18.18
<i>Planorbulina mediterraneensis</i>	6.29	3.65	9.63	4.99	23.17
<i>Rosalina bradyi</i>	5.55	3.52	7.65	4.82	27.99
<i>Miniacina miniacina</i>	4.96	3.46	9.18	4.73	32.72
<i>Elphidium macellum</i>	5.41	3.27	6.02	4.47	37.19
<i>Quinqueloculina bradyana</i>	5.34	3.09	7.28	4.22	41.41
<i>Amphistegina lobifera</i>	3.81	2.56	9.52	3.51	44.92
<i>Elphidium crispum</i>	3.68	2.32	9.92	3.18	48.09
<i>Quinqueloculina parvula</i>	3.78	2.26	7.21	3.08	51.18
E) Average similarity at 15-20 m depth in Hondoq ir-Rummien = 80.03 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Quinqueloculina disparilis</i>	2.24	8.64	32.48	10.80	10.80
<i>Amphistegina lobifera</i>	1.99	7.66	17.00	9.57	20.37
<i>Elphidium macellum</i>	1.82	6.94	14.28	8.67	29.05
<i>Miniacina miniacina</i>	2.12	6.85	6.30	8.56	37.61
<i>Peneroplis pertusus</i>	1.33	4.85	13.10	6.06	43.67
<i>Amphistegina lessonii</i>	0.96	3.64	11.98	4.55	48.22
<i>Elphidium crispum</i>	1.03	3.54	26.24	4.43	52.65
F) Average similarity at 15-20 m depth in Crystal lagoon = 77.63 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Peneroplis pertusus</i>	7.78	13.61	18.21	17.54	17.54
<i>Quinqueloculina disparilis</i>	3.85	6.76	63.58	8.71	26.25
<i>Miniacina miniacina</i>	2.42	4.04	16.57	5.20	31.45
<i>Amphistegina lessonii</i>	2.34	3.80	6.08	4.89	36.34
<i>Elphidium macellum</i>	2.44	3.66	3.26	4.71	41.05
<i>Lobatula lobatula</i>	2.32	3.60	21.17	4.64	45.69
<i>Laevipeneroplis karreri</i>	1.99	3.21	9.09	4.14	49.82
<i>Quinqueloculina bradyana</i>	1.70	2.68	4.41	3.45	53.28

SIMPER test						
G) Average dissimilarity between 2-5 m depth and 15-20 m depth in Balluta Bay = 74.77 %						
Species	Av. Abund. (2-5 m)	Av. Abund. (15-20 m)	Av. Diss.	Diss/SD	Contrib. %	Cum. %
<i>Peneroplis pertusus</i>	1.42	9.12	4.84	3.84	6.48	6.48
<i>Quinqueloculina disparilis</i>	0.57	6.78	4.01	6.74	5.37	11.85
<i>Planorbulina mediterraneensis</i>	0.51	6.29	3.91	2.32	5.23	17.07
<i>Lobatula lobatula</i>	0.82	5.95	3.33	5.97	4.46	21.53
<i>Miniacina miniacea</i>	0.00	4.96	3.30	6.27	4.42	25.95
<i>Rosalina bradyi</i>	0.64	5.55	3.16	7.48	4.22	30.17
<i>Elphidium macellum</i>	1.15	5.41	2.73	4.24	3.65	33.82
<i>Quinqueloculina bradyana</i>	1.15	5.34	2.63	3.02	3.52	37.34
<i>Sorites orbiculus</i>	0.00	3.65	2.33	4.86	3.12	40.46
<i>Quinqueloculina parvula</i>	0.59	3.78	2.25	1.82	3.00	43.46
<i>Laevipeneroplis karreri</i>	0.74	4.31	2.13	1.68	2.85	46.31
<i>Elphidium crispum</i>	0.49	3.68	2.06	5.86	2.75	49.06
<i>Amphisorus hemprichii</i>	0.24	3.30	1.95	4.20	2.61	51.68
H) Average dissimilarity between 2-5 m depth and 15-20 m depth in Hondoq ir-Rummien = 70.61 %						
Species	Av. Abund. (2-5 m)	Av. Abund. (15-20 m)	Av. Diss.	Diss/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	17.69	1.99	28.56	5.45	40.45	40.45
<i>Miniacina miniacea</i>	0.00	2.12	3.83	4.28	5.43	45.88
<i>Bulimina costata</i>	1.96	0.00	3.57	8.54	5.05	50.93
I) Average dissimilarity between 2-5 m depth and 15-20 m depth in Crystal lagoon = 87.64 %						
Species	Av. Abund. (2-5 m)	Av. Abund. (15-20 m)	Av. Diss.	Diss/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	9.32	1.47	11.59	7.49	13.22	13.22
<i>Peneroplis pertusus</i>	1.04	7.48	9.96	8.54	11.36	24.58
<i>Quinqueloculina disparilis</i>	0.00	3.85	5.67	29.58	6.47	31.05
<i>Miniacina miniacea</i>	0.00	2.42	3.58	7.37	4.08	35.13
<i>Amphistegina lessonii</i>	0.00	2.34	3.47	5.71	3.95	39.08
<i>Elphidium macellum</i>	0.17	2.44	3.32	3.58	3.79	42.87
<i>Lobatula lobatula</i>	0.24	2.32	3.05	3.56	3.47	46.35
<i>Laevipeneroplis karreri</i>	0.00	1.99	2.92	7.51	3.33	49.68
<i>Sorites orbiculus</i>	0.00	1.75	2.60	2.96	2.97	52.65

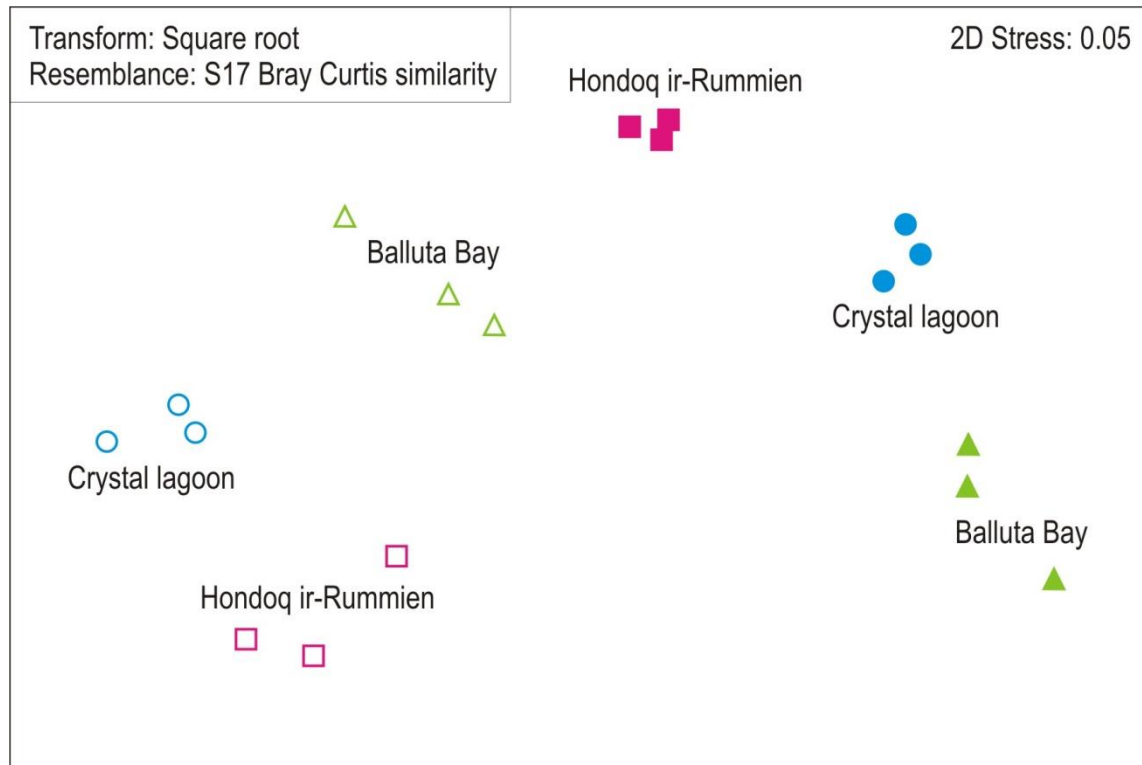


Fig. 6.2: Non-metric multidimensional scaling (nMDS) graph on modern benthic foraminiferal communities preserved in sediment samples collected at 2-5 m depth (empty symbols) and 15-20 m depth (filled symbols) from the three sampling sites of the Maltese Islands.

The same PERMANOVA design was repeated using as response variables species richness (S), Shannon index (H') and Evenness index (J'), and based on a Euclidean distance matrix. Lower values were observed for all the three response variables at 2-5 m depth (Fig. 6.3A-C). Similarly to the previous analysis, the PERMANOVAs based on Euclidean distance matrices confirmed significant differences in foraminifera community richness, diversity and evenness across depths (Tab. 6.4A-E).

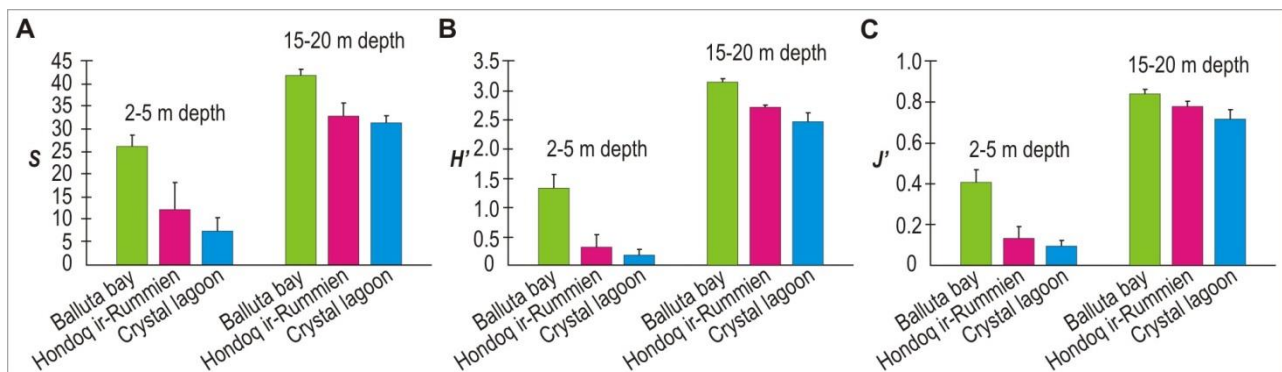


Fig. 6.3: Mean (A) species richness - S , (B) diversity - H' and (C) evenness - J' \pm standard deviation at the two sampling bathymetries (2-5 m and 15-20 m depth) in the studied sites of the Maltese Islands.

Tab. 6.4: Results of PERMANOVA tests performed on species richness - S , diversity - H' and evenness - J' : main test (A-C) and pairwise tests (D-E), based on a Euclidean distance matrix.

(A) Main test: S - species richness					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Site	2	30.418	0.0001	9948	0.0001
Depth	1	68.971	0.0989	38	0.0128
SitexDepth	2	2.2837	0.1425	9954	0.1407
(B) Main test: H' - diversity					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Site	2	54.77	0.0002	9953	0.0001
Depth	1	142.73	0.0996	38	0.0078
SitexDepth	2	5.799	0.0175	9955	0.0185
(C) Main test: J' - evenness					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Site	2	38.789	0.0003	9938	0.0001
Depth	1	68.209	0.0965	38	0.0134
SitexDepth	2	10.224	0.0024	9950	0.0024

(D) Pair-wise test: H' - diversity				
Factor 'Site', Level 'Balluta Bay'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	12.122	0.1012	10	0.0007
Factor 'Site', Level 'Hondoq ir-Rummien'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	18.743	0.098	10	0.0002
Factor 'Site', Level 'Crystal lagoon'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	20.574	0.0952	10	0.0002

(E) Pair-wise test: J' - evenness				
Factor 'Site', Level 'Balluta Bay'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	10.911	0.1011	10	0.0004
Factor 'Site', Level 'Hondoq ir-Rummien'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	16.856	0.1026	10	0.0003
Factor 'Site', Level 'Crystal lagoon'	t	P(perm)	Unique perms	P(MC)
2-5 m depth vs 15-20 m depth	18.632	0.0988	10	0.0003

All these evidences reflected the different abundances of *A. lobifera* in the communities preserved at the two sampling depths. This is also reflected by the different contribution of *A. lobifera* to the similarity of communities at 2-5 m versus 15-20 m depth (from SIMPER test, Tab. 6.3A-F). In shallower samples, where *A. lobifera* was the most important species in shaping the communities,

species richness, diversity and evenness were all low (Fig. 6.3A-C), while in deeper samples higher values of S , H' and J' corresponded to modest contribution of *A. lobifera* to the similarity among samples. In most cases, *A. lobifera* was also the species mostly contributing to the differences in shallow versus deep assemblages (Tab. 6.3G-I). All these results indicate that the potential impact of *A. lobifera* colonization is more evident in sediment samples collected at 2-5 m depth, where this NIS is actually more abundant. Consequently, in the subsequent analysis, we considered Maltese and Sicilian sediment samples collected at this bathymetry, in order to assess statistical differences between the three study areas at known different stages of invasion by *A. lobifera*.

6.3.3 Comparison across the three stages of invasion

Modern benthic foraminifera communities, preserved in bottom sediment samples from the Maltese Islands (an area corresponding to advanced stage of invasion by *A. lobifera*), Southern Sicily (medium stage) and Southeastern Sicily (early stage), were composed of a total of ~ 40 species, ~ 90 species and ~ 90 species, respectively, including the non-indigenous *A. lobifera* and the cryptogenic *A. hemprichii*, *C. arietina*, *C. hemprichii* and *S. orbiculus* (Appendix 2). The cryptogenic *A. lessonii* was not found. In the Maltese Islands, the most abundant species was *A. lobifera* that mostly contributed to the similarity of community (66%), followed by the native *P. pertusus* (7%), *E. crispum* and *E. macellum* (3%), and other epiphytic and infaunal taxa not exceeding 2% of the total contribution (SIMPER test; Tab. 6.6A). On the contrary, in Southern Sicily, the contribution of *A. lobifera* to the community similarity was much lower than expected for an area known to be at ‘medium stage of invasion’: according to SIMPER, it contributed less than 1% to the average similarity among samples. Finally, *A. lobifera* was not found in samples from the area at early stage of invasion, namely Southeastern Sicily. Both in Southern and Southeastern Sicily, the highest contributions to the similarity of community were given by native epiphytic taxa, such as *P. pertusus* (8%), *R. bradyi* (7%), *L. lobatula* (5%), *Asterigerinata mamilla* (5%) and *Rosalina macropora* (4%), together with some epifaunal and infaunal species, such as genera *Ammonia* (5%) and *Textularia* (4%), as resulted by the SIMPER test (Tab. 6.6B-C). In all three study areas cryptogenic species were very rare and their contribution to community similarity was extremely low.

The PERMANOVA based on Bray-Curtis similarities among foraminiferal community structure detected significant differences among study areas [PERMANOVA: $P(\text{perm}) = 0.0133$, $P(\text{MC}) = 0.0014$; Tab. 6.5] and significant interaction between the factors ‘Area’ and ‘Site’ [PERMANOVA: $P(\text{perm}) = 0.0001$, $P(\text{MC}) = 0.0001$; Tab. 6.5]. In particular, pairwise tests highlighted statistically significant differences between the area in advanced stage of invasion and the other two ones,

namely between the community structures of the Maltese Islands and Southern Sicily [$P(\text{MC}) = 0.0037$], and between the community structures of the Maltese Islands and Southeastern Sicily [$P(\text{MC}) = 0.0077$] (Tab. 6.5). On the contrary, no statistically significant differences emerged between the community structures of Southern Sicily and Southeastern Sicily [$P(\text{MC}) = 0.4041$]. These results confirm the pattern emerging from the nMDS graph (Fig. 6.4), showing two main clusters that represent the Maltese samples (on the left) and the Sicilian ones (on the right). In fact, if the Maltese community represented a well-separated condition, communities of Southern Sicily and Southeastern Sicily resulted to be very similar to each other. Additionally, the community structure of Southern Sicily was located in the middle respect to the Maltese and Southeastern Sicilian communities, hence representing an intermediate condition. This significant difference was also confirmed by an average dissimilarity of 83% between the community structure of the Maltese Islands and Southern Sicily, and of 89% between communities of the Maltese Islands and Southeastern Sicily (SIMPER analysis; Tab. 6.6).

Tab. 6.5: Results of PERMANOVA tests performed on benthic foraminiferal community structures of the Maltese Islands, Southern Sicily and Southeastern Sicily: main test (A) and pair-wise test (B), based on a Bray-Curtis similarity matrix.

(A) Main test					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Area	2	5.6789	0.0133	280	0.0014
Site(Area)	6	7.6511	0.0001	9888	0.0001

(B) Pair-wise test				
Factor 'Area'	t	P(perm)	Unique perms	P(MC)
Maltese Islands vs Southern Sicily	3.1757	0.0997	10	0.0037
Maltese Islands vs Southeastern Sicily	2.5594	0.0947	10	0.0077
Southern Sicily vs Southeastern Sicily	1.0245	0.3074	10	0.4041

Tab. 6.6: Results of SIMPER test performed on benthic foraminiferal community structures of the three areas: average similarity in the Maltese Islands (A), Southern Sicily (B) and Southeastern Sicily (C), and average dissimilarity between the Maltese Islands and Southern Sicily (D) and between the Maltese Islands and Southeastern Sicily (E). Cut-off level corresponds to 50%.

SIMPER test					
A) Average similarity in the Maltese islands = 54.96 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	11.05	36.59	2.64	66.58	66.58
B) Average similarity in Southern Sicily = 65.95 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Peneroplis pertusus</i>	5.13	5.32	4.51	8.07	8.07
<i>Rosalina bradyi</i>	5.08	4.74	4.63	7.18	15.25
<i>Lobatula lobatula</i>	4.19	3.66	4.41	5.55	20.80
<i>Asterigerinata mamilla</i>	3.49	3.46	4.36	5.25	26.05
<i>Rosalina macropora</i>	2.79	2.60	3.35	3.95	30.00
<i>Adelosina cliarensis</i>	2.15	2.34	3.85	3.55	33.55
<i>Ammonia inflata</i>	2.27	2.34	2.92	3.54	37.09
<i>Ammonia parkinsoniana</i>	2.02	2.18	4.21	3.30	40.40
<i>Quinqueloculina disparilis</i>	2.01	2.12	4.33	3.22	43.62
<i>Triloculina tricarinata</i>	2.16	2.11	5.58	3.20	46.82
<i>Quinqueloculina bradyana</i>	2.26	2.09	5.50	3.17	49.98
<i>Elphidium crispum</i>	1.92	2.09	3.33	3.17	53.15
C) Average similarity in Southeastern Sicily = 46.46 %					
Species	Av. Abund.	Av. Sim.	Sim/SD	Contrib. %	Cum. %
<i>Rosalina bradyi</i>	5.29	2.99	1.67	6.44	6.44
<i>Ammonia tepida</i>	3.04	2.94	3.08	6.32	12.76
<i>Ammonia inflata</i>	3.21	2.60	3.36	5.60	18.36
<i>Textularia pala</i>	2.11	2.04	2.38	4.39	22.75
<i>Lobatula lobatula</i>	3.60	1.99	1.44	4.28	27.03
<i>Elphidium advenum</i>	2.58	1.90	2.67	4.08	31.11
<i>Asterigerinata mamilla</i>	3.92	1.80	0.82	3.88	34.99
<i>Ammonia beccarii</i>	2.88	1.79	2.14	3.85	38.84
<i>Ammonia parkinsoniana</i>	2.38	1.78	1.42	3.84	42.68
<i>Elphidium complanatum</i>	2.31	1.76	2.74	3.78	46.46
<i>Quinqueloculina agglutinans</i>	1.64	1.70	2.05	3.65	50.12

SIMPER test						
D) Average dissimilarity between the Maltese Islands and Southern Sicily = 83.34 %						
Species	Av. Abund. (Mal.Isl.)	Av. Abund. (S Sicily)	Av. Diss.	Diss/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	11.05	0.49	10.27	1.93	12.32	12.32
<i>Rosalina bradyi</i>	0.37	5.08	4.30	4.26	5.16	17.49
<i>Peneroplis pertusus</i>	1.16	5.13	3.81	3.23	4.57	22.06
<i>Asterigerinata mamilla</i>	0.03	3.49	3.28	4.19	3.93	25.99
<i>Lobatula lobatula</i>	0.55	4.19	3.25	3.37	3.89	29.89
<i>Rosalina macropora</i>	0.00	2.79	2.61	3.47	3.14	33.02
<i>Ammonia inflata</i>	0.16	2.27	2.20	1.75	2.64	35.67
<i>Ammonia parkinsoniana</i>	0.00	2.02	1.97	3.39	2.37	38.04
<i>Adelosina cliarensis</i>	0.15	2.15	1.97	2.90	2.36	40.40
<i>Triloculina tricarinata</i>	0.29	2.16	1.74	3.17	2.09	42.49
<i>Ammonia tepida</i>	0.00	1.74	1.71	2.79	2.05	44.54
<i>Quinqueloculina bradyana</i>	0.46	2.26	1.65	2.23	1.98	46.51
<i>Quinqueloculina agglutinans</i>	0.00	1.75	1.59	3.08	1.91	48.43
<i>Adelosina mediterraneensis</i>	0.23	1.79	1.54	2.40	1.85	50.28
E) Average dissimilarity between the Maltese Islands and Southeastern Sicily = 89.10 %						
Species	Av. Abund. (Mal.Isl.)	Av. Abund. (S-E Sicily)	Av. Diss.	Diss/SD	Contrib. %	Cum. %
<i>Amphistegina lobifera</i>	11.05	0.00	11.44	1.61	12.84	12.84
<i>Rosalina bradyi</i>	0.37	5.29	3.75	1.93	4.21	17.05
<i>Ammonia tepida</i>	0.00	3.04	2.96	3.02	3.32	20.37
<i>Asterigerinata mamilla</i>	0.03	3.92	2.82	1.37	3.16	23.53
<i>Ammonia inflata</i>	0.16	3.21	2.75	2.95	3.08	26.61
<i>Lobatula lobatula</i>	0.55	3.60	2.44	1.99	2.74	29.35
<i>Ammonia parkinsoniana</i>	0.00	2.38	2.34	1.68	2.62	31.97
<i>Elphidium advenum</i>	0.11	2.58	2.11	2.90	2.37	34.35
<i>Elphidium complanatum</i>	0.00	2.31	2.03	3.21	2.28	36.63
<i>Ammonia beccarii</i>	0.43	2.88	2.02	1.30	2.26	38.90
<i>Cassidulina carinata</i>	0.00	2.30	1.96	1.31	2.20	41.09
<i>Quinqueloculina limbata</i>	0.00	1.68	1.79	1.11	2.01	43.10
<i>Textularia pala</i>	0.35	2.11	1.77	1.72	1.99	45.09
<i>Cibicides refulgens</i>	0.09	2.19	1.77	1.87	1.99	47.08
<i>Peneroplis pertusus</i>	1.16	2.28	1.70	2.33	1.91	48.99
<i>Quinqueloculina agglutinans</i>	0.00	1.64	1.70	2.08	1.90	50.90

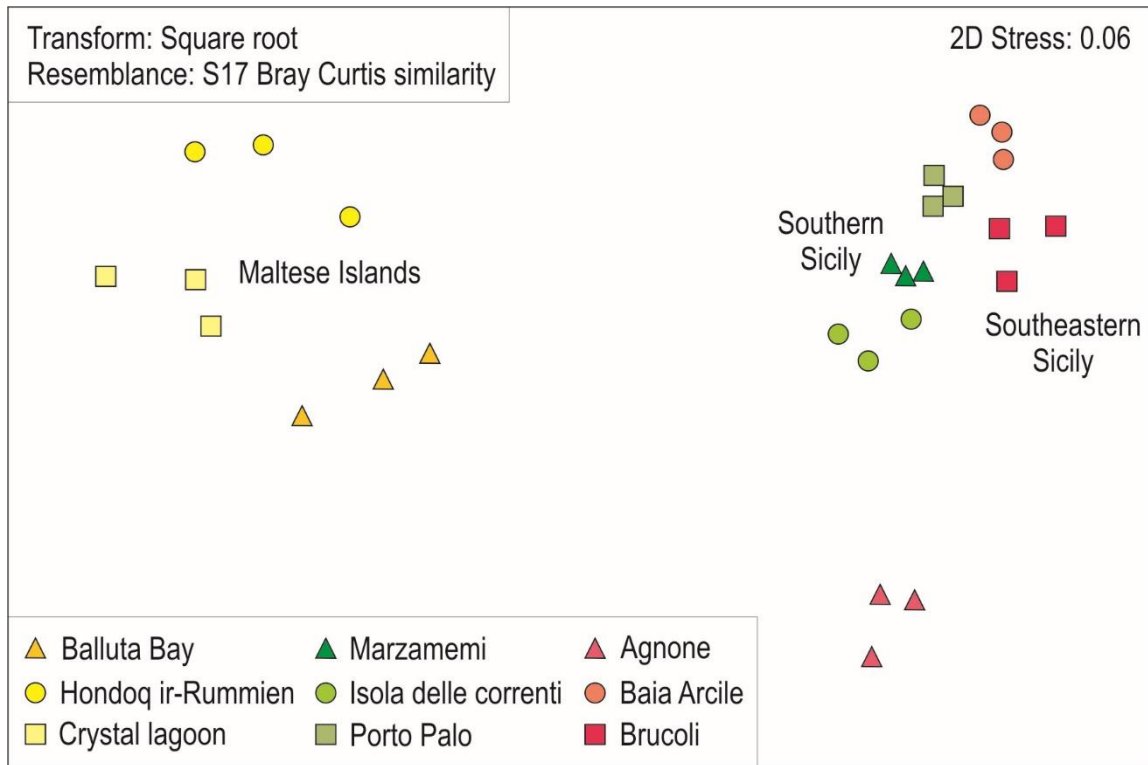


Fig. 6.4: Non-metric multidimensional scaling (nMDS) graph on modern benthic foraminiferal community preserved in sediment samples collected at 2-5 m depth from the Maltese Islands (in yellow, representing the advanced stage of invasion by *A. lobifera*), Southern Sicily (in green, medium stage) and Southeastern Sicily (in red, early stage).

Finally, significant differences between the Maltese and Sicilian communities were also supported by PERMANOVA tests based on Euclidean distances matrices calculated from species richness (S), Shannon index (H') and Evenness index (J'). All these response variables displayed significantly lower values in the Maltese Islands than in Southern and Southeastern Sicily (Fig. 6.5A-C). Contrarily to expectations, no significant differences emerged between the area identified in 'medium stage' of invasion, Southern Sicily, and the area identified as 'low stage', Southeastern Sicily (Tab. 6.7A-F).

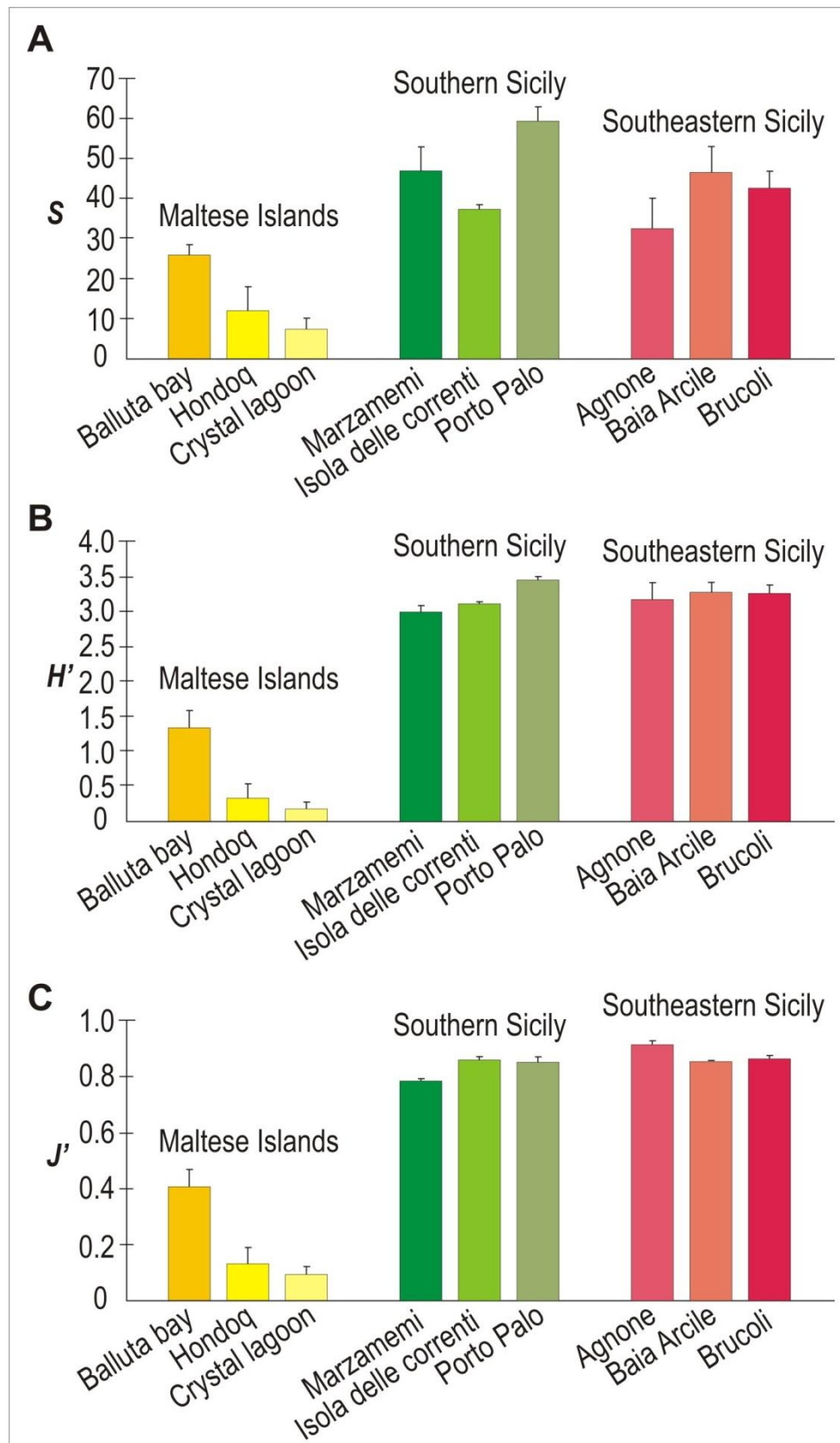


Fig. 6.5: Mean (A) species richness - S , (B) diversity - H' and (C) evenness - $J' \pm$ standard deviation across the three areas at different stages of invasion: Maltese Islands (advanced stage – in yellow), Southern Sicily (medium stage – in green) and Southeastern Sicily (early stage – in red).

Tab. 6.7: Results of PERMANOVA tests performed on species richness - S , diversity - H' and evenness - J' : main test (A-C) and pairwise tests (D-F), based on a Euclidean distance matrix.

(A) Main test: S - species richness					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Area	2	9.9895	0.0138	268	0.0133
Site(Area)	6	10.351	0.0002	9954	0.0001
(B) Main test: H' - diversity					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Area	2	45.156	0.0325	280	0.0001
Site(Area)	6	18.659	0.0001	9958	0.0001
(C) Main test: J' - evenness					
Factor	Df	Pseudo-F	P(perm)	Unique perms	P(MC)
Area	2	38.403	0.0069	280	0.0011
Site(Area)	6	32.234	0.0001	9945	0.0001

(D) Pair-wise test: S - species richness				
Factor 'Area'	t	P(perm)	Unique perms	P(MC)
Maltese Islands vs Southern Sicily	3.9025	0.0966	10	0.016
Maltese Islands vs Southeastern Sicily	3.6095	0.098	10	0.0224
Southern Sicily vs Southeastern Sicily	1.0001	0.3988	10	0.3699

(E) Pair-wise test: H' - diversity				
Factor 'Area'	t	P(perm)	Unique perms	P(MC)
Maltese Islands vs Southern Sicily	6.7011	0.1029	10	0.0023
Maltese Islands vs Southeastern Sicily	7.2279	0.1022	10	0.0015
Southern Sicily vs Southeastern Sicily	0.2357	0.8986	10	0.8226

(F) Pair-wise test: J' - evenness				
Factor 'Area'	t	P(perm)	Unique perms	P(MC)
Maltese Islands vs Southern Sicily	6.0476	0.1035	10	0.0031
Maltese Islands vs Southeastern Sicily	6.6008	0.0919	10	0.0025
Southern Sicily vs Southeastern Sicily	1.6036	0.1908	10	0.1822

The observed patterns were clearly related to the different abundances of *A. lobifera* in the three study areas. This is also reflected by the different contribution of *A. lobifera* to the similarity of communities in the Maltese Islands versus Southern and Southeastern Sicily (from SIMPER test; Tab. 6.6A-C). In the Maltese Islands, where *A. lobifera* was the most important species in shaping the communities, species richness, diversity and evenness were all low (Fig. 6.5A-C), while the higher values of S , H' and J' in Southern Sicily corresponded to very low contribution of *A. lobifera*

to the similarity among samples and corresponded to null contribution in Southeastern Sicily, where *A. lobifera* was not found. Additionally, *A. lobifera* is also the species mostly contributing to the differences in Maltese versus Sicilian assemblages.

6.4 Discussion

6.4.1 The potential impact of *A. lobifera* colonization in the Maltese Islands and Sicily

The present work is a pioneer study that aims to assess the potential impact of a non-indigenous species of benthic foraminifera on native assemblages in the Sicily Channel (Central Mediterranean). During the last decades, some authors have reported more and more evidences of the impact of amphisteginid invasions on native foraminiferal communities from both the Eastern and Central Mediterranean (e.g. Triantaphyllou et al., 2009; Langer & Mouanga, 2016), leading to consider *A. lobifera* as one of the benthic foraminiferal NIS with the highest potential impact in the Mediterranean (Tsiamis et al., 2019). However, no study has ever focused on the direct, quantitative comparison of invaded versus non-invaded communities.

The Mediterranean Sea is currently undergoing a massive and unprecedented range expansion of *Amphistegina* spp. with severe consequences on ecosystem functioning (Langer et al., 2012). In the Mediterranean, amphisteginids are known to be particularly successful invaders in shallow water environments (< 20 m depth), which represent the ideal habitats with the highest susceptibility to colonization and establishment of dense populations (Langer & Mouanga, 2016 and references therein). Based on evidences from the Eastern and Central Mediterranean, a loss of benthic foraminiferal biodiversity and alterations of foraminiferal community structures are expected where the proliferation of *Amphistegina* spp. is more preponderant. In fact, Mouanga & Langer (2014) have documented a homogenization of foraminiferal faunas due to massive invasions by amphisteginids, showing a clear correlation between abundances of *Amphistegina* spp. and the diversity loss of foraminiferal biotas. Additionally, Langer & Mouanga (2016) have documented a negative correlation between species richness and increasing abundances of *Amphistegina* spp., suggesting that these two factors are intimately linked each other.

According to the distribution map and relative abundances of *A. lobifera* recorded in the Sicily Channel, resulting from Aim 1 (see **Chapter 3** of this thesis), the colonization process of *A. lobifera* seems to be in an advanced stage in the Maltese Islands, where thus the highest impact on native assemblages was expected, while it seems to be in an early stage in Southeastern Sicily, where minor effects on native community were contrarily expected, with an intermediate condition in Southern Sicily. Based on the results of the present study, expectations were only partially met.

First, a dramatic decline in species richness, diversity and evenness of the benthic foraminiferal assemblage in the Maltese Islands was actually observed at the shallower sampling bathymetry (2-5 m depth), where *A. lobifera* showed the highest abundances, but not so much at the deeper bathymetry (15-20 m depth), where *A. lobifera* was less abundant and its effects on community structure were not so remarkable. Thus, this datum confirms that, in the Mediterranean Sea, this non-indigenous species mostly proliferates at very shallow depths, supporting the evidences that emerged from Aim 1 (see **Chapter 3** of this thesis) and literature data (e.g. Langer & Mouanga, 2016 and references therein). Differences in habitat preference may also explain the lower-than-expected relative abundances of *A. lobifera* in Southern Sicily, which had been assumed as an area at ‘medium stage of invasion’ (based on the results of recent surveys, presented in **Chapter 3** of this thesis), but which eventually resulted only modestly affected by this NIS. While preparing our sampling design, we had established to collect samples at 2-5 m depth across all areas because this was known to be the optimal habitat for *A. lobifera* in the Mediterranean (Langer & Mouanga, 2016), as also confirmed by our results from Malta. However, this was not the case in Southern Sicily, where the species was only sporadic at 2-5 m depth. In fact, most samples from Southern Sicily analysed in the framework of Aim 1 and showing appreciable abundances of *A. lobifera* had actually been collected at depths between 0 and 2 m (see Fig. 3.4), that are slightly shallower than those included in the current sampling design. Therefore, we may have missed the layer of benthic community being mostly impacted by *A. lobifera*. Since the information currently available is insufficient to derive reasonable explanations for the slightly different depth preferences of *A. lobifera*, we advocate the need of new autoecology studies on this species in the Central Mediterranean, in order to better understand its response to different environmental contexts. Unfortunately, we have not still performed the granulometric analyses of the sediment replicate collected from each sampling site that could evidence a possible relation with the substratum grain-size and composition. Nonetheless, we can suppose that in the Sicilian sampling sites, mostly collected along coasts characterised by fine sands supplied by terrigenous rocks, where probably the water turbidity is higher with respect to the Maltese areas characterised by rocky substrata only partly covered by coarse sands supplied by carbonatic rocks, *A. lobifera* prefers to inhabit shallower depths.

Secondly, many of the native species found in the Sicilian coasts were also present in the Maltese Islands but displayed much lower abundances than in Sicily. This datum suggests that all the studied areas have favourable conditions for the native assemblages, which however show gradually lower and lower abundances where *A. lobifera* is the dominant species. In fact, we clearly show that all the sites where *A. lobifera* is at an advanced stage of invasion (Balluta Bay, Hondoq ir-Rummien

and Crystal lagoon in the Maltese Islands) exhibit extremely simplified and unbalanced community structures, in contrast to neighbouring areas where *A. lobifera* has not established (yet) so successfully (Southern and Southeastern Sicily), which display rich and diverse foraminiferal communities. Additionally, in those few Maltese sites containing very few or no specimens of *A. lobifera*, the foraminiferal community structure is well diversified, similar to the situation in Sicily. This allows to conclude that, in the absence of the highly invasive *A. lobifera*, the Maltese and Sicilian communities are comparable and that the very high statistical significance of the differences observed between the invaded community and communities at an early stage of invasion are unlikely to be explained by stochastic spatial differences. We acknowledge some limitations in our experimental study, mostly due to the relatively low number of sites investigated, which is a consequence of the difficulty in achieving a larger set of samples collected in perfectly comparable habitat conditions. However, the correspondence between the invasion stage of *A. lobifera* and the structure of foraminiferal communities suggests a direct role of this invasive species in altering benthic communities. For this reason, a future decrease of benthic foraminiferal biodiversity can be expected also in areas that, according to the species distribution models for *A. lobifera* under current and future climate conditions (Figs. 3.6, 3.7 and 3.8), show high habitat suitability for the colonization and establishment of dense populations.

Finally, if *A. lobifera* dominated the benthic foraminiferal assemblage of the Maltese Islands, on the contrary, the benthic foraminiferal communities of Southern and Southeastern Sicily were well-diversified and mainly composed by several epiphytic and epifaunal species. This datum supports the evidences provided by Langer & Mouanga (2016), who documented that benthic foraminifera primarily impacted by the colonization of *A. lobifera* are epifaunal and epiphytic species, which proliferate in the same microhabitat of *Amphistegina* spp., while miliolid foraminifera seem to be less affected, probably thanks to their adaptation to a wider range of microhabitats (Langer, 1993; Hohenegger, 1994; Hallock, 1999; Hohenegger et al., 1999). Based on these evidences, competitive exclusion seems to be the major driving force that regulates species richness in communities subject to colonization of *A. lobifera* (Langer & Mouanga, 2016).

6.5 Final remarks

The Mediterranean Sea is one of the areas in the world most severely affected by invasion of non-indigenous species due to the opening of the Suez Canal in 1869, which triggered a massive migration of tropical species from the Red Sea (Langer et al., 2012). *Amphistegina lobifera* is one of the most invasive species of benthic foraminifera that, thanks to its small size (< 2 mm), expanded its colonization range remaining undetected for decades. Evidences from the Eastern and

Central Mediterranean showed that hyperabundances of amphisteginids lead to the depletion of the foraminiferal fauna, with severe impacts on biodiversity and local ecosystems. The range expansion of *A. lobifera* primarily affects the shallow coastal environments (< 5 m), causing a significant reduction of species richness and negatively affecting the community structure of benthic foraminifera along the coasts. In the Sicily Channel, one of the most affected areas is the Maltese Islands, where *A. lobifera* seems to have severely affected the diversity of native biotas, especially at very shallow bathymetry. Although the intensity of the phenomenon seems to change from one site to another, overall the Maltese Islands are strongly subject to the colonization of *A. lobifera*, which is widely and abundantly present along the whole archipelago (Guastella et al., 2019). The benthic foraminiferal species that share the same microhabitats with *A. lobifera* seem to be the most impacted by its colonization. Primarily they are epiphytic and epifaunal taxa that, in the Maltese Islands, have been replaced by the proliferating newcomer, leading to a severe disruption of the native community structure and a significant loss of species richness.

On the contrary, the Southern and Southeastern Sicily seem to be only at the beginning of this phenomenon. Although *A. lobifera* already reached the Sicilian coasts, which display high habitat suitability for the establishment of dense populations (Guastella et al., 2019), the native communities do not appear to have been significantly impacted by the colonization of *A. lobifera*, well-known for its high invasiveness in the Mediterranean Sea. The native communities are characterised by a high species richness and are mainly composed by epiphytic and epifaunal species. However, given the high habitat suitability of these areas for *A. lobifera*, it could be only a matter of time before the colonization by this species begins to affect more intensively also benthic foraminiferal communities of the Sicilian coasts, especially at very shallow bathymetry, where *A. lobifera* seems to find the ideal conditions to establish dense populations. Rising temperatures caused by climate change are known to favour the expansion of biogeographic range of tropical species in the Mediterranean Sea, through the northward moving of surface isotherms (Langer et al., 2012; 2013). Given its abundance and prolific production of carbonate, together with the rapid range expansion recorded in the Mediterranean Sea, the invasion of *A. lobifera* is likely to affect the structure of native benthic foraminiferal assemblages and to trigger changes in ecosystem functioning, ultimately leading to a homogenization of the Mediterranean benthic foraminiferal communities. However, further studies from other areas of the Mediterranean Sea are required not only to fully address the impact of *A. lobifera* colonization, in order to assess its role on the diversity on native communities, but also to evaluate its ecology, still not sufficiently understood.

CHAPTER 7

SUMMARY OF MAIN RESULTS AND FUTURE RESEARCH DIRECTIONS

7.1 Distribution of benthic foraminiferal NIS in the Sicily Channel

Few non-indigenous species (NIS) of benthic foraminifera (fora-NIS) coming from the Red Sea have entered the Mediterranean through the Suez Canal, among which the large symbiont-bearing species *Amphistegina lobifera* is the most widespread and successful, especially in the Levantine basin where it is particularly abundant and invasive (Langer et al., 2012). The Mediterranean populations of the thermophilic *A. lobifera*, whose Indo-Pacific origins have been confirmed by recent genetic studies (Schmidt et al., 2016; Prazeres et al., 2020), are known to be limited by the 14 °C winter isotherm (Hallock, 1999; Langer & Hottinger, 2000; Triantaphyllou et al., 2012). During the last decades, *A. lobifera* has been recorded also in the Central Mediterranean and in the southern Adriatic Sea, where it reached the Maltese and Pelagian Islands (Yokes et al., 2007; Caruso & Cosentino, 2014) and the southern coasts of Albania (Langer & Mouanga, 2016), respectively. Other cryptogenic benthic foraminifera have been also found in the Mediterranean Sea: *Amphistegina lessonii* (congeneric to *A. lobifera*), *Amphisorus hemprichii*, *Coscinospira arietina* and *C. hemprichii*, *Cymbaloporella squamosa*, *Heterostegina depressa*, *Pseudolachlanella slitella*, *Sorites orbiculus* and *S. variabilis*.

The first Aim of this thesis was to provide an updated overview of the current distribution and establishment status of the non-indigenous species *A. lobifera*, the cryptogenic congeneric *A. lessonii* and other cryptogenic species of benthic foraminifera in the Sicily Channel, which is centrally located in the Mediterranean Sea thus acting as an oceanographic boundary between the colder western and warmer eastern Mediterranean basins (Azzurro et al., 2014; Di Lorenzo et al., 2017). Additionally, an updated species distribution model (SDM) for *A. lobifera* was developed on the basis of the newly collected information, in order to assess its potential future distribution in the Central and Western Mediterranean Sea driven by the current and future climate conditions. To satisfy Aim 1, both sediment and algal samples were collected at different depths from five areas within the Sicily Channel and the Western Mediterranean.

7.1.1 Present patterns of fora-NIS in the Central Mediterranean

The present study provides three new records of both *Amphistegina lobifera* and *A. lessonii* in the Sicily Channel: the non-indigenous *A. lobifera* is here documented for the first time in Southern Sicily and in Pantelleria and Favignana islands; this last at present represents the westernmost limit of its distribution range in the Mediterranean basin. Likewise, the cryptogenic *A. lessonii* is

recorded for the first time in the Maltese Islands and Pantelleria and Favignana islands. In Southern Sicily, *A. lobifera* not only has markedly extended its distribution range between 2016 and 2017, colonizing a coastal area of over 150 km, but also significantly increased its relative abundance, passing from 12% to ~ 50% of the total benthic foraminiferal assemblage. The very high abundance of *A. lobifera* recorded in Pantelleria Island (~ 80% in some sites) indicates that this fora-NIS has likely arrived in the area much earlier than the first record in 2017, while the lower abundance recorded in Favignana Island (< 5%) suggests a more recent arrival. Based on our new records, an average expansion rate of ~ 13.2 km per year also resulted for *A. lobifera* in the Mediterranean Sea, a higher value with respect to previous estimates of ~ 8 km per year (Langer et al., 2013).

Interestingly, several living specimens of *A. lobifera* were found attached to algae at very shallow depths (from 50 cm to 5 cm), where radiation can be particularly intense and water temperature can exceed 30 °C. This is quite different from what has been reported from the native range of this species: in the Indo-Pacific region, in fact, *A. lobifera* is known to thrive at depths < 20 m with the highest abundances at depths < 10 m (Hallock, 1999; Langer & Hottinger, 2000; Triantaphyllou et al., 2012) and to seek protection beneath algal thalli or reef rubble when solar irradiation is too intense (Beavington-Penney & Racey, 2004; Spezzaferri et al., 2018). Our findings at very shallow depths confirm a higher light and thermal tolerance of the Mediterranean populations of *A. lobifera*, as also observed in populations of the Eastern Mediterranean (Schmidt et al., 2016; Prazeres et al., 2017; 2020; Weinmann & Langer, 2017).

According to our new records, the colonization process of *A. lobifera* seems to proceed northward within the Sicily Channel, probably driven by the surface currents that move from the Pelagian and Maltese Islands towards southern Sicily. Since living specimens of *A. lobifera* were found attached on algal thalli or seagrass leaves, it can be assumed that this fora-NIS could be passively transported via floating algae, floating rafts or marine litter (e.g. Finger, 2018) that may strongly facilitate its spread over large distances (Katsanevakis & Crocetta, 2014). Another potential vector that may be involved in the dispersal process of this fora-NIS is ichthyochory, due to the recent findings of living specimens of *A. lobifera* in faecal pellets of the herbivorous rabbitfish *Siganus luridus*, that may furtherly accelerate its spread in the Mediterranean Sea (Guy-Haim et al., 2017).

7.1.2 Range expansion of *Amphistegina* in the Mediterranean Sea: present and future

The present new records of *A. lobifera* from the Sicily Channel, combined with recent records from Tunisia (El Kateb et al., 2018) and from the southern Adriatic Sea (Langer & Mouanga, 2016), show that amphisteginids have been rapidly spreading northwestward towards the Tyrrhenian Sea

and into the Adriatic Sea. This datum strongly supports range extensions predicted by previous species distribution models (Langer et al., 2012; Weinmann et al., 2013) and allowed to refine the lower temperature tolerances of *A. lobifera*, which thrives close to its current distribution limit. Given that it has been assumed that the latitudinal distribution of amphisteginids in the Mediterranean Sea is mainly controlled by the winter SST of $\sim 14^{\circ}\text{C}$ (Zmiri et al., 1974; Betzler et al., 1997; Langer & Hottinger, 2000), the observed range extension of this thermophilic fora-NIS suggests that its northwestern spread is following the current SST increase in the Mediterranean Sea, one of the most strongly affected areas in the world by rising temperatures. According to the updated model provided by this study, suitable habitats are predicted along the coasts of Algeria, Morocco and the Alboran Sea, while in the next 50 and 100 years *A. lobifera* is expected to colonize wide areas in the Adriatic Sea, Central and Western Mediterranean, favoured by ongoing sea warming.

7.2 Invasion dynamics of *Amphistegina* spp. in Malta Island

Spatial and temporal patterns of marine bioinvasion, together with several other aspects of this phenomenon, still remain unknown in most cases, due to sporadic monitoring and incomplete taxonomic knowledge, which allowed several non-indigenous species to remain undetected for years, decades or sometimes centuries (Carlton 2009; Griffiths et al., 2010; Zenetos et al., 2019). In particular, microscopic taxa often remain unnoticed for several years after their introduction (Gómez, 2019; Guastella et al., 2019). This is the case of *Amphistegina* spp. that, thanks to its very small-size ($< 2\text{ mm}$), colonised wide areas in the Eastern and Central Mediterranean, remaining undetected for years and being recorded only after its populations had reached outstanding densities.

The second Aim of this thesis addressed this problem and investigated the first occurrence of *A. lobifera* in Malta, where it is currently widespread and highly abundant (Guastella et al., 2019), despite that it was recorded only in 2006 for the first time (Yokes et al., 2007). To reconstruct the invasion dynamics of this fora-NIS and to relate its abundance patterns with the temporal trends of sea surface temperature (SST) measured in the area, a micropaleontological approach was applied: the benthic foraminiferal content was analysed along two sediment cores chronologically constrained through radiometric dating.

7.2.1 The 70 year-long invasion history of *Amphistegina* under climate change

The present study reconstructs the 70 year-long invasion history of the Indo-Pacific species *A. lobifera* and the cryptogenic *A. lessonii* in the Central Mediterranean, where both have been

exposed to a progressive heating. According to our results, these two tropical species reached Malta at the beginning of the 1940s, several decades earlier than their first records by Yokes et al. (2007) and Guastella et al. (2019), which far exceeded our expectations. In fact, not only about a 60 year-long delay emerges in the assessment of the ‘first arrival’ of *A. lobifera* in Malta, but also a higher spreading rate for *A. lobifera* derives from the present records. If, on the basis of the results obtained from Aim 1, *A. lobifera* was estimated to have spread at a rate of 13.2 km yr⁻¹ in the Mediterranean Sea, according to new data provided by this second Aim, the species has spread at a rate of ~ 45 km yr⁻¹, considering that Malta is about 3200 km far from Port Said at the mouth of the Suez Canal and that *A. lobifera* has covered this distance in about 70 years. A remarkable spreading rate for a microscopic benthic foraminifer but consistent with the spreading rates estimated for other Erythraean invaders that move in response to climate change (Hiddink et al., 2012).

Additionally, based on our results, a strong relation between the colonization process of *Amphistegina* spp. in Malta during the last 70 years and climate change emerged. Although both *A. lobifera* and *A. lessonii* reached Malta at the beginning of the 1940s, their population explosion began only after 1990 and markedly accelerated after 2003, when the wintry SST repeatedly exceeded 15 °C. In fact, a consistent temperature increase was documented starting from 1980-1983 and a notable acceleration of the surface warming trend was reported in the Mediterranean Sea during the last two decades (Marullo et al., 2011; Pastor et al., 2017; Sakalli, 2017). The statistically significant correlation between the increasing abundances of both *A. lobifera* and *A. lessonii* along the cores and the annual/wintry SST average values and annual/wintry SST anomaly suggests that the Mediterranean surface warming caused by the ongoing climate change has promoted the development of dense amphisteginid populations in the Maltese Islands. This datum supports also the hypothesis that the increase of sea surface temperatures favours both the redistribution of marine biodiversity and the spread of thermophilic invaders (e.g. Occhipinti-Ambrogi, 2007; Walther et al., 2009; Marras et al., 2015; Molinos et al., 2015; Walsh et al., 2016), especially in the semi-enclosed Mediterranean Sea where the climatic signals can be amplified (Pastor et al., 2017).

7.2.2 The unexpected ‘early failed invasion’ of *A. lobifera*

The present study also documents an unexpected and surprising event: the presence of *Amphistegina* spp. in the Maltese Islands at the beginning of the twentieth century. However, this first attempt of colonization failed due to one of the most catastrophic geological event of the Mediterranean Sea: a tsunami wave generated by the 1908 Messina earthquake (Guidoboni et al., 2007). The analysis of the same cores collected in Marsamxett Harbour (Malta) documents the early occurrence, and subsequent disappearance, of both *A. lobifera* and *A. lessonii* in correspondence

with sedimentary signatures of tsunami deposits. The identification of an early failed invasion by a microscopic taxon in itself is very rare, if not a unique case in marine bioinvasion studies, but the present discovery is even more exceptional, because the early invasion was stopped by an extremely rare and unpredictable event: the tsunami wave generated by an earthquake. Additionally, if in sediment cores collected from deep-water settings (such as off the eastern Sicilian coasts; Smedile et al., 2011) tsunamiites are easily recognizable thanks to their peculiar sedimentary signatures, on the contrary, the deposition of tsunamiites in coastal environments is even more surprising, given that tsunami waves generally leave little or no traces in sedimentary record from coastal areas (Dawson & Stewart, 2007; Noda et al., 2007; Judd et al., 2017), because these places are continuously subject to reworking by littoral currents and anthropic activities or simply tsunamiites are mistake with tempestite layers (Nanayama et al., 2000).

The violent tsunami originated from the 1908 Messina earthquake reached the Maltese Islands about one hour later, impacting the eastern coast and causing significant damages (Borg et al., 2016; Mottershead et al., 2014; 2017). Luckily, in the naturally sheltered bay of Marsamxett Harbour, the tsunami wave caused only a going and coming of the sea during the day (Borg et al., 2016 and references therein), remobilising the sea-bottom sediments but without any significant sediment transport landwards or offshore. However, the deposition of suspended sediments buried the seagrass meadow under over 25 cm of mud, causing a drastic habitat alteration. This phenomenon directly impacted all the benthic community, including the foraminiferal species *A. lobifera* and *A. lessonii* that abruptly disappeared in Marsamxett Harbour. Both re-appeared only after 30 years, when their current, more successful, invasion process started.

In conclusion, based on the data obtained by this second Aim, the Indo-Pacific *A. lobifera* seems to have much higher colonizing capabilities than previously believed in the Mediterranean Sea. If we consider that more than a century has passed between the opening of Suez Canal occurred in 1869 and our first record of *A. lobifera* in Favignana Island in 2018 (Aim 1), an average expansion rate of ~ 13.2 km per year emerges. However, if we consider that the current colonization of *A. lobifera* in Malta started earlier at the beginning of the 1940s (Aim 2), a spreading rate of ~ 45 km per year can be calculated, a value much higher than the previous one and that could also be further increased if we consider the early invasion failed in 1908, making *A. lobifera* one of the most precocious Lessepsian invaders in the central Mediterranean Sea. This implies that any estimate of spreading rate done so far, based on the known sequence of first records of *A. lobifera* along the eastern and central Mediterranean coasts, is highly underestimated and force us to revise the species distribution models for *A. lobifera* in the entire Mediterranean basin.

7.3 Impact of *A. lobifera* colonization on native assemblages of benthic foraminifera

Alien marine species may become so invasive to have severe impacts on biodiversity, causing in some cases the decline or even disappearance of native species, with negative consequences for local habitats and relative ecosystem services (Katsanevakis et al., 2016 and references therein). Some ecological impacts of larger marine organisms (especially crustaceans, molluscs, macroalgae and fishes) have been documented (e.g. Katsanevakis et al., 2014b), but very little is known about the impact of microscopic taxa, such as benthic foraminifera, on native communities. *Amphistegina lobifera* has been considered as one of the benthic foraminiferal NIS with the highest potential impact (Tsiamis et al., 2019), because profound ecological changes have been observed in the Eastern Mediterranean (Langer et al., 2012; 2013; Mouanga & Langer, 2014), where it has become the dominant foraminiferal species altering the fluxes of carbonate deposition and forming biogenic sands, and thus modifying benthic habitats (Hyams et al., 2002; Meriç et al., 2008; Triantaphyllou et al., 2009; Çinar et al., 2011; Langer et al., 2012; Schmidt et al., 2015). However, in the Central Mediterranean, where *A. lobifera* was more recently recorded (Yokes et al., 2007; Caruso & Cosentino, 2014; Guastella et al., 2019), the real effects of its colonization on native assemblages are as yet unexplored.

The third Aim of this thesis addressed the potential impact of the highly invasive *A. lobifera* on native benthic foraminiferal assemblages in the Sicily Channel (Central Mediterranean). In particular, experimental designs aimed at evaluating differences across areas, sites and depths by using species composition, richness, diversity and evenness as response variables, in order to compare benthic foraminiferal communities among three areas at different stages of invasion. Collected results document a conspicuous decline in foraminiferal richness, diversity and evenness where *A. lobifera* is more abundant. In fact, lower values of community indices are recorded in the Maltese Islands, where *A. lobifera* is widely and abundantly present along the entire archipelago, especially at 2-5 m depth, where this fora-NIS dominates the native assemblage. On the contrary, the foraminiferal community is significantly more diversified in Southern and Southeastern Sicily, where *A. lobifera* still shows low to null abundances. Therefore, the present data support a correlation between the invasion process of *A. lobifera* and the alterations of foraminiferal community structure, given that a significant loss of benthic foraminiferal biodiversity and the consequent homogenization of the foraminiferal faunas were recorded where *A. lobifera* is more invasive. As already documented in the Adriatic Sea (Langer & Mouanga, 2016), epiphytic foraminiferal species (mainly the temporary attached taxa; Langer, 1993) seem to be primarily impacted by the invasion of *A. lobifera*, probably because they share the same microhabitat at

shallow water ecosystems, while small-size non-symbiont bearing miliolid (epiphytic permanently motile) seem to be less affected, probably due to their adaptability to a wider range of microhabitats (Mouanga & Langer, 2014; Langer & Mouanga, 2016).

7.4 Open questions

During these three years of doctorate, I tried to answer several questions shedding light on some aspects of bioinvasion phenomenon by non-indigenous benthic foraminifera in the Sicily Channel (Central Mediterranean), especially for the highly invasive *A. lobifera*. Although many results have been successfully achieved, other questions are still open and need further investigations.

Firstly, the understanding of possible impact on native assemblages achieved here is currently based on a limited set of data and should be supplemented with additional samples and granulometric analyses. This was actually foreseen in the original experimental design, but was subsequently reduced due to time constraints and a re-allocation of the thesis Aims. Similarly, impact studies could be conducted not only across space, but also across time, by studying the foraminiferal communities along sediment cores. Finally, impacts on benthic meiofauna (other than foraminifera) and macrofauna are yet to be explored, in order to better understand the effects of this invasion on the whole benthic habitat.

In this thesis it was shown that *Amphistegina lobifera* is amongst the most precocious NIS in the Mediterranean Sea that have reached the central basin: the core samples, in fact, provide evidence that *A. lobifera*, together with *A. lessonii*, had already occurred in Malta at the beginning of the twentieth century with an early invasion, then failed. However, this result is very limited in space and is not enough to understand the overall pattern of *A. lobifera* invasion from the Eastern Mediterranean towards the central basin. Therefore, it would be fundamental to collect and analyse other sediment cores from different areas of both Eastern and Central Mediterranean, in order to correctly date the sequence of *A. lobifera* first occurrences throughout the Mediterranean basin and to compare its temporal invasion histories across the spatial scale.

Moreover, since *A. lobifera* seems to possess spreading capability much higher than previously supposed, even the most updated species distribution model provided earlier in this thesis (Aim 1) will need to be revised accordingly. Furthermore, it is not to be excluded that the currently known distribution range in the Mediterranean Sea, with the current westernmost limit located in Favignana Island (Guastella et al., 2019) and its northern limit located in the southern Adriatic Sea (Langer & Mouanga, 2016), has actually been already overcome. Therefore, it would be fundamental to carry out further monitoring activities in those areas where *A. lobifera* has not been

recorded yet but is expected to have already arrived. Auto-ecological studies are also required to better understand the preferred habitat of Mediterranean populations of amphisteginids. The present study, in fact, highlighted preference of *A. lobifera* for very shallow depths, which somehow contradicts observations conducted in the native area (Hallock, 1984; 1999). Similarly, thermal tolerance of the Mediterranean populations may be responsible for their invasion success and should be studied in detail.

Finally, another still unresolved question concerns the origin of the Mediterranean populations of *A. lessonii*, which is currently to be considered cryptogenic. If the Indo-Pacific origin of the Mediterranean *A. lobifera* has been proven thanks to recent genetic analyses (Schmidt et al., 2016; Prazeres et al., 2020), little is known on the congeneric *A. lessonii*. This species, in fact, could be a Lessepsian immigrant entered by the Suez Canal together with *A. lobifera*, or alternatively an Atlantic species entered through the Strait of Gibraltar, or even a relict species that survived in ecological refuges since the Pliocene. In order to clarify this, it would be fundamental to carry out also for the species *A. lessonii* the same genetic studies conducted to assess the Indo-Pacific origin of *A. lobifera*. However, in the Mediterranean Sea, *A. lessonii* seems to occur more rarely and often with smaller-sized specimens compared to the congeneric *A. lobifera* (Titelboim et al., 2019). Consequently, a higher sampling effort is required in order to collect *A. lessonii* populations from different geographical areas of the Mediterranean Sea.

CHAPTER 8

THE STATE-OF-THE-ART BEFORE AND AFTER THE PHD PROJECT

The biological invasions of non-indigenous species (NIS) in the marine environment are redefining the biogeography of the oceans and continental seas with negative effects on native communities and local ecosystem functioning, thus representing one of the most significant components of global change (Occhipinti-Ambrogi, 2007; Butchart et al., 2010; Early et al., 2016). The dispersal of marine NIS is favoured by the opening of artificial canals and new gateways, which connect previously separated basins and allow NIS to pass by natural spread or facilitated also by shipping traffic. The Mediterranean Sea is a semi-enclosed basin highly affected by a biogeographic phenomenon called ‘Lessepsian invasion’: due to the opening of the Suez Canal, an artificial waterway completed in 1869 by the businessman Ferdinand de Lesseps, several Red Sea and Indo-Pacific marine species have massively entered the Mediterranean during the last century, causing a dramatic shift of marine communities (Givan et al., 2018 and references therein). In the last decades, the progressive enlargements of the canal, culminated with the excavation of a second navigation lane in 2016 and the drop of physiographic and ecological barriers such as the progressive salinity decrease of the Bitter Lakes, have even more dramatically increased this phenomenon, also favoured by the current global warming that is driving the so called ‘tropicalization’ of the Mediterranean Sea.

The most widespread and successful NIS of benthic foraminifera coming from the Red Sea is the thermophile *Amphistegina lobifera*. This species, whose Lessepsian origin has been recently demonstrated by genetic data (Schmidt et al., 2016; Prazeres et al., 2020), is highly invasive in the Eastern Mediterranean, where it is known to have modified native habitats and to have altered benthic community structure (Hyams et al., 2002; Meriç et al., 2008; Triantaphyllou et al., 2009; Çinar et al., 2011; Langer et al., 2012; Schmidt et al., 2015). Another ‘new appearance’ in the Mediterranean, although so far only very sporadic, is represented by *Amphistegina lessonii*, congeneric of the previous species. Although its exact origin is unknown, hence it should be considered cryptogenic instead of non-indigenous, this species is also worth attention. Amphisteginids have been rapidly expanding towards the Central Mediterranean and, more recently, have been recorded in the Sicily Channel (Yokes et al., 2007; Caruso & Cosentino, 2014) and in the southern Adriatic Sea (Langer & Mouanga, 2016). However, the real status of invasion of both *A. lobifera* and *A. lessonii* in the central Mediterranean basin is still poorly known, as well as their temporal colonization dynamics and the possible effects on native benthic foraminiferal communities.

The present PhD project aimed to fill these gaps of knowledge, starting from the evaluation of the current distribution of *A. lobifera* and other cryptogenic benthic foraminifera (*A. lessonii*, *A. hemprichii*, *C. arietina*, *C. hemprichii* and *S. orbiculus*) in the Sicily Channel, thus allowing to update the current establishment status of these poorly known small-sized marine organisms in the Central Mediterranean. This study provided new records of both *A. lobifera* and *A. lessonii* in Southern Sicily, along the coast between Ragusa and Siracusa, and Pantelleria and Favignana islands. Moreover, it allowed to document three different stages of invasion in the Sicily Channel: more advanced in Pantelleria and the Maltese islands, where *A. lobifera* was highly abundant and widespread in most of the studied sites, medium stage in Southern Sicily, where it occurred in most of the studied sites but with lower abundances, and early stage in Favignana Island, where only sporadic specimens were found. These new records, combined with other recent findings from the Central Mediterranean (Langer & Mouanga, 2016; El Kateb et al., 2018), show that amphisteginids have been rapidly spreading northwestward towards the Tyrrhenian Sea and into the Adriatic Sea, following the current SST increase in the Mediterranean Sea as clearly simulated by the updated species distribution model here presented and discussed. Therefore, the new data on distribution and establishment status of *A. lobifera* and *A. lessonii* show that both species have already colonized most of the Sicily Channel, reaching the oceanographic boundary with the Tyrrhenian Sea, and are ready to colonize also the western Mediterranean basin (assuming they have not already done so) given the current sea surface temperature increase.

The present PhD project also aimed at reconstructing the invasion dynamics of both *A. lobifera* and *A. lessonii* in Malta, which is centrally located within the Sicily Channel and where both species are widespread and *A. lobifera* is also highly abundant. The lack of historical records, in fact, has so far prevented determining the timing of their real first occurrence in the island. To this purpose, a ‘micropaleontological approach’ was applied for the first time through the analysis of sediment cores radiometrically dated and assessment of the abundance of the target foraminiferal species along the core. This approach successfully allowed reconstructing the temporal dynamics of the current invasion of both *A. lobifera* and *A. lessonii* in Malta, which started at the beginning of the 1940s and remained undetected for about 60 years. The studied cores provided also an unexpected finding: an early failed invasion, making it possible to backdate the first occurrence of amphisteginids in Malta to the beginning of the twentieth century, about one century earlier than the first record of *A. lobifera* (Yokes et al., 2007) and more than one century earlier than the first record of *A. lessonii* (Guastella et al., 2019). The invasion failure was triggered by an unpredictable geological event: the 1908 Messina earthquake that caused an abrupt tsunami. The destructive waves reached the Maltese coasts causing damages and stopping the first invasion attempt of both

target species. Additionally, the collected data explain that ocean warming probably drove the *Amphistegina* spp. outbreak since their arrival in the early 1940s. A clear positive correlation between the abundances of *Amphistegina* spp. along the record and the sea surface temperature values recorded in the Central Mediterranean Sea was statistically demonstrated. This supports once again the hypothesis that the current global warming is driving the colonization success of tropical amphisteginids in the Mediterranean Sea (Langer et al., 2012; 2013). These surprising data, although limited to only one locality of the Sicily Channel, overturns what was known so far about the colonization process of amphisteginids in the Mediterranean Sea, consequently leading to reconsider their colonizing capabilities, which seem to be much higher than previously supposed, and to revise the species distribution models for *A. lobifera* in the entire Mediterranean basin. Therefore, the applied micropaleontological approach deserves to be included in the toolkit for studying of invasion dynamics by the smallest marine organisms with mineralised remains.

Finally, the present PhD project is the first study that quantitatively assessed the potential impact of *A. lobifera* invasion on native assemblages of benthic foraminifera in the Sicily Channel. To this purpose, community structure variables were estimated in three areas of the Sicily Channel that were known to be at different stages of invasion, on the basis of the data collected in Aim 1 (Maltese Islands: advanced stage; Southern Sicily: medium stage; Southeastern Sicily: early stage). Results document a severe loss of biodiversity in the Maltese Islands, where *A. lobifera* is highly abundant, while in the Southern and Southeastern Sicily this phenomenon seems to be only at the beginning. This last result provides a dramatic picture of what could happen in the next future in the whole Sicily Channel, as well as in the rest of the Mediterranean, where many coastal areas could be invaded by *A. lobifera* as predicted by the updated species distribution model, which now is known to have largely underestimated the spreading capability of this species.

In conclusion, the unprecedented multidisciplinary approach applied in my PhD research, which combines for the first time methods commonly used in Marine Ecology with those used in Micropaleontology, resulted successful and useful for the investigation of marine bioinvasions. Therefore, my PhD project is a pioneer study that could serve as a trailblazer for further research on invasion by small-size marine NIS, of which it is evident that very little is still known.

CHAPTER 9

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