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DISTRIBUZIONE E CARATTERIZZAZIONE DI *SAVALIA*
***SAVAGLIA* (BERTOLONI, 1819) (CNIDARIA: ANTHOZOA) NEL**
MEDITERRANEO

DISTRIBUTION AND CHARACTERIZATION OF *SAVALIA*
***SAVAGLIA* (BERTOLONI, 1819) (CNIDARIA: ANTHOZOA) IN THE**
MEDITERRANEAN SEA

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Riassunto

Savalia savaglia (Bertoloni, 1819) (Cnidaria, Anthozoa) è uno zoantario appartenente alla famiglia Parazoanthidae, presente in Mediterraneo e in alcune zone del NE Atlantico. È considerata una specie rara e, dato il suo caratteristico scheletro di natura proteica, è comunemente nota come “Corallo dorato” o “Falso corallo nero”. Benchè le sue caratteristiche biologiche ed ecologiche siano ancora poco chiare, alcuni aspetti rendono questa specie estremamente interessante.

La capacità di deporre un proprio scheletro è rara tra gli zoantidi e risulta peculiare in *S. savaglia* soprattutto considerando che questa specie è solita crescere parassitando i rami di altri cnidari, in particolare gorgonie: una volta ricoperto interamente l'ospite, *S. savaglia* inizia a deporre il suo scheletro in sottili anelli visibili in sezione. Questo tipo di deposizione ha permesso di stimare l'età di alcune colonie a più di 2000 anni, rendendo questa specie una delle più longeve sulla Terra: *S. savaglia* potrebbe essere usata per ricostruire le condizioni paleoclimatiche su scala millenaria.

In questa tesi è stata aggiornata la distribuzione di *S. savaglia* in Mediterraneo e, applicando modelli predittivi, sono state evidenziate le aree ancora poco studiate del bacino che potrebbero ospitarla, allo scopo di fornire un valido strumento per pianificare futuri monitoraggi sul campo. Una volta raccolti questi dati, abbiamo valutato se popolazioni geograficamente distanti di *S. savaglia* possano essere caratterizzate in base ad alcuni aspetti della loro morfologia e biologia, analizzando campioni raccolti in sei località del Mediterraneo: Portofino, Ischia, Ustica, Gallipoli, Baia di Kotor (Montenegro) e stretto dei Dardanelli.

La raccolta di osservazioni di *S. savaglia* nel Mediterraneo è stata effettuata sfruttando diverse fonti: la letteratura scientifica, inclusa la *grey literature*, progetti di Citizen Science e risorse del World Wide Web. Utilizzando il software QGis 3.4.7, è stato possibile elaborare delle mappe, per comprendere le dinamiche della distribuzione della specie come la profondità o il numero di colonie. Sui dati di presenza di *S. savaglia* sono stati poi applicati tre tipi di modelli predittivi e le performances confrontate tra loro e valutate in base alle caratteristiche ecologiche attualmente note.

In merito alla caratterizzazione delle sei popolazioni campionate, è stato preso in considerazione ed analizzato lo cnidoma, un carattere morfologico applicato spesso in tassonomia con risultati molto variabili in base alla specie. Successivamente sono stati analizzati i sedimenti incorporati e il contenuto in collagene nei tessuti molli. L'incorporazione dei sedimenti è un processo che accomuna molte specie di zoantari, nonché di poriferi. Le nostre analisi hanno permesso di verificare (1) se in *S. savaglia* esista un meccanismo di selezione dei sedimenti incorporati, per taglia e/o per composizione (2) se questo comportamento abbia lo scopo di stimolare l'espressione del gene per la sintesi del collagene, come osservato nella demospongia *Chondrosia reniformis*. I sedimenti incorporati sono stati analizzati, in termini sia qualitativi che quantitativi, in tutte e sei le popolazioni campionate, ed i risultati ottenuti confrontati con la mineralogia dei siti; per tre popolazioni – Ischia, Gallipoli e baia di Kotor – è stato quantificato anche il collagene.

Dalla raccolta delle osservazioni di *S. savaglia*, è emerso che esiste ancora una grande lacuna nelle conoscenze sulla distribuzione nel bacino orientale del Mediterraneo e che ulteriori indagini sul campo dovrebbero essere effettuate. Nella pianificazione di futuri monitoraggi, il nostro modello predittivo può essere un utile strumento per capire quali aree meritino attenzione prioritaria.

In merito alla caratterizzazione di popolazioni distinte di *S. savaglia*, dalle nostre analisi è emerso che queste si differenziano sia in base allo cnidoma che alla quantità e composizione di sedimenti incorporati. In particolare, *S. savaglia* presenta sette diverse tipologie di nematocisti distribuite in diverse parti del polipo: in termini sia di localizzazione che di dimensioni e abbondanza, si osserva una variabilità non solo tra popolazioni, ma anche all'interno della stessa popolazione. Queste osservazioni invitano a proseguire nelle indagini, per capire quali fattori ambientali, biologici ed ecologici influiscano sulla plasticità dello cnidoma.

I sedimenti incorporati, se da una parte appaiono selezionati in base alla granulometria, favorendo una taglia compresa tra i 10 e i 50 μm , dall'altra riflettono abbastanza bene la mineralogia tipica della località in cui le popolazioni campionate si trovano. Le analisi effettuate sul collagene, infine, hanno evidenziato una relazione tra l'abbondanza di quarzo incorporato e l'abbondanza di questa proteina. Ulteriori indagini su più popolazioni

serviranno, in futuro, a comprendere meglio i meccanismi di questo processo e a individuare altri fattori che influenzano la produzione di collagene in *S. savaglia*.

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1. Introduction

Savalia savaglia (Bertoloni, 1819) (Cnidaria, Anthozoa) is a zoantharian species belonging to the family of Parazoanthidae, distributed in the Mediterranean Sea, as well as in some areas of the N-E Atlantic Ocean. The scarce knowledge about its biology and ecology has led to various mistakes in its definition and classification. *S. savaglia* is considered rare and, owing to its ability to build a hard proteinaceous skeleton, is commonly known as “Gold coral” or “False black coral”. Although little is known about this genus, however, it’s clear that *S. savaglia* is in many aspects a peculiar species.

Savalia spp. are among the few zoanthids able to secrete their own skeleton: the structure is of a hard, dense, layered proteinaceous material, a unique form among skeletal accreting animals. The zoanthid usually starts growing on the stem of gorgonians or Antipatharians and, when the host is completely engulfed, it starts secreting its own skeleton (Bell, 1891; Zibrowius, 1985). In some area also other strategies have been reported. Where the typical hosts are absent or do not offer the appropriate support, the colony may grow directly on other substrates, forming a basal plate on which it can produce the skeleton (Ocaña and Brito, 2004). Anyway, these patterns of growth need to be better explored.

The trunk is deposited in small, 5-10 μm thick bands, visible in thin sections. Radiocarbon-based analyses suggest that particulate organic carbon (POC) is a major source of carbon to the skeleton and allowed Druffel *et al.* (1995; cited as *Gerardia savaglia*) to estimate the age of some specimens at almost 2000 years. Other studies report specimens with an even longer lifespan (2742 years, in Roark *et al.* (2006)), making the species of this genus among the most long-living on Earth. These data suggest a potential role in the paleoenvironmental reconstruction on millennial time scales, by using the layered trunk as a record of surface

productivity and nutrients vertical fluxes (Druffel *et al.*, 1995; Prince and Kheshgi, 1998; Roark *et al.*, 2009).

Savalia savaglia also appears to incorporate sediments within its tissues, a behaviour that has only been observed in zoanthid species (e.g. *Palythoa* spp. as observed by Haywick and Mueller, 1997) and sponges. Among Porifera it is a widespread phenomenon, even in phylogenetically non-related species, suggesting a polyphyletic origin (Cerrano *et al.*, 2007). While in sponges this behaviour has been better analysed, data regarding zoanthids are still missing and further studies are needed in order to understand the mechanism and its possible physiological roles.

Zoanthids of the genus *Savalia* have been reported to grow in a wide depth range. In the Mediterranean Sea, *S. savaglia* has been observed from 10-20 m along the Montenegro's coasts (Kružić, 2007) and down to 900 m near the Balearic Islands (OCEANA, 2010); however, most of the sightings refer to the depth range typical of the mesophotic zone (Giusti *et al.*, 2015). This species can form monospecific facies of hundreds of colonies, as observed in Montenegro and, between 27 and 70 m, in the Canary Islands (Brito, 1983; Ocaña Vicente *et al.*, 1995; Ocaña and Brito, 2004).

Being a long-living organism able to produce a three-dimensional hard skeleton, *S. savaglia* can be considered an ecosystem engineer (Jones *et al.*, 1994). Ecosystem engineers are increasingly recognized as relevant ecological drivers of diversity and community composition; those that create new habitats or microhabitats, like *S. savaglia*, seem to have stronger effects than those that modify habitats or cause bioturbation (Romero *et al.*, 2015). Many studies have demonstrated that the structural complexity of the marine habitat plays an essential role in the life history of invertebrate species and fishes, both in shallow and

deep waters (Jones, Lawton and Shachak, 1994; Baker, Puglise and Harris, 2016; Andradi-Brown *et al.*, 2019; Holstein *et al.*, 2019; Reimer *et al.*, 2019). Cerrano *et al.* (2010), by studying a dense population of *S. savaglia* near the Portofino promontory, demonstrated that the species enhances the surrounding biodiversity, by providing shelter and feeding areas for many organisms, as well as deposition of bioavailable substrates. Although this role can also be ascribed to the neighbouring gorgonian forests, the extreme longevity of *S. savaglia* suggests that its effects can be relevant on longer temporal scales than those observed for shorter-lived species, such as gorgonians. Another effect of these animal forests on habitats may be the creation of a so-called “buffer zone”, where environmental conditions are more stable and the effects of stressors are mitigated (Valisano *et al.*, 2016; Holstein *et al.*, 2019).



Figure 1 A colony of *Savalia savaglia* among gorgonians. Messina Strait, photo by Santi Cassisi.

Lately, an interest in the ecology and biology of deep-sea corals, and in the habitat they provide for other organisms, has increased in response to growing concerns due to the threat of physical destruction caused by pelagic and benthic fisheries. Many studies report that *S. savaglia*, mostly living in areas strongly impacted by fishing activities, is severely threatened by physical damage from fishing gear, mainly ghost nets and fishing lines (Zibrowius, 1985; Pais *et al.*, 1992; Boudouresque *et al.*, 1996; Bavestrello *et al.*, 1997; Calado, 2006; Cerrano *et al.*, 2007; Barrajon Domenech *et al.*, 2008; Altuna, Sinniger and Aldrey, 2010; Torchia *et al.*, 2016; Noel and Francour, 2017; Enrichetti *et al.*, 2019; Manna and Sarà, 2019); excessive sedimentation could also damage the colonies, as reported by Topçu *et al.* (2019)

observing effects of such event caused by construction and landfill operations in the Sea or Marmara. Recreational Scuba diving maybe another threat, since colonies could be damaged due to direct contact of diver equipment

S. savaglia is too often collected to exhibit its beautiful skeleton as a souvenir (Pais *et al.*, 1992; Barrajon Domenech *et al.*, 2008) or used to make jewellery (Artuz *et al.*, 1990; Öztürk and Bourguet, 1990). The harvesting of *Savalia savaglia* in the Mediterranean was a very common practice a few decades ago: in 1960, a renowned magazine about SCUBA diving even published an article in which the best methods to collect it were explained (Mondo Sommerso, March 1960). In several areas of the Pacific Ocean, until recently, species of genus *Savalia* were sold at approximately \$400/kg. Even though specific weight quotas and size limits were already enforced, based on estimates of maximum sustainable yields and optimum yields, a serious depletion of the *Savalia* beds had to be prevented by NOAA's National Marine Fisheries Service (NMFS), which in 2008 implemented a moratorium on the harvesting of Gold Coral throughout the U.S. western Pacific (Cooper *et al.*, 2011). A *Savalia* sp. fishery in Turkey was abandoned as well after learning that it served as substratum for several shark species to lay their eggs (Öztürk and Bourguet, 1990).

S. savaglia should be specifically protected, not only for its rarity and vulnerability, but also because it has a prominent role in sustaining high levels of biodiversity and ecosystem functioning in the surrounding benthos of the mesophotic zone (Cerrano *et al.*, 2010). At present, *S. savaglia* is included in Annex II of Specially Protected Areas of Mediterranean Importance (SPAMI-Barcelona Convention) for the protection of threatened species and their habitats and in Appendix II of the Berne Convention on the Conservation of European Wildlife and Natural Habitats.

1.1. Taxonomy of *Savalia savaglia*

Savalia savaglia is the only representative of the genus *Savalia* in the Mediterranean. Although the species has been well known for a long time, it is not well described in literature and its classification, as well as its name, have been debated for many decades. It was first described as *Gorgonia savaglia* by Bertoloni in 1819, who had seen it near the Canary Islands. In 1844, Nardo classified the species in the genus *Savalia*. Haime later, in 1849, named the species as *Leiopathes lamarcki*. In 1864, Lacaze-Duthieres introduced the genus name *Gerardia* and placed it in the order of Antipatharia, based on anatomic criteria, mainly due to the presence of a hard skeleton. Carlgren then merged the species into the Parazoanthidae family in 1895. Finally, in 1951, Roche and Tixier-Durivault reclassified the genus within the order of Zoantharia, based on the anatomy of the polyps and cnidome. Although the genus name *Gerardia* is still often used in recent literature, this thesis will use the genus name *Savalia*, since it has taxonomical priority over junior synonym *Gerardia* (Haussermann, 2003).

1.1.1. Class of Anthozoa (Ehrenberg 1834)

The class Anthozoa belongs to the phylum Cnidaria and contains over 7000 accepted species of marine animals (Worms, 2020), including sea anemones and other anemone-like groups with and without skeleton, as well as sea pens, sea fans, blue coral and black coral (Fautin and Romano, 2000). Most anthozoan orders have colonial species, such as the famous reef-building corals, but solitary organisms are also present, especially among sea anemones (Haussermann and Försterra, 2003). The polyp of an anthozoan has a soft tube-shaped body ending with a single mouth. The body wall consists of two epithelial layers, the ectoderm and the endoderm, which are separated by an extracellular matrix, called mesoglea, and arranged around a central gastrovascular cavity, called coelenteron. The tentacles

surrounding the mouth are typically hollow and carry nematocyst cells (Fautin and Romano, 2000).

The anatomy of Anthozoans is based on two main structures: the actinopharynx and the mesenteries (*Figure 2*). The actinopharynx is a pharyngeal tube that connects the mouth with the coelenteron; it contains at least one specialized longitudinal channel, called siphonoglyph, which is used to drive water into the coelenteron. Most species have two siphonoglyphs opposite to one another (Fautin and Romano, 2000). The mesenteries, or septa, are folds of endoderm that run vertically along the body, dividing the coelenteron into compartments (Berking, 2007; Berking and Herrmann, 2010). The mesenteries, besides providing body support, also guarantee a greater surface for the gastrodermis (digestive layer); moreover, they contain longitudinal muscles which enable the polyp to retract. The arrangement of these muscles is unique for each order of Anthozoa, therefore it is used as diagnostic character (Chevalier *et al.*, 1987; Fautin and Romano, 2000). Septa,

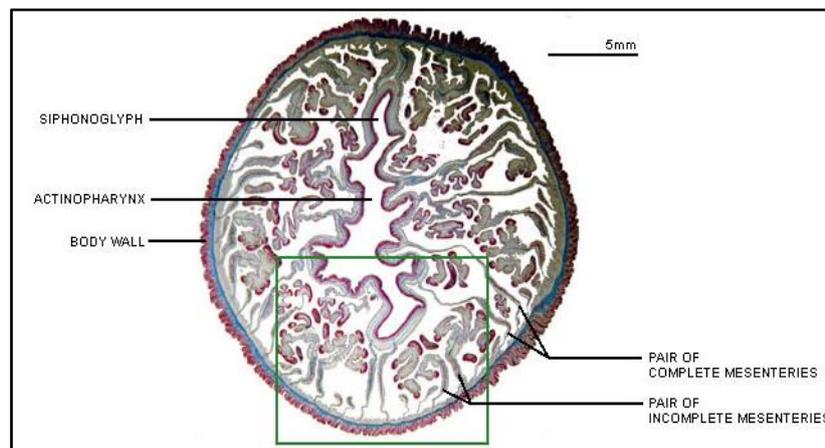


Figure 2 Cross-section of an anthozoan species at the level of the actinopharynx (from Fautin and Romano, 2000)

siphonoglyphs and all the associated structures give the anthozoan polyp a bilateral symmetry (Berking, 2007; Berking and Herrmann, 2010). The anthozoan life cycle generally includes a larval stage, free and ciliated, but not a medusa stage (Chevalier *et al.*, 1987).

Anthozoa have been divided into two subclasses, based on both morphological features, such as the number and form of the tentacles (Fautin and Romano, 2000), and molecular data:

— Octocorallia: the polyp has eight pinnate tentacles and eight mesenteries, therefore the space between each couple of mesenteries extends into one tentacle. The subclass is further divided into three orders, which are Alcyonacea, Helioporacea and Pennatulacea (World Register of Marine Life, 2020)

— Hexacorallia: the individual polyp is typically characterized by a multiple of six tentacles and mesenteries; metamorphosis from the planula larva typically results in a polyp with six evenly spaced mesenteries and six tentacles, one communicating with each inter-mesenterial space. Mesenteries and tentacles are added as the polyp grows, but the placement of new structures differs among taxa and radial symmetry is frequently altered. The subclass is further divided in six existing orders, being Actiniaria, Antipatharia, Ceriantharia, Corallimorpharia, Scleractinia and Zoantharia (Fautin, Romano and William, 2000).

Savalia savaglia belongs to the subclass Hexacorallia and the order Zoantharia.

1.1.2. Order Zoantharia (Milne Edwards 1857)

Zoantharia is the third largest order of Hexacorallia (Sinniger *et al.*, 2005) and species (zoanthids) are found in most marine environments, from shallow tropical waters to the deep sea. Systematic of Zoantharia appears to be very problematic due to the absence of appropriate descriptions and the lack of easily accessible and informative morphological characters, including the ones traditionally used for taxonomic diagnosis (Ocaña and Brito, 2004; Reimer *et al.*, 2007; Sinniger, Reimer and Pawlowski, 2010; Swain and Swain, 2014). In addition, organisms belonging to this order show high levels of intraspecific morphological variation (Swain and Swain, 2014; Low, Sinniger and Reimer, 2016). The

main characters traditionally used for species identification were the number of tentacles or septa, the colour, the shape and position of the sphincter muscle and the size and distribution of different types of nematocysts (Sinniger *et al.*, 2005); in a recent study by Swain and Swain (2014), the form of the marginal musculature emerged as a potential informative character to differentiate taxa and to better understand phylogenetic relations between genera. Molecular approaches have the potential to expand our understanding of complex taxonomic trees, but sometimes, and that's the case for Zoantharia, there are some obstacles. Despite the intensive application of nucleotide sequence-based molecular phylogenetics since early 2000s, the evolutionary relationships among zoanthid species have evaded our understanding until very recently (Low, Sinniger and Reimer, 2016; Swain, 2017). The main problem was that the most used nucleotide sequences are invariant or inconsistently differentiated between congeners; for example, the mitochondrial cytochrome oxidase subunit I gene (COI) (Swain and Swain, 2014; Swain, 2017). Poliseno *et al.* (2020) made a huge step ahead in the understanding of Zoantharian taxonomy by sequencing for the first time the complete mt-genomes of members of eight of nine zoantharian families and recovered a fully resolved tree. The study reported relatively high nucleotide diversity in mt-DNA regions other than the standard regions traditionally considered for DNA barcoding of this group, as well as variations among mt-genomes' sizes, substitution rates and introns. However, many studies underlined the need to integrate our knowledge of modern molecular phylogeny with classical microanatomy and ecology (Sinniger *et al.*, 2005; Swain and Swain, 2014; Poliseno *et al.*, 2020); without thorough morphological descriptions it is impossible to discern species boundaries and correctly apply taxonomic names to similar specimens, even if the DNA is accessible (Ryland and Lancaster, 2003; Reimer *et al.*, 2007; Poliseno *et al.*, 2020). Ecological characters may be of help in discerning similar zoanthid

species as well: most authors suggest, as potential diagnostic features, the associated substrate, biogeography and depth, but further investigations are needed to assess the validity of such characteristics (Sinniger *et al.*, 2005; Swain and Swain, 2014).

Zoanths are characterized by a double row of tentacles and a single ventral siphonoglyph (Sinniger *et al.*, 2005). Most zoanths live in colonies that are connected by living tissue called coenenchyma (also known as coenosarc) and incorporate sand grains and detritus in their ectoderm and mesoglea to help form their structure (Herberts, 1972; Previati *et al.*, 2010) while *Savalia* is the only genus of Zoantharia known to secrete its own skeletal structure (Sinniger, Reimer and Pawlowski, 2010). Zoantharia are divided into two suborders based on the organization of the fifth pair of mesenteries: Macrocnemina, which have a complete fifth pair of mesenteries, and Brachycnemina, which have an incomplete fifth pair of mesenteries (Figure 3). Molecular phylogenetic analyses confirm this division,

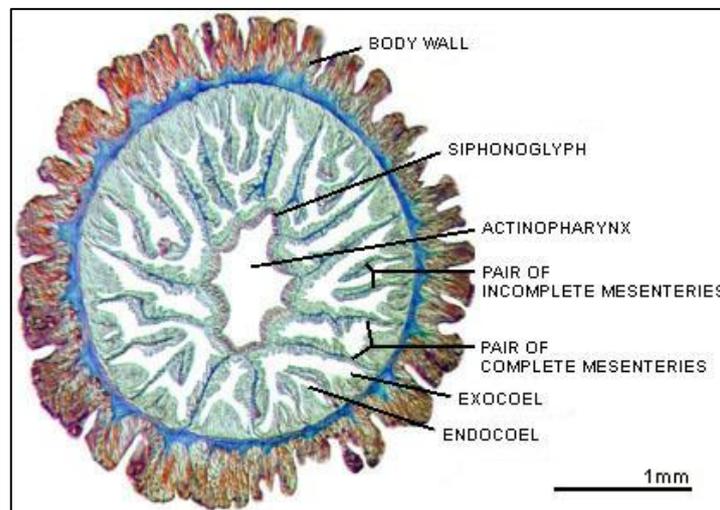


Figure 3 Cross-section of a juvenile sea anemone with three cycles of mesenteries; section near the base (from Fautin and Romano, 2000).

but they also indicate that, whereas Brachycnemina is a monophyletic suborder, Macrocnemina is paraphyletic (Sinniger *et al.*, 2005; Poliseno *et al.*, 2020). The suborders also differ in terms of reproductive cycle: Brachycnemina are commonly hermaphroditic and

produce planktonic larvae (Semper's larvae), while Macrocnemina are usually gonochoric and their larval stage is yet to be discovered (Ryland *et al.*, 2000). Regarding their ecology, Brachycnemina are found in tropical waters, mostly in the intertidal zone and shallow sublittoral of coral reefs, and usually contain zooxanthellae; in contrast, Macrocnemina species tend to occur deeper and lack zooxanthellae (Ryland and Lancaster, 2003; Previati *et al.*, 2010).

The two suborders are currently divided into nine families (Low, Sinniger and Reimer, 2016; Poliseno *et al.*, 2020); three families (with six genera) belong to Brachycnemina:

- Neozoanthidae Herberts, 1972
- Sphenopidae Hertwig, 1882
- Zoanthidae Rafinesque, 1815

while Macrocnemina are divided into six families (with twenty genera):

- Abysoanthidae Reimer and Fujiwara, 2007
- Epizoanthidae Delage and Hérourard, 1901
- Hydrozoanthidae Sinniger, Reimer and Pawlowski, 2010
- Microzoanthidae Fujii and Reimer, 2011
- Nanozoanthidae Fujii and Reimer, 2013
- Parazoanthidae Delage and Hérourard, 1901.

Savalia savaglia belongs to the suborder Macrocnemina and family of Parazoanthidae.

1.1.3. Family Parazoanthidae (Delage and Hérourard 1901)

The family of Parazoanthidae is polyphyletic, due to the placement of genus *Isozoanthus* outside the group. It is a widespread family that uses a lot of different organisms as substrate

and its taxonomy appears to be particularly problematic (Sinniger, Reimer and Pawlowski, 2010). Several studies have suggested morphological or histological characters to distinguish between different genera and species, such as the cnidome (Ryland and Lancaster, 2004b) and the sphincter muscle anatomy (Lwowsky, 1913). The latter, in particular, was confirmed to be a valuable diagnostic character by molecular analyses performed by Polisenio *et al.* (2020). The marginal musculature arrangement within the order Zoantharia can be divided into three main types: endodermal, transitional, and mesogleal. The ancestral state and subsequent transitions of this arrangement were studied and appear to be consistent with the phylogenetic relationships estimated through mitogenomic analyses. Sinniger, Reimer and Pawlowski (2010) suggested to include ecological and biogeographical parameters (e.g. locality, environmental data, depth) as taxonomic characters – primarily substrate selectivity, since Parazoanthids are often associated with other organisms used as substrates. More sampling, however, is necessary to gain a clearer picture of the range of species used as substrate by each genus (Low, Sinniger and Reimer, 2016).

The family of Parazoanthidae is currently divided into the following genera (Reimer and Sinniger, 2020):

- *Antipathozoanthus* Sinniger, Reimer & Pawlowski, 2010
- *Bergia* Duchassaing & Michelotti, 1860
- *Bullagummizoanthus* Sinniger, Ocaña & Baco, 2013
- *Corallizoanthus* Reimer in Reimer Nonaka Sinniger & Iwase, 2008
- *Hurlizoanthus* Sinniger, Ocaña & Baco, 2013

- *Isozoanthus* Carlgren in Chun, 1903
- *Kauluzoanthus* Sinniger, Ocaña & Baco, 2013
- *Kulamanamana* Sinniger, Ocaña & Baco, 2013
- *Mesozoanthus* Sinniger & Haussermann, 2009
- *Parazoanthus* Haddon & Shackleton, 1891
- *Savalia* Nardo, 1844
- *Umimayanthus* Montenegro, Sinniger & Reimer, 2015
- *Zibrowius* Sinniger, Ocaña & Baco, 2013.

Savalia savaglia belongs to the genus *Savalia*.

1.1.4. Genus *Savalia* (Nardo 1844)

Savalia has been a taxonomically problematic genus. The wide use of two different names for the same genus in literature (*Savalia* by Nardo, 1844 and *Gerardia* by Lacaze-Duthiers, 1864) has led to taxonomic confusion, which was enhanced by the reclassification of *Savalia* species within. Currently, *Savalia* is estimated to contain two species:

- *Savalia lucifica* Cutress and Pequegnat 1960
- *Savalia savaglia* Bertoloni 1819.

While the first is distributed along the North Eastern Pacific, the latter is present in the Mediterranean Sea and North Atlantic; Sinniger, Reimer and Pawlowski (2008) suggested that *S. lucifica* and *S. savaglia* may be conspecific.

The genus *Savalia* comprises slow-growing and extremely long-living species (Druffel *et al.*, 1995; Roark *et al.*, 2006, 2009) that are colonial and lack zooxanthellae (Low, Sinniger

and Reimer, 2016). *Savalia* can be found in the Mediterranean, north Atlantic and north-eastern Pacific, depending on the species, and is characterized by a wide bathymetric distribution, with depths ranging from 10 to 900 m (Cerrano *et al.*, 2010). The colonies may exceed one meter in height. *Savalia* spp. is the only known cnidarian thus far to produce ajugasterone-C. It produces an endogenous, black skeleton: a rigid and thick structure made of densely layered proteinaceous material that contains large amounts of histidine (Druffel *et al.*, 1995; Cerrano *et al.*, 2010). The organism only starts producing this skeleton when it has fully engulfed the host substrate on which it grows – Gorgonians or Anthipatarians (e.g. *Paramuricea* spp., *Eunicella* spp., *Anthipathes* spp and *Leptogorgia sarmentosa*) (Cerrano *et al.*, 2010; Low, Sinniger and Reimer, 2016). *Savalia* is also characterized by polyps having a cyclically transitional marginal musculature (Swain and Swain, 2014; Poliseno *et al.*, 2020) and lacking a mesogleal canal system in the column. The reproduction of *Savalia* can be sexual and asexual. Asexual reproduction leads to the formation of large colonies that grow on the host. It is hypothesized that the sexual reproduction follows the seasonal variations in sea water temperatures (Waller and Baco, 2007; Previati *et al.*, 2010), but larval stages and their dispersal strategies are still unknown (Ryland *et al.*, 2000; Waller and Baco, 2007; Previati *et al.*, 2010).

Some other species were previously considered to be part of this genus, since they use other Anthozoans as substrate and appear to be able to secrete their own skeleton. *Antipathozoanthus macaronesicus* Ocaña & Brito 2003 was initially baptized *Gerardia macaronesica* (Ocaña and Brito, 2004), but the authors later amended this classification (Ocaña Vicente *et al.*, 2007), and Sinniger, Reimer and Pawlowski (2010) showed through molecular analyses that it belonged to the new genus *Antipathozoanthus*, named according to the substrate specificity of its species. *A. macaronesicus* also seems to be able to outgrow

its host by at least a few centimetres (Low, Sinniger and Reimer, 2016). The genus is distributed in Eastern Atlantic (Cape Verde and Principe Islands), Madagascar, Japan and Galapagos. Another skeleton-secreting zoantharian that was misplaced in the genus *Gerardia* is *Kulamanamana haumea*, found on the Hawaiian Seamounts (Sinniger, Ocaña and Baco, 2013). Ecological features, such as depth, and molecular characters can be used to distinguish these genera (Low, Sinniger and Reimer, 2016).

1.2. Species description

1.2.1. Morphology

Savalia savaglia forms colonies with many branches, often arranged in a single plane (thus creating a fan-shaped colony), and can reach heights of over 1 m; Bell (1891) reported the finding of a specimen almost 2 metres high and over 2 metres wide, the main trunk 42,5 cm in circumference. Since *S. savalia* usually grows on colonies of gorgonians or black corals, Zibrowius (1985) suggested that its colonies may have the same type of ramification as the host's overgrown colony. When the species generates its own skeleton, it grows on a basal plate through asexual reproduction, until the colony is fully grown (Ocaña and Brito, 2004). The endogenic skeleton plays an important role in the biology of *S. savaglia* (Ocaña and Brito, 2004): the skeletal material seems similar to other structural proteins, such as bone collagen, both in terms of elemental composition and in the amino acids content. The main difference between the *Savalia* skeletal protein and collagen is that while the latter contains large amounts of Hydroxyproline, the zoanthid trunk is richer in histidine, the only amino acid with a sidechain that can be variably charged under normal biological conditions. The structure of its skeleton allows *S. savaglia* to withstand strong currents (Druffel *et al.*, 1995).

The coenenchyma is wide in the well-developed areas, but finer and less developed in the distal parts of the branches. The polyps, closely set on the axis, are more or less cylindrical and vary in size between 10-60 mm high and 20-50 mm wide (measurements of preserved polyps by Ocaña and Brito, 2004, and Altuna, Sinniger and Aldrey, 2010); the texture is rough, due to the sand incrustations in the ectoderm and mesoglea (Ocaña and Brito, 2004). The tentacles are smooth, between 21 and 27 in number and disposed into two distinct circles around the mouth. *S. savaglia* can be bright to pale yellow in colour, as well as white-pinkish, apparently without any distinction in terms of geography, gender or reproductive stage (Altuna, Sinniger and Aldrey, 2010).

Mesenteries are 26 to 28, with a macrocnemic disposition; there is a single, large siphonoglyph, with a short pharynx, and endodermal sphincter muscles do not seem well developed. Retractor muscles are also small, with a cyclically transitional marginal musculature, and tentacular muscles are rather weak (Ocaña and Brito, 2004). The ectoderm

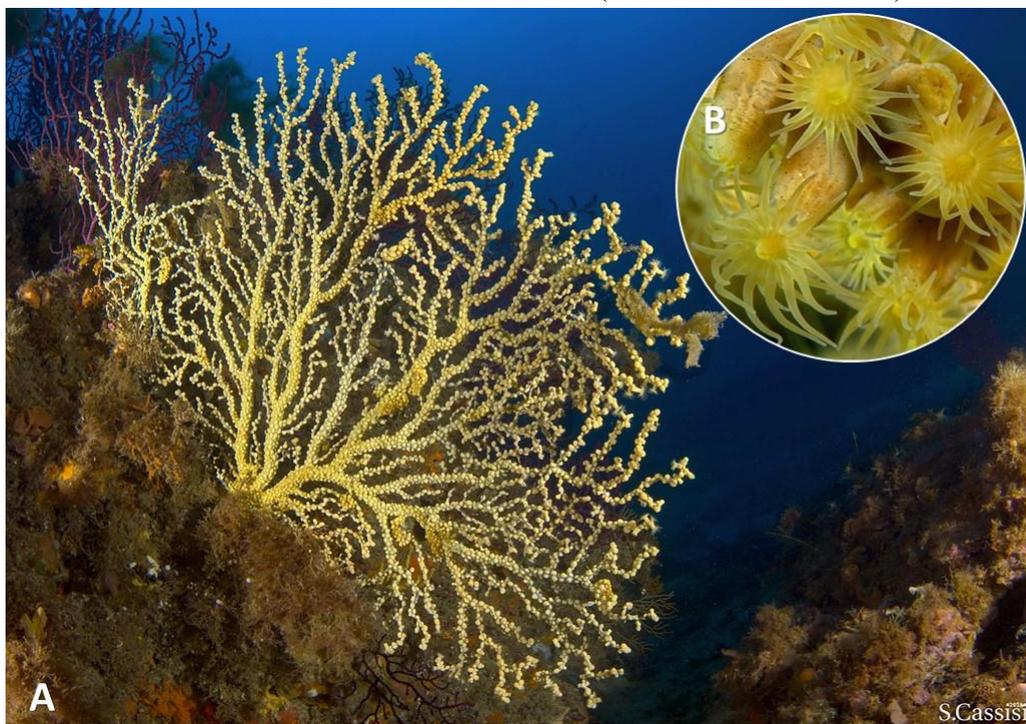


Figure 4 A: (A) A colony of *S. savaglia* and (B) a macro of its polyps. Favignana (TP), 45m, photo by Santi Cassisi.

can reach 75 μm in thickness in the column, 100 μm in the tentacles; the mesoglea of the column wall can be 125 μm thick and is more developed here than in the mesenteries and pharynx, where it is hardly noticed (Ocaña and Brito, 2004). Different types of cnidae can be found in the column, pharynx, filaments and tentacles; at present, only Ocaña and Brito (2004) and Altuna, Sinniger and Aldrey (2010) have analysed the cnidome in *S. savaglia* from specimens coming from Ceuta (Straits of Gibraltar) and Banyuls sur Mer (Southern France) and from the Galician coasts (North-Western Spain), respectively. With only these data, variability between colonies is still unknown, but the two papers actually show some divergences both in size and type of nematocysts detected.

1.2.2. *Biology*

Radiocarbon measurements from basal cross-sections of the skeleton show that *Savalia savaglia* has growth rates ranging from 5 to 45 $\mu\text{m yr}^{-1}$ (Druffel *et al.*, 1995; Roark *et al.*, 2009), probably with much higher values (5-8 cm yr^{-1}) in the early life stages, when it is covering its host's skeleton – this behaviour would be advantageous for the establishment of the colony (Zibrowius, 1985; Druffel *et al.*, 1995). Once the host is completely overgrown, *S. savaglia* starts secreting its own skeleton and the growth gets slower (Previati *et al.*, 2010). Such slow growth rates, however, are probably due to the relatively sparse diet and slow metabolic rate of this species (Prince and Kheshgi, 1998).

Cold water corals and zoantharians can feed on a wide range of food sources, including detritus, phytoplankton and different size spectrum of zooplankton; food particles are captured and stuffed into the mouth by the tentacles (Orejas and Jimenez, 2019). It is believed that organic-rich particles are an important food source for deep-sea zoantharian and corals (Druffel *et al.*, 1995; Roark *et al.*, 2009). The diet of *S. savaglia* is still relatively

unknown: some studies report the finding of several species of copepods inside the coelenteron of dissected polyps, suggesting that *S. savaglia* may be a planktonic captor (Ocaña and Brito, 2004; Altuna, Sinniger and Aldrey, 2010).

S. savaglia can reproduce both sexually and asexually. Ocaña and Brito (2004) observed a form of asexual reproduction by budding, which allows the zoanthid to colonise and grow on its hosts and form large colonies. The sexual reproduction has been only recently described by Previati *et al.* (2010): *S. savaglia* appears to be gonochoric and, analysing the sex ratio of a population in the Portofino MPA, females seem to be strongly predominant (M/F = 0,3). The species has an annual cycle, developing gametocytes in May and spawning eggs and sperms in December; oocytes tend to occupy most of the gastric cavity of the polyps, while male gametes are densely packed in spermatids. Sexual reproduction, however, has been observed only in colonies that have already entirely engulfed their host; prior to that, *S. savaglia* only reproduces asexually. Larval stages are still unknown as are their dispersal strategies.

S. savaglia has been found to contain large amounts of ecdysteroids, a family of hormones with a structure similar to that of Cholesterol. Ecdysteroids were first discovered as the moulting hormones present in many insect groups; they were then unexpectedly found in large quantities in terrestrial plants. Since these findings, extensive studies rapidly showed the general occurrence of phytoecdysteroids (Dreier, 1987) in many plant species, as well as many zooecdysteroids in all arthropods, but also in many invertebrates groups and in tunicates (Lafont and Koolman, 2009). *S. savaglia* is the first animal and the only marine organism observed thus far to produce Ajugasterone-C, an ecdysterone previously known exclusively from terrestrial plants (Druffel *et al.*, 1995). Further studies showed that this

species also contains 20-hydroxyecdysone, Ecdysone and the new ecdysteroid Gerardiasterone (Suksamrarn *et al.*, 2002).

1.2.3. Ecology and Distribution

S. savaglia is an important component of the deeper section of the Mediterranean Sea's coralligenous assemblage (Zibrowius, 1985; Ballesteros, 2006; Giusti, Innocenti and Canese, 2014) and is mostly present on the continental platform, but several occurrences at higher depths have been reported (Gili, National and Barange, 1987; Artuz, 1990; Ocaña and Brito, 2004; Cerrano *et al.*, 2010; Giusti *et al.*, 2015). It lives preferentially in areas interested by moderate but constant hydro-dynamism (Ocaña and Brito, 2004; Giusti, Innocenti and Canese, 2014) and stable environmental conditions, as it appears – like many other Anthozoans – to be rather sensitive to extreme environmental changes such as salinity drops, temperature rises or episodes of excessive sedimentation (Topçu *et al.*, 2019). It is usually associated with Gorgonians (*Paramuricea clavata* Risso 1826, *P. grayi* Johnson 1861, *Eunicella cavolinii* Koch 1887, *E. singularis* Esper 1791, *E. verrucosa* Pallas 1766, *Leptogorgia sarmentosa* Esper 1789) or Antipatharians (*Antipathella subpinnata* Ellis & Solander 1786), which also serve as its preferential growth substrate; together, these species can form the so-called “gorgonian forests”, very peculiar, three-dimensional habitats offering shelter and feeding resources to many organisms. These ecosystems can be

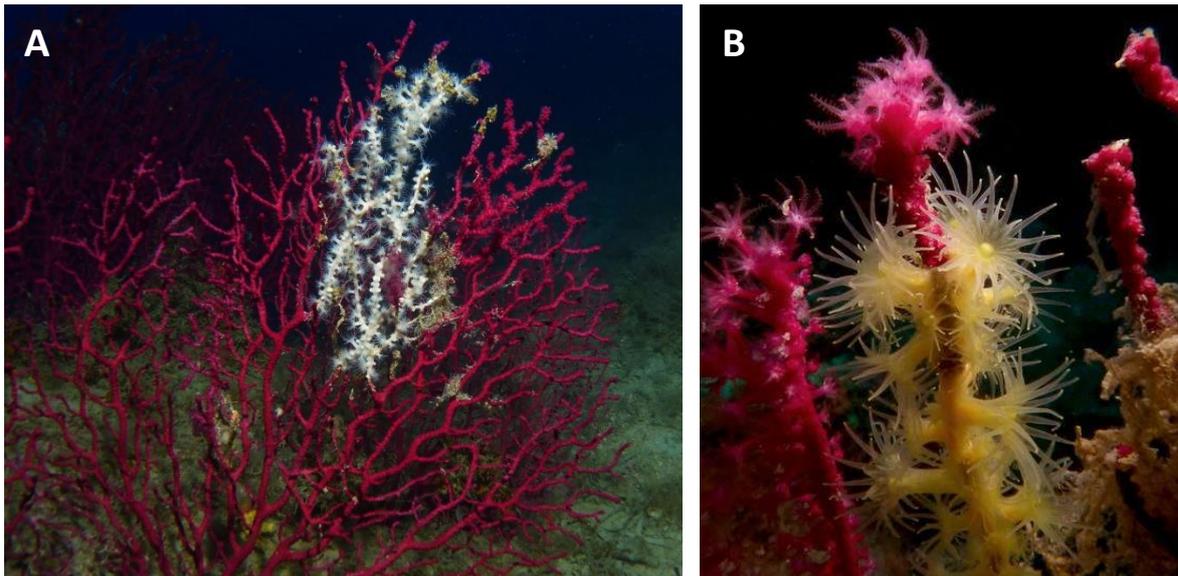


Figure 5 (A) A colony of *Paramuricea clavata* partially covered by *S. savaglia*. (B) Detail of the necrotic tissue of the gorgonian due to *Savalia*'s attack. Secca di Costacuti, Anzio (RO), 40 m, photo by Alessandro Diotallevi.

considered as a transition zone in which typically deep species are encountered together with shallower counterparts (Cerrano *et al.*, 2010). Other associated organisms are the decapod *Balssia gastii* (Balss, 1921) (cited as *Balssia ghassti*), which is often found also living on gorgonians as a commensal (Zibrowius, 1985; Ocaña Vicente *et al.*, 1995), and the parasitic crustacean *Laura gerardiae* Lacaze-Duthiers, 1865 (Noel and Francour, 2017). Generally, *S. savaglia* colonies host a rich community of molluscs and gastropods (Ocaña Vicente *et al.*, 1995; Cerrano *et al.*, 2007).

S. savaglia is typically found in the Mediterranean Sea (Haussermann, 2003) and in the Eastern Atlantic, although in the past, because of some taxonomic mistakes (see Section 1.1), it was thought to be present in the Pacific as well. The species is widely distributed in the Mediterranean, especially in the North-western basin, and has a thermophilous chorology (Rossi, 1958). Its first description, however, was given by Bertoloni in 1819 (named *Gerardia savaglia*) from specimens sampled in the Canary Islands, and later Johnson (1899) described it as *Savaglia lamarcki* from Madeira (Figure 6).



Figure 6 Area of distribution of *Savalia savaglia*. *World Register of Marine Species* (2020)

The presence of *S. savaglia* in the Macaronesian Islands has been long debated: Brito (1983) and Ocaña *et al.* (1995) collected samples from several locations in the Canary Islands, Cape Verde, Madeira and Azores, initially identifying them as *Gerardia savaglia*, but further analyses showed that at least some of those specimens belonged to another species, eventually identified as *A. macaronesicus* (Ocaña and Brito, 2004; Ocaña Vicente *et al.*, 2007; Sinniger, Reimer and Pawlowski, 2010; see Section 1.1.4). *S. savaglia*, however, is certainly present in numerous sites in Gran Canaria, Lanzarote, Tenerife, Madeira, Cape Verde and near Assilah, on the Atlantic coast of Morocco (Ocaña Vicente *et al.*, 2007). Here the species can be found growing both on its hosts and on its own skeleton, between 20 and 40 m, on flat rocky bottoms as well as on vertical walls.

S. savaglia has been recently found along the Atlantic coast of Spain as well: Altuna, Sinniger and Aldrey (2010) reported the presence of four colonies in the Ría de Arousa, in Galicia, at 26-29 m depth. The colonies, one of which is yellow and the others white-pinkish, probably engulfed specimens of *Paramuricea grayi*, a common gorgonian species in that area. Although this coastal tract is rather frequented by both divers and fishermen, *S.*

savaglia was never spotted before; since suitable habitats for its growth extend deeper in the area, the authors suggest that other colonies could be present.

The latest organic information about the distribution of *Savalia savaglia* in the Mediterranean can be found in two recent studies: one by Giusti *et al.* (2015) and one by Di Camillo *et al.* (2018). The first one considers the entire Mediterranean basin and its authors analysed three different datasets: images from ROV transects conducted by ISPRA between 2009 and 2013, observations made by volunteer SCUBA divers within the Coastal Environmental Monitoring Project (<http://www.progettomac.it>) and information regarding the presence of the species found in literature – both scientific papers and reports from field surveys were considered. The second study is mainly focused on the Central-Eastern Mediterranean Sea, about which there is a general lack of knowledge in terms of benthic biodiversity. The authors analysed large datasets obtained from literature and Citizen Science projects, but also from more complex data sources: the World Wide Web contains conspicuous information about marine local biodiversity, scattered among diving centres' websites, social network pages, underwater photography blogs, online communities sharing platforms and so on. If adequately collected and organised, this material can be used to integrate or validate scientific data about the distribution of any marine species. The paper identifies this kind of information as Web Ecological Knowledge (WEK).

1.3. The Mediterranean Sea: structure and biodiversity

The Mediterranean Sea is the largest and deepest enclosed sea on Earth, located at the crossroads of Africa, Asia and Europe. It is connected to the Atlantic Ocean in the west through the Straits of Gibraltar, to the Sea of Marmara and the Black Sea in the East through

the Straits of Dardanelles and the Suez Canal links it to the Red Sea and Indian Ocean in the Southeast since 1869. Due to its isolation and the scarce riverine inputs, the Mediterranean is an evaporitic basin, characterized by high mean salinity and sea surface temperatures, which show a high seasonality. The resulting pressure gradient pushes relatively cool, low-salinity shallow water from the Atlantic across the Mediterranean basin. This water mass warms up to the east, where it becomes saltier and then sinks in the Levantine Sea; it then flows back to the west and exits through the Strait of Gibraltar below the ingoing water flux. A 400 m deep ridge in the Straits of Sicily divides the Mediterranean into two main sub-basins, the Western Mediterranean and the Central Eastern Mediterranean. From West to East, several environmental gradients can be observed: (1) mean salinity and (2) temperature increase eastwards, while (3) nutrient concentration and (4) productivity are lower in the Levantine basin (Coll *et al.*, 2010; Lavigne *et al.*, 2015).

The current marine biota is composed of endemic species, which are estimated to represent a quarter of the total biodiversity, Atlantic species (from warm-temperate, boreal or subtropical regions), widely distributed species and Indo-Pacific species, which mostly entered through the Suez Canal and established in the Eastern basin (Bianchi *et al.*, 2012). The Suez Canal actually caused an exponential increase of alien species, particularly in the Eastern basin, which represent a serious threat to the conservation of indigenous species. The high biodiversity of the Mediterranean Sea may be explained by historical, paleogeographic, and ecological reasons; in fact, the geological history of the basin has been fairly turbulent, with the isolation from the world oceans and near drying out during the Messinian crisis (5.96 million years ago) and more recent drastic changes in climate, sea level, and salinity (Coll *et al.*, 2010; Lejeune *et al.*, 2010; Bianchi *et al.*, 2013). These extreme environmental changes exerted high evolutionary pressures on the biota, leading to

either extinction or speciation; Lejeusne *et al.* (2010) defined the Mediterranean as a factory designed to produce endemics.

In general, species richness shows a decreasing gradient from Northwest to Southeast (*Figure 7*), which may be ascribed to the influx of Atlantic species (which are confined to the Western basin by the “frontier” of the Straits of Sicily) and the wide range of physicochemical conditions in the Western basin (Coll *et al.*, 2010). Some suggest, however, that this gradient could reflect a lack of data concerning the biodiversity in the Levantine basin, since the vast majority of studies are focused on the Western Mediterranean (Coll *et al.*, 2010; Bianchi *et al.*, 2012; Di Camillo *et al.*, 2018; Ponti, Turicchia and Costantini, 2019). The western basin shows more biological similarity with the Atlantic Ocean, hosting a higher number of cold-temperate species, while the eastern basin shows more biological similarities with the Indo-Pacific, and hosts a larger number of subtropical species (Coll *et al.*, 2010). At a regional scale, the distribution pattern of the Mediterranean marine biota is more heterogeneous, to the point that Bianchi *et al.* (2013) argue that the expression “Mediterranean species” is hardly informative. The constant climate warming and related changes in water circulation, however, seem to be causing some alterations in the species distribution; the Straits of Sicily, which used to act as a filter bridge between the western and eastern basins, now appears to be allowing warm-water species, either exotic or native, to

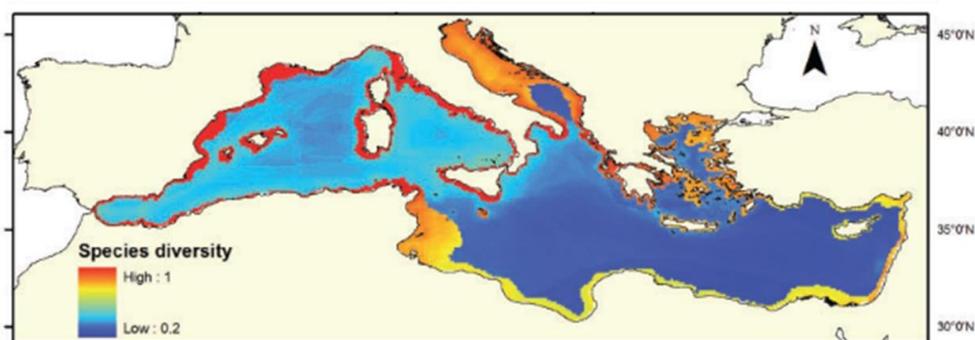


Figure 7 Species diversity in the Mediterranean Sea. From Coll *et al.* (2012)

cross the Straits of Sicily and to penetrate in sub-basins where they were formerly absent. Should this trend continue in the future, Bianchi *et al.* (2013) hypothesize that the Mediterranean would undergo a generalised process of biotic homogenisation.

1.4. Distribution modelling and its applications

Monitoring the biotic components of marine ecosystems is prohibitively expensive in terms of both time and money, especially when collecting point source data of species distributions over large spatial scales: this hurdle paved the way for the implementation of Species Distribution Models (SDM) (Robinson *et al.*, 2017). SDM, also known as Habitat Suitability models or Bioclimatic Envelopes, are correlative approaches that use full spatial coverage data of environmental variables to explain patterns of species distribution (Elith and Graham, 2009). Recent developments in marine habitat mapping, using remote sensing tools as well as satellite-based observations, provide information on a variety of physical parameters on a global scale, allowing to build predictive large-scale maps of the distribution of species or habitats based on relatively few occurrence data. SDMs are largely applied in terrestrial ecosystems, while their use in the marine environment is still in its early stages, because it is harder to acquire the biological and environmental data essential to build a sufficiently accurate model. As mentioned above, biological sampling is a long and expensive process and data are often biased, being mostly condensed in shallow sites close to the coast (Phillips *et al.*, 2009). As for environmental data, they are the fundamental basis of Distribution Modelling and, although continuous progress in data acquisition techniques and analytic power is made, those of marine ecosystems generally have a low spatial resolution and are scattered in non-homogeneous local datasets, precluding their smooth integration (Reiss *et*

al., 2015). Especially for the benthic layer, relevant predictors are only available at local scale.

In recent years, however, the advent of cutting-edge spatial interpolation lead to the creation of public marine datasets, with environmental data available in uniform extent and resolution at a global scale, increasing in quality day by day, such as Bio-Oracle (Tyberghein *et al.*, 2012; Assis *et al.*, 2018), EmodNet (<http://www.emodnet-seabedhabitats.eu/>) or Marspec (Sbrocco and Barber, 2013).

SDM performance depends on a variety of factors. It is influenced by the ecological and biological characteristics of the target species, such as niche width, dispersal range, species interactions: in general, it performs better for species with narrow ecological niches and distribution areas. The choice of environmental variables is also fundamental: these geoclimatic predictors should ideally represent limiting factors, resources or disturbances, linked to the focal species (or its habitat) by cause-effect relationships. Sometimes, particularly for benthic habitats, the influence of environmental variables on the distribution is not understood in detail; distribution models should then be considered as useful indicators of possible causalities, triggering further research on these relationships (Reiss *et al.*, 2015).

Moreover, the modelling approach should be carefully evaluated: comparative studies showed that the performance of different models applied on the same variable system can vary considerably (Valle *et al.*, 2013). The choice of a specific model should depend on the available biological data, on the eco-biological features of the target species, and on the spatial and temporal scale and the objective of the analysis.

The potential applications of SDM in marine spatial planning are multiple. Distribution models provide information to better plan monitoring programs, allowing to focus the

sampling effort on specific areas. When applied to species of special interest for conservation, they can be used to plan and designate future Marine Protected Areas and other means of habitat protection. Habitat suitability models of alien species are early warning systems useful to prevent their further spreading. Considering climate change scenarios, DMs are applied to forecast changes in the distribution of a species or habitat, allowing to implement protection measures effective in the long-term (Reiss *et al.*, 2015). When DMs are applied in ecosystem management, a threshold probability level can be applied to set the cut-off value beyond which a species is expected to be present; the threshold is chosen based on the specific objective, for instance if the actual presence of the species is more important than identifying all its potential habitats, a threshold lower than 0.5 should be used.

1.5. Cnidome analysis and taxonomic value

Cnidocysts, or cnidae, are membrane-enclosed cellular organelles or secretions of the Golgi apparatus, consisting of a capsule and an eversible tubule (Östman, 2000); they represent

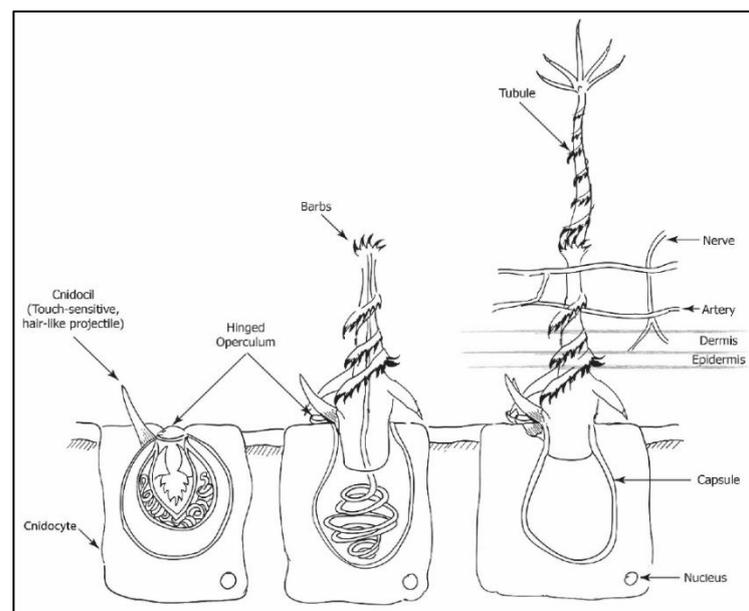


Figure 8 The general structure and mechanism of a penetrating nematocyst. Modified from Montgomery *et al.* (2016)

one of the defining characteristics of Cnidarians and are considered an important systematic character within the phylum.

Each microscopic capsule is secreted by the Golgi apparatus of a cell specialized for this function, called nematoblast. With an appropriate chemical and/or mechanical stimulus, a nematocyst discharges and the tubule that had been coiled and twisted inside the capsule everts from one end of the capsule, which opens as part of the discharge process (*Figure 8*). This discharge is among the fastest cellular processes known so far. (Fautin, 2009). Cnidarian types, characteristics, and capsule dimensions have been used for taxonomic purposes for more than a century (Ryland and Lancaster, 2004a). Weill (1934) classified them in two major categories, nematocysts and spirocysts, the latter being exclusive of the Anthozoa. Spirocysts have a thin capsule wall and strongly coiled, spine-free tubules which secrete a unique adhering hygroscopic substance: they are glutinant cnidaria. Nematocysts are solvent or penetrant cnidaria, they are conspicuously diverse and can be found in all Cnidarian classes; while Weill (1934) identified sixteen categories of nematocysts, several studies subsequently added new types and today we have over thirty varieties and sub-varieties. Many nomenclatural systems have been developed over the decades, but generally nematocyst classification is based on that of Weill (1934), with following modifications and improvements, especially thanks to improved visualisation techniques and instruments (see Östman (2000) for details on the evolution of nematocyst classification). The nematocysts of Hydrozoa and Scyphozoa are more similar to each other than to those of Anthozoa.

Ryland and Lancaster (2004a) described, between holotrichs, atrichs and mastigophores, seven different types of nematocysts for the Zoanthidea; the cnidome analysis in this order, however, appears to be complex, because of the high intraspecific diversity observed; the

taxonomic value of nematocyst data has thus been questioned more than once (Ryland and Lancaster, 2003; Sinniger and Häussermann, 2009; Sinniger, Reimer and Pawlowski, 2010). Few species have yet been studied in detail, but in other hexacoral orders procedures have become more robust in recent years and have been also proven useful for discerning some zoanthid species (Ryland and Lancaster, 2003). Further analyses on different Zoantharia should be done in order to properly evaluate cnidome diversity within the order and the level of inter-colony variation (Ryland and Lancaster, 2004a).

1.6. Biomineralization

Biomineralogy is the science that studies biomineralization processes. The mineral and the living worlds are compatible—since the latter arose from the former—and still coexist (Giovine *et al.*, 2013). The first studies on biominerals were conducted by zoologists who described skeletons, either external – like carapaces, shells, and concretions formed by invertebrates and protozoa, or internal – like bones in vertebrates and spicules in the form of long needles or that of fenestrated stones in many invertebrates (Bouligand, 2004). Later, the concept of biomineralogy has been used referring not only to how living organisms can handle mineral elements to build frameworks, but also to how ambient mineral elements affect living organisms (Cerrano *et al.*, 2007). While several metazoans use foreign bodies to set up skeletons, only in demosponges and zoanthids foreign bodies are actively incorporated in their tissues as they grow (Herberts 1972; Wood 1991).

1.6.1. Sediment embedding in Demospongiae: the study case of Chondrosia reniformis
Most Demospongiae have a siliceous skeleton formed by variably shaped spicules, while also incorporating sand grains and foreign spicules. This behaviour has been observed both

on hard and soft bottoms; sponges often select either carbonate materials or silica, depending on the species (Cerrano *et al.*, 2007). The demospongia *Chondrosia reniformis* (Nardo, 1833) has been object to many studies related to this matter. It has no autochthonous spicules: the support of the body is guaranteed by the close association between collagen fibers and sediment grains and exogenous spicules, which the sponge actively incorporates in its ectosome (Giovine *et al.*, 2013). The availability of particles depends on environmental conditions, but several studies show that *C. reniformis* is able to actively select siliceous particles (Bavestrello *et al.*, 1995; Cattaneo-Vietti *et al.*, 2004; Cerrano *et al.*, 2007; Giovine *et al.*, 2013). However, detachment of the animal or parts of it from the substrates results in the loss of the ability to select particles until a new settlement is found, making stabilization a priority (Cerrano *et al.*, 2007). Once incorporated, quartz and amorphous silica don't share the same fate: while quartz particles are strongly etched and made uniform in size, the hydrated silica remains unaltered; this remarkable selectivity is probably due to cellular mechanisms of particle recognition and compartmentation. Bavestrello *et al.* (1995) reported that ascorbic acid, the reducing agent in proline hydroxylation during collagen biosynthesis, can partially dissolve the surface of quartz, increasing the concentration of soluble silica in the surrounding medium; this reaction is favoured by the specific distance between surface silanols (Si-OH) in crystalline quartz, but not in amorphous silica (Fenoglio *et al.*, 2000). The etching of quartz particles and release of silicates have been proved to positively affect the expression of the collagen gene, inducing collagen production in *C. reniformis* (Nickel and Brümmer, 2003; Pozzolini *et al.*, 2012). Quartz dissolution by benthic organisms could be a mechanism involved in silica turnover in the marine environment, making dissolved silica available from a previously crystalline condition.

1.6.2. Sediment embedding in Zoantharians: the study case of *Palythoa* spp.

Many Zoanthids present an inlay of foreign particles; these grains seem to be selected according to their dimensions, rather than their composition. Herberts (1972) reports that *Palythoa capensis* Haddon & Duerden 1896 selects mostly 125 μm sized particles, while specimens of *Parazoanthus axinellae* Schmidt 1862 growing on sponges incorporate mainly spicules at the base of the column and elements of different nature on the distal part. Sediment embedding in zoanthids was addressed by Haywick and Mueller (1995, 1997) in relation to species of the genus *Palythoa*. *Palythoa* spp. contain significant amounts of foreign material within their mesogleas, reaching an average of 30-65 wt % sediment; according to the authors, sediment enters the mesoglea via a transepithelial mechanism, which they define as sediment assimilation in order to distinguish it from the passive trapping of sediments typical of most sponges overgrowing material. This study also supports Herberts (1972) hypothesis that grain size is the major factor regulating this mechanism; the best size sorting appears to occur within the tissue comprising the tentacles, while coenosteal tissue presents a wider range of sediment sizes, including coarse sand (500 μm). Petrographic evidence also suggests that active biomineralization occurs within *Palythoa* spp. Aragonite crystal aggregates were found in layers, especially in the coenosteal tissue; their L/W ratio, their nucleation on other sedimentary particles and their orientation imply that they grew in place. The purpose of the incorporation of sediments in *Palythoa* spp. is still uncertain: it might be the strengthening of tissues, in order to make *Palythoa* spp. more resistant to waves and currents.

1.7. Collagen biosynthesis and structure

Collagens are a family of fibrous proteins found in all multicellular animals, representing the major constituent of many connective tissues. They are neutral macromolecules of low immunogenicity, used in many pharmaceutical applications, and their properties spurred intense research on a sustainable and safe source of these proteins. The characteristic feature of a typical collagen molecule is its long, stiff, triple-stranded helix, in which three collagen polypeptide chains are wound around one another in form of a rope-like superhelix. These peptides are extremely rich in proline and glycine, both of which are important for the formation of the collagen-specific helical structure (Swatschek *et al.*, 2002). Collagens from invertebrates are numerous, but very few data are available on their molecular structure. Pozzolini *et al.* (2012) reported the complete cDNA sequence of a nonfibrillar collagen (COLch) isolated from the marine sponge *Chondrosia reniformis*, using a PCR approach.

2. Aim of the study

This study had the general objective of updating the distribution of *S.savaglia* in the Mediterranean Sea, as well as our understanding of certain ecological and biological features that are still unclear and poorly investigated. More in detail, the research questions that shaped the structure and approach of this work, with the relative hypotheses and study methodologies, are the following:

1. What is, at present, the known distribution of *S. savaglia* in the Mediterranean Sea?

We speculated that the species distribution currently found in scientific literature does not report all the available records of the occurrence of *S. savaglia*, which are scattered among many different sources. To update the map of presence of *S. savaglia* in the Mediterranean, we adopted several approaches for collecting occurrence records of the species, then organized our findings in a complete dataset and built our distribution map.

2. Which areas of the Mediterranean basin may potentially host this species, based on their geoclimatic setting, and thus deserve further investigations? We hypothesised that many potential habitats of *S. savaglia* may still exist undetected, especially in areas of the Mediterranean Sea characterized by a general lack of knowledge on the local biodiversity. To address this matter, we applied Species Distribution Modelling techniques to our newly collected dataset and elaborated predictive maps of suitable habitats for *S. savaglia*.

3. Can different populations of *S. savaglia* be characterized by distinct biological and morphological features? Considering the great variability already observed for this species, we speculate that geographically distant populations of *S. savaglia* might differ, and thus be characterized by morphological features as well as biological processes. We addressed this topic by performing some analyses on samples of *S. savaglia* from six different stations in

the Mediterranean: we surveyed and compared the cnidome as well as the incorporated foreign material in the tissues of the organism, trying to find patterns linked to the geographical setting of each population.

4. What is the purpose of embedding foreign inorganic material in *S. savaglia*? Our hypothesis was that *S. savaglia* incorporates sediments and etches quartz crystals with a mechanism similar to that of the sponge *Chondrosia reniformis*, and with the same objective of enhancing the expression of the collagen gene. We then proceeded by analysing, in quantitative and qualitative terms, the embedded sediments as well as the content in collagen in two of the sampled populations of *S. savaglia*.

3. Materials and Methods

3.1. Distribution Map

In this work we aimed at updating the distribution map of *Savalia savaglia* along the entire Mediterranean Sea, starting from the distribution data presented in Giusti *et al.* (2015) and Di Camillo *et al.* (2018). The data we tried to collect for each sighting were:

- location, depth and number of colonies observed
- observation period
- whether in each site the number or size of the colonies is increased or decreased compared to the past
- the presence of one or more typical hosts of *S. savaglia*: *Paramuricea clavata*, *P. grayi*, *Eunicella cavolinii*, *E. verrucosa*, *E. singularis*, *Leptogorgia sarmentosa*, *Anthipatella subpinnata*
- The substrate on which the colony/colonies are growing
- The disposition of the colonies in each site, distinguishing whether they're isolated (only one colony), sparse in a vast area (colonies are more than 10 m apart from one another) or aggregated (less than 10 m from one another).

In this study, data were collected by resorting to the same sources as the two previously cited studies, but also by adopting a more active approach. A poster was created to invite anyone interested to share their observations of *S. savaglia* through a mail contact (*Figure 9*). A survey was also created using Google forms, with the intent to collect more organized and complete data. The survey asked general information about the respondent and then focused on the questions listed above.

In order to share these products with as many people as possible, two main strategies were followed: one was to directly contact SCUBA diving operators and Diving Centers of the interested countries. The Diving centers were traced down on Google Maps, by searching the query “diving” in the interested regions. The message included a brief explanation of the project, the link to fill the survey and a picture of a colony of *S. savaglia*. The other strategy was to share the poster on social media, focusing on Facebook pages and communities related to SCUBA diving, marine biology and underwater photography. The poster was introduced by a caption describing the project and the importance of gaining knowledge on the distribution of sensitive species, followed by the link to the Google survey.



Figure 9 The poster created to sponsor the project on social media and collect more data.

The Google survey, an example of the email sent to diving centres and details on the sources of the distribution data can be found in the Supplementary Data SA.

All the collected data were organised in a dataset on Excel (Microsoft Corporation, 2019): coordinates, abundances and depths were harmonized to make analyses and comparisons easier, and distribution maps were created using QGIS 3.4.7 (QGIS Development Team, 2019). The occurrence data were integrated with environmental data layers obtained from

open-source platforms: Bio-Oracle for climatic rasters (Tyberghein *et al.*, 2012; Assis *et al.*, 2018), Emodnet for substrate layers (EMODnet broad-scale seabed habitat map for Europe (v2019)) and GEBCO for the bathymetry raster (GEBCO Compilation Group, 2015).

Bio-Oracle provides ecologically relevant surface and benthic layers for both present conditions and future climate change scenarios. Data layers for present conditions are produced with climate data collected between 2000 and 2014 by combining satellite and *in situ* observations at regular spatial grids. All data layers are available at a common spatial resolution of 5 arcmin; given that focal cells included a range of depth values, the benthic layers are available for the minimum, average and maximum depths (Assis *et al.*, 2018).

The EMODnet Seabed Habitats website provides a permanent single portal for accessing seabed habitat data in Europe. This includes the EMODnet broad-scale seabed habitat map for Europe (EUSeaMap) and habitat maps from surveys across Europe. The data layers, with cell size of approximately 100 m, are freely available in a GIS format for downloading.

Finally, Bathymetric data were obtained from the Global Bathymetric Chart of the Oceans (GEBCO) 2015 grid, with a resolution of 30 arcsec. The data set is largely based on a database of ship-track soundings with interpolation between soundings guided by satellite-derived gravity data; where they improve on the model, data sets generated from other methods are included.

Data on the distribution of marine protected areas of the Mediterranean, and more generally on sites of interest to the conservation of the marine environment, were also retrieved from the MAPAMED GIS Database, developed by MedPAN.

3.2. Distribution Modelling

The occurrence dataset of *Savalia savaglia* (Section 3.1) was also used to develop Species Distribution Models in the Mediterranean, with the main objective to assess which areas deserve priority in future field surveys. The model development was carried out using R software (R Development Core Team; www.r-project.org) and the *zoon* R package (Golding *et al.*, 2018). Environmental data were obtained once again from the Bio-Oracle dataset, using the *sdmpredictors* package specifically developed for R, while the substrate layer (EMODnet broad-scale seabed habitat map for Europe (v2019)) was manually uploaded in the software.

Zoon encodes SDM analyses in as a simple workflow of five key steps and associated functions:

1. Obtaining occurrence data
2. Obtaining covariate data
3. Applying processes to these data
4. Fitting one or more models
5. Generating outputs.

Since absence data on *Savalia savaglia* were not collected in this phase of the project, we adopted a presence-only modelling approach. To compensate the lack of absence data and better fit our models, we randomly selected 100 background data in the study region. We wanted to apply different models to our dataset and compare the results. Considering the characteristics of our dataset and the available environmental data, we decided to use three SDMs: MaxEnt, Random Forest and Logistic Regression.

Maximum entropy (MaxEnt) modelling is amongst the highest performing modelling techniques for presence-only modelling (Ross and Howell, 2013), developed by Phillips *et al.* (2004): it estimates the distribution of a species by finding the distribution which has maximum entropy (i.e. is closest to geographically uniform) subject to constraints derived from environmental conditions at recorded occurrence locations (Phillips *et al.*, 2017). The MaxEnt output is a logistic probability with values between 0 (low probability) and 1 (high probability).

Random Forest is a regression model that works on bootstrapped datasets and generates multiple decision trees with a randomised subset of predictors. Each split is based on a single value of predictor, picked among the subsetted predictors to maximize the differences between the offspring branches. The final output is generated by averaging the model outputs of all trees. The number of trees used in this study is 500 and the model tried two predictors at each split (Wei *et al.*, 2010).

Logistic Regression is another regression model normally applied to presence-absence modelling: a binary response variable is related to one or more predictor variables through the logistic, or sigmoid, function. A Logistic Regression estimates the probability of an event occurring versus the probability of that event not occurring, depending on the values of the independent variables. In other words, it predicts the probability of the outcome variable, which can have only two possible types of values: “yes” or “no”, “presence” or “absence”. In this study, LR model was adapted for use with presence-only data by treating the background data as pseudo-absence data. The output of this function is always between 0 and 1 (Gogina and Zettler, 2010).

We tried different combinations of environmental variables, prioritizing those potentially having an effect on the distribution of *S.savaglia*, but also considering their quality and the level of information they provide at such large spatial scale.

After downloading the selected variables from the *sdmpredictors* package, we created a unique raster and cropped it at the extent of our study region. Before adding the substrate layer to the raster, we had to define the same projection, extension and resolution as the environmental data. Since R interpreted the substrate layer numerical values as continuous data instead of discrete data, we had to indicate the substrate layer as factorial. At this point the substrate data were ready to be stacked together with the environmental data. We assessed the correlation between these variables through a Pearson's correlation matrix and decided to exclude variables having a level or collinearity above 0.6.

Some cleaning of our occurrence data was also carried out: records lacking coordinates were removed, as well as duplicate records with equal latitude and longitude; sample data were further reduced to one data point per cell of environmental data, leaving us with 492 records. Finally, the occurrence layer was plotted on the environmental layer.

A preliminary assessment of how well the selected covariates explain *S. savaglia* distribution was carried out by extracting the environmental data related to the occurrence records and performing a Principal Component Analysis, or PCA.

The *zoon* workflow was then used to develop the three models. As mentioned above, a hundred background datapoints were randomly picked to increase the models performance; furthermore, a two-fold cross-validation was set.

Usually, the occurrence dataset is split in two parts: the first is used to build the model and is called training dataset, the second part is used to test the accuracy of the model on new data. A k-fold Cross validation is a good solution when the occurrence dataset is too small and the training dataset cannot be reduced. The dataset is divided into k subsets and the data analysis is repeated k times: each time, k-1 subsets are used for training and one k subset is used for testing. This process significantly increases the fitting of a model.

As outputs for each model, we requested a predictive map, the response curves for each covariate and the performance measures: all these outputs are tools to evaluate the performance of our models and we used them firstly to select the best combination of environmental variables to predict *S. savaglia* distribution, and then to compare the results obtained with the three applied models.

When assessing for each point the occurrence probability of the target species, an SDM can produce four results:

1. The model says the species is present, and it is actually present (True Positive, TP)
2. The model says the species is present, but is actually absent (False Positive, FP)
3. The model says the species is absent, and it is actually absent (True Negative, TN)
4. The model says the species is absent, but it is actually present (False Negative, FN).

Sensitivity expresses the ability of a model to produce TP, while specificity indicates its ability to produce TN. These two parameters are inversely proportional and depend on the threshold set on the predictive model: with a high threshold, the output will be highly specific but will lack in sensitivity, and vice versa. Since the models created in this project are based on a presence-only data, sensitivity values are more reliable than those for specificity.

By plotting the TP rate against the FP rate and varying the set threshold, it is possible to obtain a threshold-independent performance measure, the AUC, by calculating the area under the obtained curve. The area under the receiver operating characteristic curve, or AUC, is a highly effective coefficient giving the rate of successful classification of a model.

These parameters are calculated for any kind of SDM, but each type of model also provides other features to understand how it worked and how to read its results. MaxEnt provides a table with the percentage contribution given by each covariate to the prediction, so as to easily interpret the results. Random Forest gives a confusion matrix, which shows how many of our training data were correctly predicted as presence and absence (or pseudo-absence, in our case) and how many were not, representing clearly the sensitivity and specificity levels of the model. Lastly, Logistic Regression provides the coefficients of each covariate, which give us an idea on how much did each of them influence the prediction: coefficients are negative for covariates inversely proportional to the occurrence probability and positive if they are directly proportional. The higher the coefficient, the stronger is its influence on the outcome.

The covariates we ultimately selected to build the models are mean benthic salinity, long term minimum benthic dissolved oxygen, long term maximum benthic temperature, substrate and slope.

Substrate nature is a fundamental parameter for the distribution of benthic species and it is clear that *S. savaglia* only grows on rocks or other hard substrates. Moreover, it is believed that *S. savaglia* does not tolerate high temperatures and cannot bear sea temperatures above 22°C for long, hence the choice to include the long term maximum benthic temperature. Based on information on the ecology of this species, effects of hydro-dynamism and

sedimentation rates could also be important, but data on these processes are scarce and unreliable and it was not possible to include them in the models.

Recently, Topçu *et al.* (2019) reported the effects of excessive sedimentation on colonies of *S. savaglia* in the Sea of Marmara: they suggested that high sedimentation rates may not be the direct cause the degradation of its health state, but may indirectly provoke it by strongly reducing the dissolved oxygen in the benthic layer. Following their hypotheses, we included data on minimum dissolved oxygen as proxy of sedimentation rates.

Slope has also been included as a proxy for other variables, in particular mean current velocity in the benthic layer, sedimentation rates and food availability.

We often included mean bathymetry in our covariates, since it is an essential predictor for the composition of bottom communities. The bathymetry, however, also has a direct bearing on physical parameters acting on the seabed such as substratum, wave energy, salinity, and temperature and it always resulted strongly correlated with one or more of these covariates. Since these predictors are more informative and functionally relevant, we preferred to rule out the bathymetry.

The R script for our modelling can be examined in the supplementary material S3.

3.3. Material examined

To find the main peculiar characters able to describe intraspecific differences among different Mediterranean populations of *Savalia savaglia*, several features have been selected. In particular, we considered the cnifome, the abundance, size and composition of the embedded particles, the density of collagen.

The analyses to assess morphological and physiological diversity between different populations were performed on samples from six locations throughout the Mediterranean, collected in the last five years by different SCUBA diving operators. Little branches were carefully removed and preserved in 96% Alcohol.

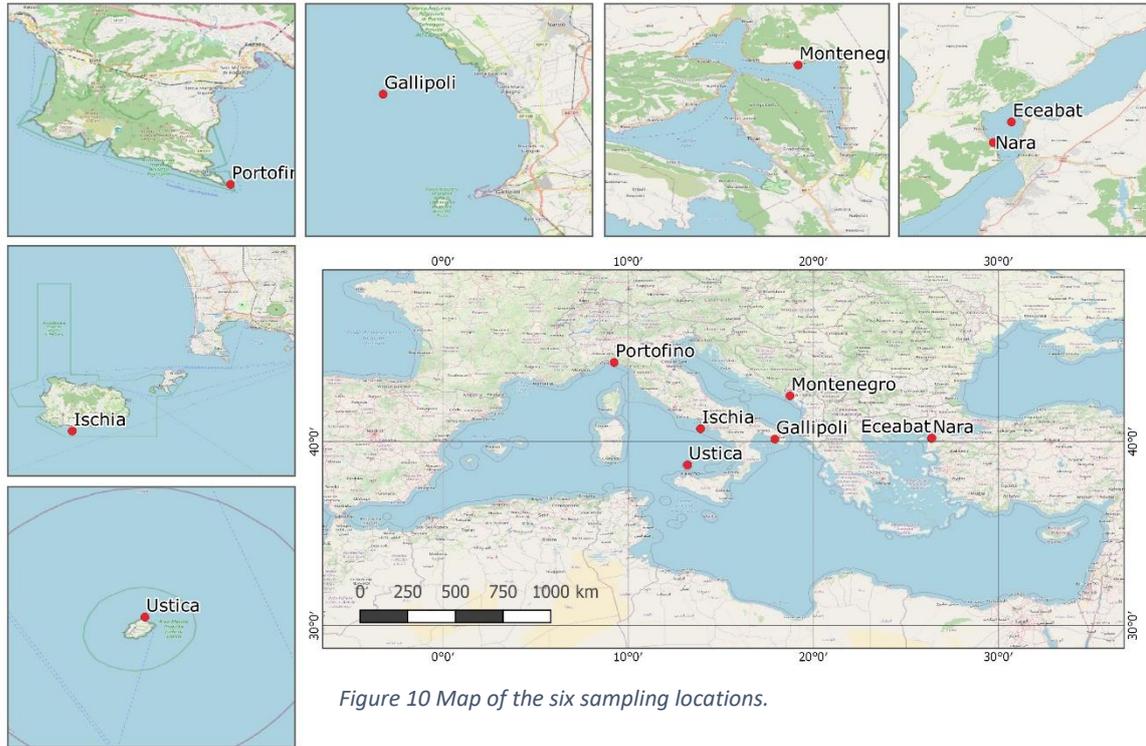


Figure 10 Map of the six sampling locations.

3.3.1. Ischia

Punta Sant'Angelo (40° 41' 30.76" N; 13° 53' 37.17" E), 06/21/2018, 36-38 m, some branches from 5 (IS 1-5) colonies growing on *Paramuricea clavata*.

The island of Ischia is located off the coasts of the Miseno promontory, about 35 km from Napoli, and is one of the historically active volcanic complexes in the bay. The morphology of the island is dominated in the central part by the Monte Epomeo, around which are located numerous other reliefs of volcanic nature. The southern coast of the island is characterised by some promontories; between the Punta del Chiarito and the S. Angelo Promontories well-

stratified volcanic deposits crop out and a large debris avalanche created the continental slope below (Aiello, 2017). The flank starts with a steep upper slope, locally cut by nearly vertical scarps and canyon heads, and covers an area of 400 km², extending up to water depths of 1000 m (Chiocci and de Alteriis, 2006). The slope is composed of alkali-potassic volcanic rocks (trachytes, latites and alkali-basalts) (Poli *et al.*, 1987).

A small population of *Savalia savaglia* can be observed off the coast of Punta S. Angelo, on a vertical cliff at 36-38 m depth. The colonies grow inside a wide mixed forest of the gorgonians *Paramuricea clavata* and *Eunicella cavolini*.

Water circulation is influenced by the currents along the water column and the complex bottom topography of the Naples bay, which is furrowed by a lot of submarine canyons (Giordano *et al.*, 2006; Cianelli *et al.*, 2015); along the southern coasts of Ischia a moderate to intense current is always present.

The hydrology in the Gulf of Naples presents a seasonal pattern characterized by the summer stratification of the water column, determining the formation of a surface mixed layer 30-40 m thick; by contrast, the intense winter mixing involves the entire water column (Cianelli *et al.*, 2012). Temperature also shows seasonal variations, ranging from 14°C down to 150 m in winter to 25°C down to 75 m during summer (Cianelli *et al.*, 2012).

The Gulf of Naples is subject to an intense anthropic pressure, determining a strong impact on the marine ecosystem (GNRAC, 2006, as cited by Cianelli *et al.*, 2012). Human activities range from touristic enterprises, urban settlements to industrial areas located on the coast, to intense maritime traffic, resulting in the potential discharge of sewage, industrial pollutants and hydrocarbons. In addition, from east the gulf receives the land runoff of the very polluted river Sarno (Cianelli *et al.*, 2012).

The habitat in which *S. savaglia* is found is under protection within the National Marine Protected Area “Regno di Nettuno” created in 2007, in a no take Zone B: bathing, sailing, rowing and diving are regulated, but allowed, while fishing, spearfishing and anchoring are forbidden. The area is also part of a Natura 2000 site since 1995 (“Fondali marini di Ischia, Procida e Vivara”).

3.3.2. Gallipoli

Santa Caterina (40° 07' 34.1" N; 17° 54' 29.8" E), 07/14/15. 65 m; some branches from 5 (GP A11-15) colonies from a coralligenous assemblage on a rocky outcrop, also hosting the gorgonians *P. clavata* and *Eunicella cavolini*.

The coastal area between Santa Maria di Leuca and Porto Cesareo has been shaped by the alternating Sea level increase and reduction, as proven by the several fossils of marine species found on land. The landscape is characterized by a series of terraces descending towards the sea: many caves are also present. The underwater landscape reflects the one on land, with submerged terraces of variable extension constituting the Ionian platform around 50-60 m depth. These terraces are delimited by coralligenous concretions and show a homogenous distribution along the coast up to Taranto (Damiani *et al.*, 1988).

A numerous population of *Savalia savaglia* is observed in front of Santa Caterina (Nardò), about 7 km offshore. The area is dominated by *Paramuricea clavata*, and *Eunicella cavolinii* and *Parazoanthus axinellae* Schmidt 1862 are also present in lower amounts. Sponges are quite abundant too, especially the genera *Axinella* sp and *Jaspis* sp. Colonies of *P. clavata* partially or completely covered by *S. savaglia* can be observed. These two anthozoans are both colonized by a rich community of epibionts, notably *Neopycnodonte cochlear* Poli 1795 e *Pteria hirundo* Linnaeus 1758 of the order Ostreida. These epibionts appear to weigh down

the colony branches and to increase friction against currents, sometimes to the point of breaking them and leading to the death of the Cnidarian.

The Ionian basin does not receive much water inputs from land and meteoric waters rapidly penetrate the soil through fissures, directly supplying the groundwaters. Sea surface temperature shows a strong seasonality, while salinity is rather stable. The predominant superficial circulation flows from SE to NW (Damiani *et al.*, 1988). No localized sedimentation phenomena are observed, but rather a wide dispersion of the dissolved material. The predominant lithotypes are limestones and dolomites.

The area facing Gallipoli is particularly subject to anthropogenic pressures: during the sampling activities several fishnet residuals were observed in the entire area, and these often get entangled in the colonies causing damage to both *P. clavata* and *S. savaglia*. At present, no measures of protection and conservation are enforced in this specific site.

3.3.3. Montenegro

Dražin Vrt, Kotor Bay (42° 28' 58.9" N; 18° 43' 56.2" E), 12/19/19, 10-12 m; 6 small branches (MN 1-6) from colonies of a dense population (200-300 specimens) of *Savalia savaglia* dominating a coralligenous assemblage, which also hosts other cnidarian species (*Cladocora caespitosa* Linnaeus 1767, *Lophogorgia* cf *sarmentosa*, *Parazoanthus axinellae* Schmidt 1862). Yellow and white colonies are observed.

The eastern border of the Adriatic Sea is characterized by fragmented coastlines, islands, coastal bays, that interact with the main basin influencing it and being influenced by it in terms of circulation patterns and freshwater supply. One of the most important features along the entire Adriatic is the bay of Boka Kotorska, where the sea enters inland for over 20 km. The Bay is composed by three main basins: Herceg Novi, Tivat and Morinj-Risan-Kotor,

which is the innermost basin and can be divided into two major sub-triangular embayments to the NW and to the SE (Morinj-Risan and Kotor bays, respectively) (Bortoluzzi *et al.*, 2017).

Savalia savaglia is present in significant number in the Morinj-Risan-Kotor bay in 2 localities: in Strp, between 15 m and 19 m of depth, and in Drazin Vrt (where the examined material was collected), in depths from 9 m to 17 m. The uniqueness of these sites is related both to the abundance of this rare species and to its superficial bathymetric distribution, when generally it ranges from 20 m to more than 600 m depth. In Drazin Vrt the number of colonies of *S. savaglia*, in an area extended over a surface of about 130 m x 30 m, can be estimated in about 200-300 specimens. The populations form a Facies with *Leptogorgia sarmentosa* in a typical coralligenous habitat. Given the complex geomorphology of the seafloor (e.g. hard and soft substrata, natural and artificial ones, slope of the seabed) this habitat is present in mosaic within other biocenoses (Badalamenti *et al.*, 2011).

The superficial distribution observed in this locality can be explained by the peculiar hydrogeological features of the Boka Kotorska Bay. The water circulation is mainly driven by meteorological conditions and freshwater inputs: rainfall in the Boka Kotorska, mainly concentrated in the rainy season in autumn (Badalamenti *et al.*, 2011), reaches the point of the highest precipitation in Europe (4584 mm per year near Crkvice). Strong surface currents are due to the action of winds, while bottom currents are controlled by freshwater inputs from numerous sources. Up to 5 m of depth there is almost only freshwater at lower temperature; below this superficial layer, water temperature is lower (annual average of 19,4°C at the surface and of 17,4°C at the bottom) compared to what is observed in the open

sea. In general, temperature, salinity and current patterns have a strong seasonality (Bortoluzzi *et al.*, 2017).

The geology of the Montenegrine coasts is characterized by Cretaceous limestones, anydrites and dolomites, and by Eocene-Oligocene flysch deposits and carbonates; these units are in direct contact with the sea in the northern sector of Kotor Bay.

The Kotor Bay is interested by several anthropogenic impacts. The areas of Dražin Vrt and Strp, hosting *S. savaglia*, are especially affected by excessive scuba diving activities, although touristic diving is more focused in the coasts facing the open sea, discharging of solid waste, water pollution from domestic sewage and fishing activities. Fishery is regulated in the bay (bottom trawls, floating trawls and encircling large-scale fishing nets are prohibited), however some illegal activities still seem to exist. Global warming may be the greatest threat to this habitat, given that its survival mostly depends on submerged cold springs and riverine inputs keeping temperatures low (Badalamenti *et al.*, 2011).

The Kotor Bay has been included in the UNESCO list of World Heritage Sites in 1979 and a National Marine Protected Area, the Kotorsko-Risanski Zaliv, has been designated to cover the entire Morinj-Risan-Kotor basin (MAPAMED, 2017).

3.3.4. *Marmara Sea*

Nara station (40° 10' 4.61" N; 26° 22' 20.55" E), 12/12/15, 37m, 1 small branch (MA 4A) from a colony of little less than 1 m height. Eceabat (40° 11' 32.07" N; 26° 23' 36.98" E), 12/12/15, 33-38 m, 2 small branches from colonies of about 50 cm height (MA 4B-4C). Both sites are situated on vertical rocky shores hosting gorgonian species.

The Sea of Marmara is connected to the Aegean Sea by the Çanakkale Strait (Dardanelles) and to the Black Sea by the Istanbul Strait (Bosphorus). Dense assemblages of benthic sessile suspension feeders were reported in the Sea of Marmara, including dense populations of gorgonians and the zoanthid *Savalia savaglia* (Topçu *et al.*, 2019).

In this study, samples from two populations of *S. savaglia* were analysed. Both populations are situated in the Çanakkale Strait, in Nara Station and Eceabat, about 20 km away from the opening on the Aegean Sea, on vertical rocky shores between 33 and 38 m depth.

The environmental conditions are highly influenced by a two-layer stratification, with a brackish upper layer originating from the Black Sea flowing southward and a lower Mediterranean layer flowing northward. The two layers are separated by a permanent halocline, located at 15–30 m depth depending on the season and location. The colonies are just below the superficial layer, which shows seasonal variations in temperature and salinity: in the lower layer the salinity is about 38.5 psu and the temperature is stable at 14–15°C throughout the year, with narrow fluctuations (1,2°C) (Topçu *et al.*, 2019). In addition, there are significant vertical mixing points, especially in narrow passages as here (Artuz *et al.*, 2019). In general, the area where the sampled *Savalia* populations are located is characterized by moderate currents and low sedimentation rates.

The Çanakkale Strait is a narrow valley that presumably resulted in the Pliocene as a consequence of compressional events generated by faulting activities. The lithology of this area is characterized by Miocene strata of sandstone, siltstone and mudstone, deposited in fluvio-lacustrine environments, and of marls; these formations are overlaid by conglomeratic sandstone and poorly sorted carbonated arenaries. Detritic material 0.5 to 14 m thick, depending on the morphology of the shoreline, covers the seabed (Yaltırak *et al.*, 2000).

Over the years, the Sea of Marmara has been under pressure in terms of pollution from domestic and industrial wastewater sources, owing to a rapid increase in industrialization and urbanization, particularly along its southern coasts. Therefore, water quality has degraded significantly and biodiversity has been compromised (Artuz *et al.*, 2019). Recently, a massive die-off of benthic suspension feeders, including *S. savaglia* was observed along Prince Islands coasts, in the north-eastern Sea of Marmara: this event has been linked to excessive sedimentation caused by anthropogenic activities, specifically construction and landfill operations (Topçu *et al.*, 2019). Another significant threat to the populations of *S. savaglia* in the basin has been the excessive collection for jewelry production, as reported by Artuz (1990) and Öztürk and Bourguet (1990).

Currently, in the Sea of Marmara no specific protection and conservation measures for marine biodiversity are enforced.

3.3.5. *Ustica*

Northern side of the island (38° 40' N; 13° 13' E), 08/03/2018, 34 m, 11 small branches (US 1-11) from colonies growing on a rocky substrate, which also hosts gorgonian species (*P. clavata*, *Eunicella singularis*).

Ustica is situated in the southern Tyrrhenian Sea, about 60 km north of Palermo. The island represents the remnant of a complex volcanic edifice that was built up by Middle-Late Pleistocene intraplate volcanism, but there are many open questions about its geomorphological evolution. Its volcanic activity began underwater, until the top of the seamount emerged forming the subaerial volcano Guardia dei Turchi (Barberi and Innocenti, 1980). Ustica is surrounded by a very narrow insular shelf with an enhanced asymmetrical shape: along the south-eastern sector the slope ranges between 4-10°, while in the north-

western side the shelf is steeper. The north-western sector also hosts the largest concentration of volcanic cones, as well as fault escarpments and landslide scarps (Furlani *et al.*, 2017).

Savalia savaglia occurs both on the south and on the north of the island. In the south, several colonies grow at 45-55 m depth, on the vertical walls of pinnacles also hosting the gorgonians *Paramuricea clavata* and *Eunicella cavolinii*. The site is interested by constant strong currents. In the northern site are localised the colonies that were sampled for this study. There are 11 colonies at about 34 m depth, on vertical rocky walls among abundant sponge species and sea fans.

The geology of the island is typically volcanic and mainly characterised by pyroclastic deposits of alkali-basaltic composition (Barberi and Innocenti, 1980).

The temperature profiles do not show, during summer, a well-established thermocline. The temperature fluctuates between 15.0 and 28.5 °C. The same particular tendency is noticeable in the salinity trend, which over the year ranges between 37.1 and 38.0 psu. The reason of this particular trends could be related to the geographical position of the island (Sanfilippo *et al.*, 2016). Small-scale mixing processes prevail over the large-scale ones and topographic effects could be effective on deviating the currents trajectories, both on surface as on depth (Sanfilippo *et al.*, 2009); in general, the coasts of the island are frequently interested by moderate to intense currents.

“Isola di Ustica” MPA was founded in 1986 and represents, with Miramare (Trieste), the first MPA in Italy. Our site of interest lies in the B zone, the so-called buffer zone surrounding the strict nature reserve: here bathing, diving activities, sailing and rowing are allowed, as well as anchoring, while artisanal and professional fishing must be authorised and spearfishing is prohibited. The island is also a Site of Community Interest (SCI), under

the European Habitat Directive (92/43/EEC of 2.05.1992), for its environmental and biodiversity value (Sanfilippo *et al.*, 2016). The major threat to the integrity of these habitats is the excessive touristic diving.

3.3.6. Portofino

Faro di Portofino (44° 17' 55.2" N; 9° 13' 13.8" E), 10/12/19, 62 m, 5 small branches (PF 1-5) from a population of 23 colonies on a small rocky shoal among sea fans of the species *Paramuricea clavata*, *Eunicella cavolini*, and *E. verrucosa*.

The Portofino Peninsula covers 18 km² and has a 13 km long coastline; the orography is characterised by a mountain ridge with different elevations and the promontory breaks the continuity of the coast between Genoa and La Spezia with its vertical cliffs, that continue underwater down to 50–60 m depth (Faccini *et al.*, 2018). On a detritic bottom, at 67 m depth and about 50 m distant from the base of the cliff, there is a dense population of the gold coral *Savalia savaglia*. Colonies grow up from a small rocky shoal (1.5 m high and 5 m long) and are surrounded by the sea-fans *Paramuricea clavata*, *Eunicella cavolinii*, and *E. verrucosa*. The whole population counts 23 colonies. Amongst sponges, *Cacospongia* sp., *Axinella damicornis* overgrown by *Parazoanthus axinellae*, and *Sarcotragus* sp., are the species more represented (Cerrano *et al.*, 2010).

The area is subject to the typical cyclonic circulation of the Ligurian Sea, with a seasonal cycle related to the seasonal variations of atmospheric forcing: hot summers, long periods of insolation, mild winters and rainy autumns. Close to the coastal area of interest the circulation, indeed, runs northwest-ward with only short periods of reversal (Cerrano *et al.*, 2010).

The geology of the Portofino Natural Park is characterised by sedimentary rock formations: the Conglomerate of Portofino, dated back to about 30 million years ago, along the southern slope, while in the northern part the Flysch of Mt Antola formed about 90-55 million years ago. The limestones of Mount Antola consist of alternating layers of limestones and dark grey marly limestones, calcarenites, marls and shales. The Conglomerate of Portofino is characterised by pebbles and coarse fragments of different nature and composition: primarily marly limestone and sandstone, while other lithotypes such as ophiolites, cherts, gneiss and schists are less frequent. These clasts are bound together by a cement of carbonate nature. Clasts of pure calcite, of unknown origin, are also present. The powerful and selective erosive action of meteoric and marine waters uncovered along the coast the strongest layers, richer in Calcium carbonate (Assandri , 2014; Faccini *et al.*, 2018).

The peninsula is an internationally acknowledged area of great landscape value for its historical settlements, vegetation and geomorphological features has been a protected area for more than 80 years: since 1995 it has been managed by the Ente Parco Portofino Authority. The Portofino Protected Marine Area, comprising the entire coast of the Promontory, was created in 1999. The population of *S. savaglia* here described lies inside the zone C of the MPA, where the restrictions are milder: diving activities, mooring, recreational and artisanal fishing are allowed, but regulated, while spearfishing is forbidden in all the MPA (Faccini *et al.*, 2018).

3.4. Sediment analysis

3.4.1. Sediment extraction

A portion of each sample was cut, taking note of the number of polyps; the central axis was removed, and the tissue weighed; the tissue was then placed in a stove at 60°C for approximately 24 hours for drying. The dry tissue was weighed and transferred in pyrex tubes with about 4 mL of 30% Hydrogen Peroxide, for the tissue dissolution; the solution was incubated at 60°C and sometimes boiled on a Bunsen burner to accelerate the process.

The incorporated sand resulting from this process was washed once with deionized water and twice with 96% Alcohol.

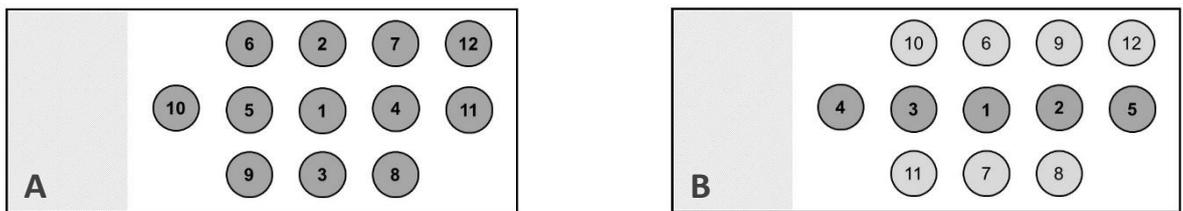
3.4.2. Sediment quantification

For each site, three replicates were analysed. Once extracted and washed as described in the previous Section, the sediments were evenly distributed on one or more slides, depending on their abundance. The slides were closed with a synthetic resin (Eukit). For each slide, 12 identical fields were observed under a light microscope (Nikon Eclipse Ni compound microscope), using a 10x objective. All sediment particles of each field were counted, as well as all the spicules and all the other biogenic particles.

The number of particles observed was related to the total area of the fields (twelve for samples mounted on a single slide, twenty-four or thirty-six for samples distributed on more slides) and then related to the total area of the slide – or slides – of each sample. Once obtained the estimated total abundance of sediments, spicules and other biogenic particles, they were related to the dry weight of the samples, so as to obtain comparable measures expressed in number of particles per milligrams of dry tissue weight.

3.4.3. Granulometry

The granulometric analysis was performed on the same slides used for quantification; the slides were observed using a light microscope (Nikon Eclipse Ni compound microscope), with a 20x objective and a Differential Interference Contrast (DIC) filter. The DIC microscopy technique allows to enhance the contrast in unstained, transparent samples, based on the principle of interferometry. For each slide, a minimum of 5 fields, evenly distributed on the slide, were considered; all the particles of each field were measured, for a minimum of 100 particles per slide. For some samples, the extracted sediments were rather poor and it was necessary to consider more than 5 fields on each slide, in order to reach the minimum number of 100 particles measured.



Fields distribution on the slide surface for: A) quantitative analysis and, B) granulometry. The darker fields were always considered, while the lighter ones were only considered if with the first 5 the minimum number of 100 measures was not reached.

For each sample, the percentage frequencies of the following size classes were calculated:

- $\leq 5 \mu\text{m}$
- $5 \div 10 \mu\text{m}$
- $10 \div 50 \mu\text{m}$
- $50 \div 100 \mu\text{m}$
- $> 100 \mu\text{m}$.

3.4.4. Sediment characterization

For the qualitative analysis of the incorporated sand, a Scanning Electron Microscope (SEM) and a diffractometer were used.

A SEM uses an electron flux to scan an object and produce its image; the interaction between the electrons and the atoms at the surface of the object generates a signal whose intensity depends on the topography and composition of that object. A detector converts the signal into an electric one, producing an image. Since the wavelength of electrons is smaller than the wavelength of photons, the resolution of an electronic microscope is about a thousand times higher than that of a light microscope.

Two samples for each site were analysed using a SEM (TESCAN Vega 3), performing Energy-Dispersive X-Ray Spectroscopy (EDS) analyses. The EDS is used for the elemental analysis and chemical characterization of a single point of a sample. The atoms of an object possess unexcited electrons in discrete energy levels; by focusing an electronic beam on a point of the object, an electron of the inner energy level of an atom may get excited and eject, thus creating an electron hole in that level. Then, an electron from an outer and higher energy level will fill the hole releasing its extra energy in the form of X-Rays. The number and energy of the X-Ray emissions depend on the atomic structure of an element, in other words they are distinctive of that element, allowing the elemental and chemical characterisation of a single point of a sample.

The extracted sediment was dried on a heating plate and then placed on an aluminium stub; in order to obtain clearer images, the samples were sputter-coated in low-vacuum with an ultrathin layer of electrically conducting material, specifically gold.

For high-quality photos of the sand grains, a ZEISS Supra40 SEM was used.

A diffractometer is based on the X-Ray diffraction; the diffraction of a wave is determined by the sum of all the electromagnetic waves generated by atoms of the same wavefront. The instrument produces an X-Ray flux which hits the sample's surface: its atoms, interacting

with the flux, will produce a scattering pattern which allows to identify the structure of the sample. Only crystalline solids can be analysed with this technique. The X-Ray spectrum of the sample obtained with this procedure can be compared with standard spectra of all mineral substances for identification. This technique requires a consistent amount of sample, which unfortunately could not be obtained from most of the samples analysed in this study. It was possible, however, to characterize the embedded sediments in samples from Ischia and Gallipoli, which were the most abundant, and a BRUKER D8 diffractometer was utilised.

3.5. Collagen quantification

These analyses were performed at Università degli Studi di Genova in Genova. Three samples for each site were analysed. The collagen content was quantified in a branch fragment of about 2,5 cm, by estimating hydroxyproline content using a modified method based on chloramine-T reaction (Reddy and Enwemeka, 1996). Polyp tissue was removed from the skeleton and weighted; collagen was then isolated following the protocol described by Swatschek *et al.* (2002).

3.5.1. Collagen Isolation

The wet tissue was put in pyrex tubes with a 100 mM Tris-HCl buffer solution (pH 9.5; 10 mM EDTA, 8 mM Urea, 100 mM 2-Mercaptoethanol) in a 1:5 ratio, then homogenized using a blender (Kenwood BL 901, new Lane, Havant Hants, England). The tubes were constantly stirred at room temperature for 24 hours; the pellet was then discarded, while 2,5 mL of supernatant were transferred in Eppendorf tubes and centrifuged (5000xg; 5'; 2°C: Sorvall RC-5B, Du Pont Instruments, Wilmington, USA).

2,4 mL of the resulting supernatant were collected and centrifuged with 800 µL Acetic Acid to adjust pH to 4 (20000xg; 30'; 2°C); collagen was thus precipitated. After discarding the

supernatant, the pellet was resuspended in 1 mL of distilled water for washing (the pink colouring must be reduced to improve the accuracy of the spectrophotometer measurements) and then centrifuged again (20000xg; 30'; 2°C). The supernatant was once again discarded.

3.5.2. *Hydroxyproline assay*

The resulting insoluble pellet was hydrolyzed in a 2M NaOH solution. A white (200 µL distilled water; 200 µL NaOH 4M) and four standard solutions with different concentrations of 10mg/mL cis-4-hydroxy-L-proline (Sigma, USA) (200 µL Hypr solution; 200 µL NaOH 4M) were prepared. The samples, white and standard solution were autoclaved at 120°C for 20 minutes. 200 µL of each of these solutions were transferred in Eppendorf tubes with one volume of 3M HCl, in order to lower the pH. 100 µL of the resulting solution were collected and diluted threefold with milliQ water; 200 µL of 0,056M Chloramine T reagent (141 mg Chloramine T; 8 mL acetate-citrate buffer; 1 mL 50% n-propanol) were added and the Eppendorf tubes incubated at room temperature for 20'. 200 µL of 1M Ehrlich's reagent (1,5 g pDAB; 6 mL n-propanol, 2,6 mL 60% perchloric acid) were added and the solutions were incubated at 60° for 30'.

Absorbance of each standard and sample were read against white at 550 nm with a Beckman DU 640 spectrophotometer (Beckman Coulter Inc, Brea, USA).

The Hydroxyproline concentration of each sample was expressed as Hydroxyproline content per milligram of wet tissue weight; the collagen content was estimated assuming 10% of collagen is Hydroxyproline. Collagen has never been isolated and characterized in *S. savaglia*: the weight percentage of hydroxyproline is therefore yet to be determined for this species. The choice of estimating a 10% of collagen is hydroxyproline was made based on

the values indicated for other Cnidarian species whose collagen has already been isolated (Barzideh *et al.*, 2014; Miki *et al.*, 2015).

3.5.3. *Bradford protein assay*

In order to estimate the total protein content of the samples, the remaining 500 μL of the supernatant obtained after centrifuging the tissue homogenate, as described in the Section 3.5.1, was analysed following the protocol described by Bradford (1976).

The supernatant was centrifuged again (13000 RPM; 5'; 2°C) to ensure a good separation of the pellet. 10 μL were then transferred in Eppendorf tubes and diluted in distilled water in a 1:10 ratio; a second dilution was performed with a ratio calibrated according to the turbidity of the solution (either 1:10, 1:5 or 1:20). A white (100 μL distilled water) and four standard solutions with different concentrations of 0,1 mg/mL BSA solution (Sigma, USA) were prepared. 900 μL of filtered Bradford Reagent (100 mg Coomassie Brilliant Blue G-250; 50 mL 95% Ethanol; 100 mL 85% w/v Phosphoric Acid; 850 mL distilled water) were added to all the samples and standards and to the white.

Absorbance of each standard and sample were read against white at 595 nm with a Beckman DU 640 spectrophotometer (Beckman Coulter Inc, Brea, USA).

The total protein concentration of each sample was expressed as total protein content per milligram of wet tissue weight.

3.6. Cnidom analysis

The characterisation of the cnidom was performed in order to make comparisons between specimens grown in different conditions. The results were also compared to the ones obtained by Ocaña and Brito (2004) and Altuna *et al.* (2010). Nematocysts from the tentacles, pharynx, mesenteries and column were measured and classified following the example of these papers: the nomenclature is after Schmidt (1972, 1974) as adapted by Ryland and Lancaster (2004b) for Zoanthid species. The following table (*Table 1*) shows, for each nematocyst type identified by Ryland and Lancaster, the corresponding name used by Brito and Altuna, who preferred a nomenclature after den Hartog (1980, 1993).

Table 1 Correspondence between the cnidae nomenclature after Ryland and Lancaster (2004), adopted in this study, and that after den Hartog (1980), which was used by Ocaña and Brito (2004) and Altuna et al. (2010).

Ryland & Lancaster, 2004	Den Hartog, 1993
Atrich	Atrich
Small Holotrich	Homotrich
Large Holotrich	Penicillus E
Basitrich	Spirula
p-Mastigophore	Penicillus A
Microbasic b-Mastigophore	Special Spirula

Three samples per site and two polyps per sample were analysed; the conditions of the tissue of the samples from Ustica were not ideal and thus they were not analysed. Fragments of 2 mm of each tissue of the polyp were mounted on microscope slides and delicately squashed, while maintaining the hydration with distilled water. The material being conserved in Alcohol and not fresh, it was rarely possible to find ejected cnidae, so the identification was performed on the basis of the form of the capsule and the structure of the tubule. In some cases, the conservation status of the samples also made it difficult to separate the different tissues and a certain risk of contamination is to be taken into account. Cnidae whose tubule was not visible through the capsule were not considered and only capsules lying entirely on

the focal plane were measured. The slides were observed with a Light microscope (Nikon Eclipse Ni compound microscope) with a x100 oil immersion objective. The whole surface of the slides was observed in order to estimate the general abundance of the different cnida types: the frequencies given are based on subjective impressions. The general abundance is indicated as such: 1 = rare; 2 = uncommon; 3 = rather common; 4 = common; 5 = very common. The survey also presents means (with standard deviation) and ranges of length and (maximal) width of nematocyst capsules in micrometres. When measures are less than five, only ranges are indicated. The results for each site are arranged in Tables, following the examples of Ocaña and Brito (2004) and Altuna *et al.* (2010).

In an attempt to facilitate the identification of *Savalia savaglia* specimens based on the cnidome, a summary table of all collected data is also presented.

4. Results

4.1. Distribution Map

A total of 985 records have been collected in the course of this project: to facilitate the interpretation of these data and make the reading of maps easier, however, close single-colony occurrences having the same source were integrated in a single occurrence record, leaving us with 515 occurrences.

In *Figure 11* a map of the distribution of *Savalia savaglia*, created by incorporating our dataset into the Directive Habitat 10 km grid (retrieved from the European Environment Agency website), is presented. It is clear that the collected records are not evenly distributed in the study area, but they are mainly confined into the European coasts of the Mediterranean: very few data are located in the Southern part of the basin.

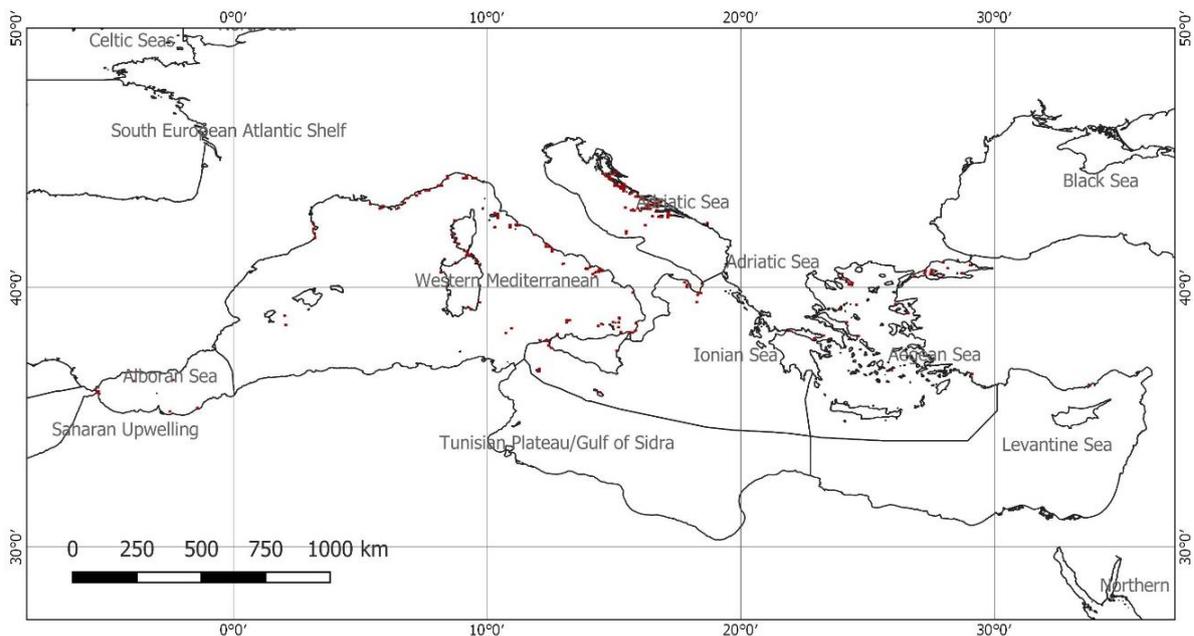


Figure 11 The updated distribution of *Savalia savaglia*, in red, in the Mediterranean Sea. Data are integrated in the Habitats Directive 10km grid.

The three data source types considered for this study performed differently from one another (*Figure 13*): of the total records, 127 were obtained from Scientific Literature in general, including reports and proceedings (SCI), 43 from other sources in the Web (WWW) and 345

from Citizen Science resources, including the survey and poster (CS). The Reef Check database alone provided 181 records, representing 35,1% of the total records. The WWW only provided 8,3% of the total data. Of 1353 Diving Centres operators contacted via mail or other means of communications, only 94 answered.

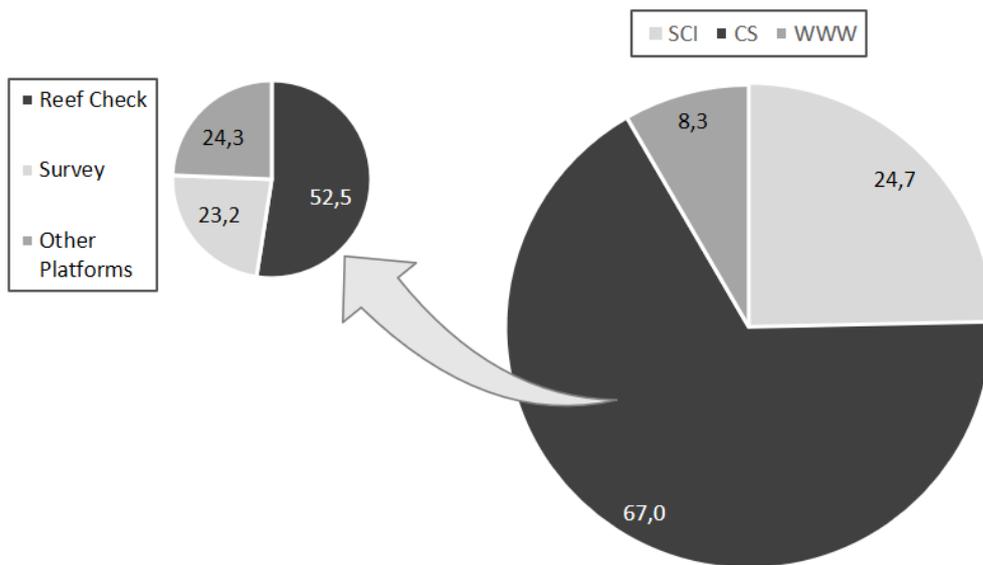


Figure 13 Contribution in percentage of each source type to the distribution dataset and detail of contribution percentages from CS sources.

Records from the different source types also differ in their distribution in the study area (Figure 12): data from CS are mainly located in Italy, Croatia and France, while occurrences in the Aegean Sea and Sea of Marmara mostly come from Literature.

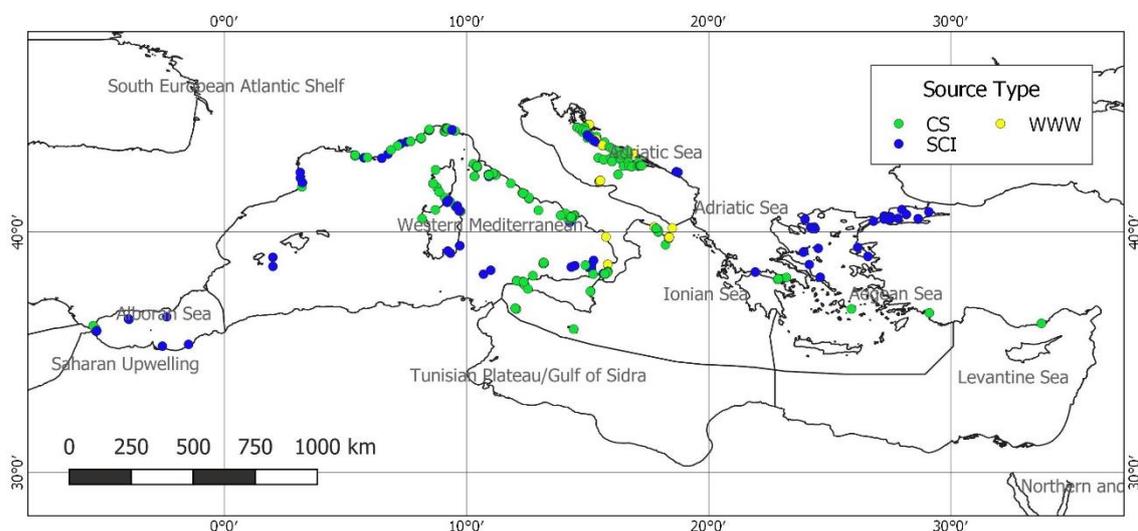


Figure 12 Distribution of *S. savaglia* based on source type.

The *S. savaglia* poster (Figure 9; Section 3.1) was shared on Facebook 60 times by different users, including pages dedicated to SCUBA diving and underwater photography with a discrete number of followers.

The Google survey was filled 153 times, with 89 positive answers to the question whether *S. savaglia* had been seen in the Mediterranean Sea. 27 respondents provided photos and videos, through which it was possible to discard erroneous identifications; other answers were not considered due to the lack of enough precision in the localisation of their sighting (e.g. answers reporting only the name of the region or the country in which the observation was made). This survey provided 80 validated occurrence records.

Looking at the depths of our records (Figure 14), most of them are above 40 m (66,5%). Very few exceed 50 m depth (11,2%) and only 20 records (3,9%) refer to depths higher than 70 m; these deeper records were mostly retrieved from Scientific Literature and are more or less evenly distributed in the Northern Mediterranean. The shallower records are particularly abundant in the Adriatic Sea: here in the Kotor Bay (Montenegro) occurrences above 20 m depths were obtained. 50 records lack information on the depth of the colonies.

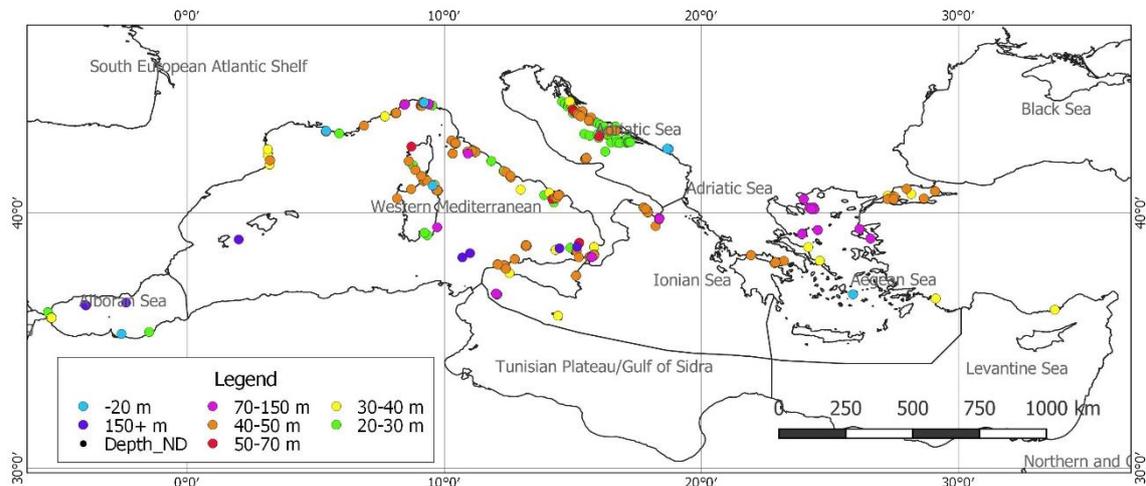


Figure 14 Distribution of *S. savaglia* based on depth ranges.

Records reporting the higher number of colonies come from the Sea of Marmara and Aegean Sea, the Corinthian Strait, Kotor Bay, the Croatian coasts, and several locations along the coasts of Italy: Capo Caccia and Tavolara MPAs in Sardinia, the Portofino and Punta Manara promontories in the Ligurian Sea, the Gulf of Naples, the Sicilian islands of Favignana,

Ustica and Pantelleria, the Strait of Messina and the Apulian coasts facing the Ionian Sea. Dense populations are also reported in the Parc National des Calanques, near Marseille, and in the Alboran Sea, in front of Almería (Figure 15). 82 records lack information on the abundance of colonies.

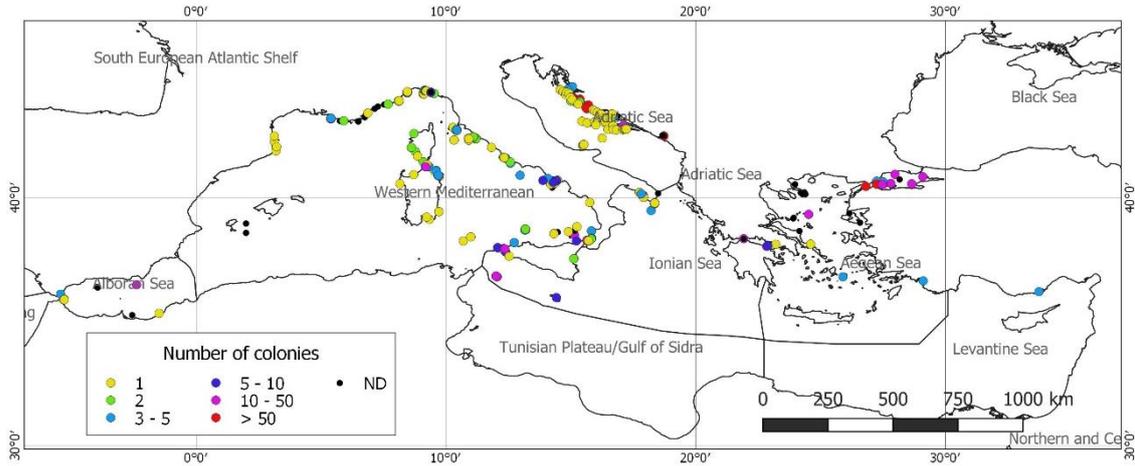


Figure 15 Distribution of *S. savaglia* based on number of colonies.

When collecting occurrence records, the type of aggregation of *S. savaglia* colonies was also investigated. Very few records hold this information: mainly those acquired by directly contacting observers, using the poster or the Google survey. Aggregation types were defined

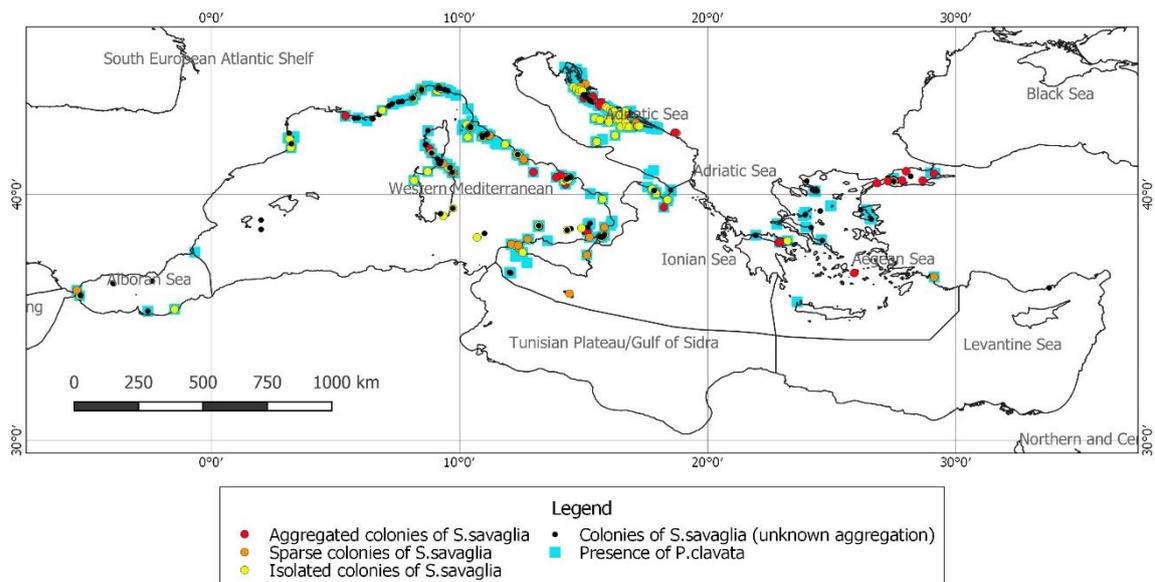


Figure 16 Distribution of type of aggregation of *S. savaglia*, correlated to the distribution of *P. clavata*. Occurrence data of *P. clavata* were obtained from the Reef Check Web-GIS database.

as such: “isolated”, when a single colony is present; “sparse”, when *S. savaglia* colonies are sparse in a vast area, in single colonies or in small groups more than 10 m distant from one another; “aggregated” when colonies form a dense population condensed in a restricted area. *Figure 16* displays the findings of this inquiry. Isolated colonies are far greater in number, but this is mainly due to the fact that records holding a number of colonies equal to “1” could be easily classified as having a “isolated” type of aggregation, although not specified by the original source.

Additional maps displaying environmental conditions are provided in the Supplementary material SB.

4.2. Distribution Model

In *Figure 17* the predictive distribution maps for *Savalia savaglia* in the Mediterranean are displayed.

Some differences between the three distribution maps can be observed: the MaxEnt output map (A), includes very few areas with a high probability of *S. savaglia* presence, in contrast with the Random Forest (RF; B) and Logistic Regression (LR; C) models, which both present areas with very high occurrence probability. In the MaxEnt and RF models, the areas indicated as more suitable for *S. savaglia* are roughly the same, while they are much more spread in the LR model, including most of the Western Mediterranean basin, the Gulf of Gabès, the Sea of Crete and the northern Adriatic Sea. In the RF model, suitable areas are mostly restrained to the coasts.

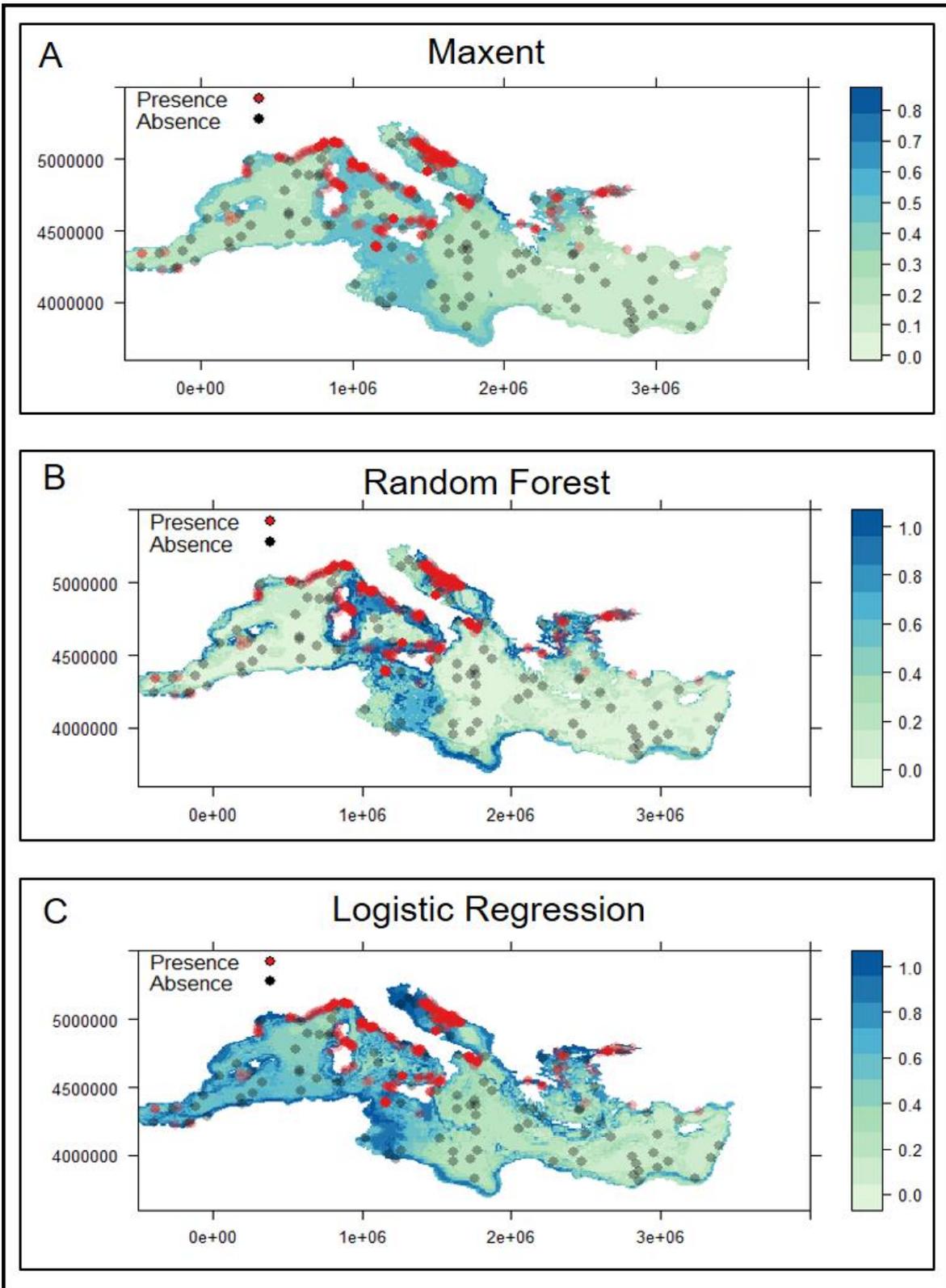


Figure 17 Habitat Suitability maps created with MaxEnt (A), Random Forest (B) and Logistic Regression (C). The red dots indicate our occurrence data, while black dots indicate the background data randomly selected during the workflow.

The Pearson's correlation matrix (Figure 19) shows that the selected covariates have low collinearities, the highest being between the long-term maximum benthic temperature and the long-term minimum benthic dissolved Oxygen. Looking at the PCA (Figure 18), the variable predictors seem to well represent all our data and to all have a rather strong influence on the distribution.

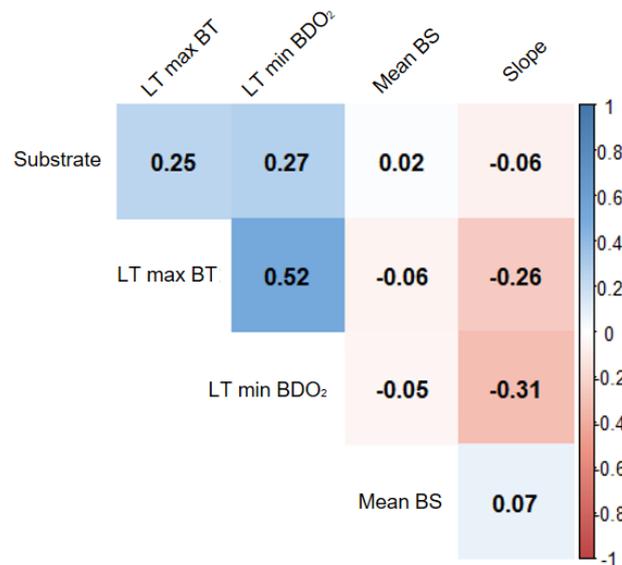


Figure 19 Pearson's correlation matrix resulting from the selected covariates for the SDM. Values range from -1 (strong negative correlation) to 1 (strong positive correlation).

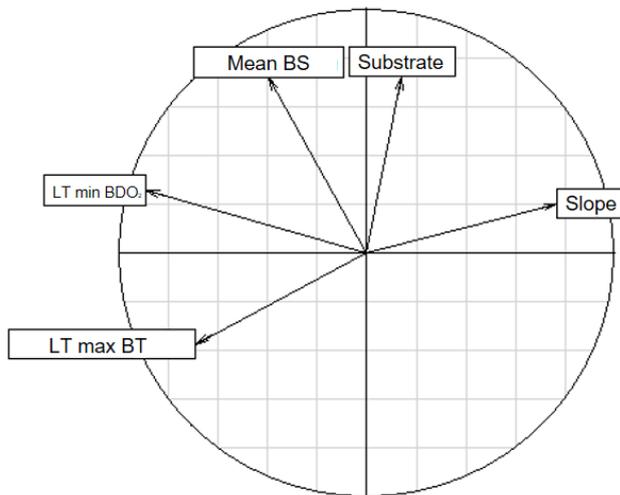


Figure 18 PCA performed on the predictors in relation to the occurrence data. From the correlation circle, we try to select variables on different quadrants or anyway not overlapping. If variables in the same quadrant have different lengths, we should choose the longer one, as that means it varies more over the environmental space. Orthogonal predictors are unrelated, while parallel arrows indicate positively (same direction) or negatively (opposite direction) correlated variables.

Tables 2 to 4 provide the parameters used to evaluate the performance of each model.

MaxEnt (Table 2) based its predictions mainly on the long term minimum dissolved Oxygen (29%), the long term maximum

Table 2 Percentage contributions of each covariate to the prediction in the MaxEnt

	%
LT min BDO ₂	29
LT max BT	28.9
Mean BS	28.5
Slope	9.6
Substrate	3.9

benthic Temperature (28.9%) and the mean benthic salinity (28.5%). Contribution from the substrate type is the lowest (3.9%).

The RF confusion matrix (*Table 3*) shows that the model misclassified 8 times Actual Presence (AP) data out of 492 total presence data, and 24 times Actual Absence (AB) data out of 100 pseudo-absence data.

Table 3 Confusion matrix for the Random Forest model. PA, Predicted Absence; PP, Predicted Presence; AA, Actual Absence; AP, Actual Presence.

	PA	PP	error
AA	76	24	0.24
AP	8	384	0.020408

LR covariates coefficients (*Table 4*) show that the prediction was mostly influenced by slope, which appears positively correlated to occurrence probability (3.90558). Long term minimum benthic dissolved oxygen (0.06601) and substrate (0.01767) also positively influence the occurrence probability, but at much lower levels; mean benthic salinity is the second most important covariate, but it has a coefficient of only -0.6604, one order of magnitude lower than that of slope. Salinity as well as long term maximum benthic temperature negatively affect probability occurrence.

Table 4 Coefficients for the covariates in the Logistic Regression model.

	Coefficients
Mean BS	-0.6604
LT max BT	-0.02704
Substrate	0.01767
LT min BDO2	0.06601
Slope	3.90558

We can directly compare the performances of all the models with the parameters presented in *Table 5*. The model having the highest AUC is RF (0.973), which also presents the highest

Table 5 Values of AUC, sensitivity and specificity for each model.

	MaxEnt	RF	LR
AUC	0.912	0.973	0.82
Sensitivity	0.722	0.982	0.969
Specificity	0.92	0.7	0.51

sensitivity rate (0.982); in terms of specificity, however, its performance is less optimal (0.7). MaxEnt has an AUC above 0.9 as well (0.912), but it displays a lower sensitivity rate (0.722); by contrast, specificity is high (0.92). LR has the lowest values for AUC (0.82) and specificity (0.51), but it performed better than MaxEnt in terms of sensitivity (0.969).

4.3. Lithology of the sampling sites

Here are provided tables showing the minerals found in the rocks characterizing the geology of each sampling site (*Table 6*): these data are compared with the results of our qualitative analyses of the sediments incorporated by each population of *Savalia savaglia*.

Table 6 Typical lithology of the sampling sites.

Ischia	Alkali-potassic volcanic rocks	
Sanidine	$K(AlSi_3O_8)$	alkali-feldspar
Anorthoclase	$(Na,K)AlSi_3O_8$	feldspar
Plagioclase	$(Na,Ca)(Si,Al)_4O_8$	feldspar
Biotite	$K(Mg,Fe^{2+})_3[AlSi_3O_{10}(OH,F)_2]$	silicate
Pyroxene	$XT(Si,Al,Fe^{3+})_2O_6$	silicate
Olivine	$(Mg,Fe)_2SiO_4$	silicate
Met. Oxides	$(Fe,Ti)O_3$	
Augite	$(Ca,Mg,Fe^{2+},Fe^{3+},Ti,Al)_2(Si,Al)_2O_6$	silicate
Quartz	SiO_2	very poor
Montenegro	Flysch deposits: limestones, sandstones, marls, dolomites.	
Anhydrite	$CaSO_4$	evaporitic
Calcite	$CaCO_3$	carbonate
Dolomite	$(Mg,Ca)(CO_3)_2$	carbonate
Muscovite	$KAl_2(Si_3Al)O_{10}(OH,F)_2$	silicate
Biotite	$K(Mg,Fe^{2+})_3[AlSi_3O_{10}(OH,F)_2]$	silicate
Caolinite	$Al_2Si_2O_5(OH)_4$	silicate
Quartz	SiO_2	abundant
Ustica	Pyroclastic deposits of alkali-basaltic composition	
Sanidine	$K(AlSi_3O_8)$	alkali-feldspar
Anorthoclase	$(Na,K)AlSi_3O_8$	feldspar
Plagioclase	$(Na,Ca)(Si,Al)_4O_8$	feldspar
Olivine	$(Mg,Fe)_2SiO_4$	silicate
Augite	$(Ca,Mg,Fe^{2+},Fe^{3+},Ti,Al)_2(Si,Al)_2O_6$	silicate
Quartz	SiO_2	very poor
Marmara	Sedimentary rocks: siltstones, mudstones, marls	
Calcite	$CaCO_3$	carbonate
Dolomite	$(Mg,Ca)(CO_3)_2$	carbonate
Muscovite	$KAl_2(Si_3Al)O_{10}(OH,F)_2$	silicate
Biotite	$K(Mg,Fe^{2+})_3[AlSi_3O_{10}(OH,F)_2]$	silicate
Caolinite	$Al_2Si_2O_5(OH)_4$	silicate
Pyroxene	$XT(Si,Al,Fe^{3+})_2O_6$	silicate
Plagioclase	$(Na,Ca)(Si,Al)_4O_8$	feldspar
Quartz	SiO_2	abundant
Gallipoli	Sedimentary rocks: limestones and dolomites	
Calcite	$CaCO_3$	carbonate
Dolomite	$(Mg,Ca)(CO_3)_2$	carbonate
Muscovite	$KAl_2(Si_3Al)O_{10}(OH,F)_2$	silicate
Biotite	$K(Mg,Fe^{2+})_3[AlSi_3O_{10}(OH,F)_2]$	silicate
Caolinite	$Al_2Si_2O_5(OH)_4$	silicate
Quartz	SiO_2	abundant
Portofino	Sedimentary and metamorphic rocks: marls, sandstones, cherts, ophiolites, gneiss, schists	
Calcite	$CaCO_3$	carbonate
Dolomite	$(Mg,Ca)(CO_3)_2$	carbonate
Biotite	$K(Mg,Fe^{2+})_3[AlSi_3O_{10}(OH,F)_2]$	silicate
Caolinite	$Al_2Si_2O_5(OH)_4$	silicate
Plagioclase	$(Na,Ca)(Si,Al)_4O_8$	feldspar
Muscovite	$KAl_2(Si_3Al)O_{10}(OH,F)_2$	silicate
Pyroxene	$XT(Si,Al,Fe^{3+})_2O_6$	silicate
Olivine	$(Mg,Fe)_2SiO_4$	silicate
Amphibole	$W_{0-1}X_2Y_5(Al^{3+},Si^{4+})O_{22}(OH,F)_2$	amphibole
Serpentine	$Mg_3(Si_2O_5)(OH)_4$	silicate
Quartz	SiO_2	abundant

4.4. Embedded sediments

4.4.1. Quantification of incorporated material

A characterization of the incorporated foreign material in the tissues of *Savalia savaglia*, in terms of abundance and size, has been carried out on samples from all six sites: the results, presented in *Figure 20*, show a certain variability between the populations. In all the Graphs displayed below, the different populations are indicated as such: Ischia, IS; Portofino, PF; Ustica, US; Gallipoli, GP; Montenegro, MN; Marmara, MA.

The abundance of all incorporated material, sand, grains, sponge spicules (whole or fragmented) and other biogenic fragments (i.e. shells of Foraminifera and Radiolaria) was quantified (*Figure 20A*) and expressed as number of granules per milligram of dry weight (dw) tissue. These data show the greatest inter-population variability, with samples from Portofino and Marmara exceeding the other sites of one order of magnitude for total incorporated material (315342 and 212748 granules/mg dw on average, respectively); the samples from Ustica are the poorest, with averagely 17668 granules/mg dw. While in all the other samples sand grains constitute at least 95% of the total incorporated material, in samples from Gallipoli they only represent little more than 47% (meanly 13121 out of a total of 27799 grains/mg dw), and sponge spicules appear extremely abundant (14630 spicules/mg dw, one order of magnitude more than in Ischia and Ustica samples, two more than Marmara and Montenegro samples). In all sites, abundance of other biogenic materials in the tissues is not significant.

High variability, however, can be observed between populations in different sites, but also between colonies of the same population.

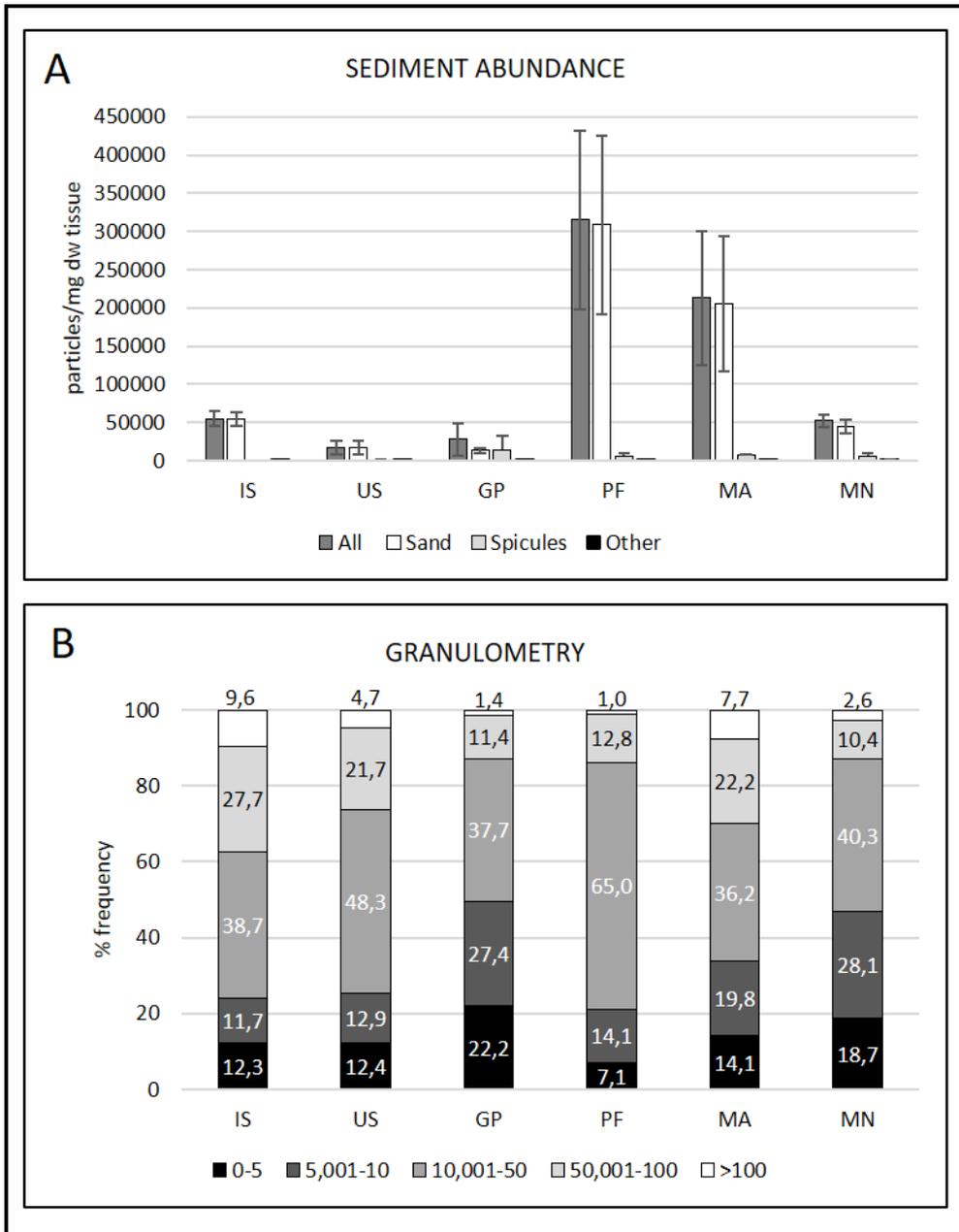


Figure 20 (A) Mean content of embedded foreign material. All, total sediment particles; Sand, sand granules; Spicules, whole or fragmented spicules of all symmetry types; Other, other biogenic material, i.e. shells of Radiolaria and Foraminifera. (B) Percentage frequency of sand grains size classes.

The granulometric distribution (*Figure 20B*) of sand grains, on the other hand, is rather consistent among the populations, with the 10-50 μm size class being predominant in all of them. In samples from Portofino it gets to represent 65% of the total, whereas the finest (0-5 μm) and largest (>100 μm) size classes are very scarcely present (7,1% and 1% of the total,

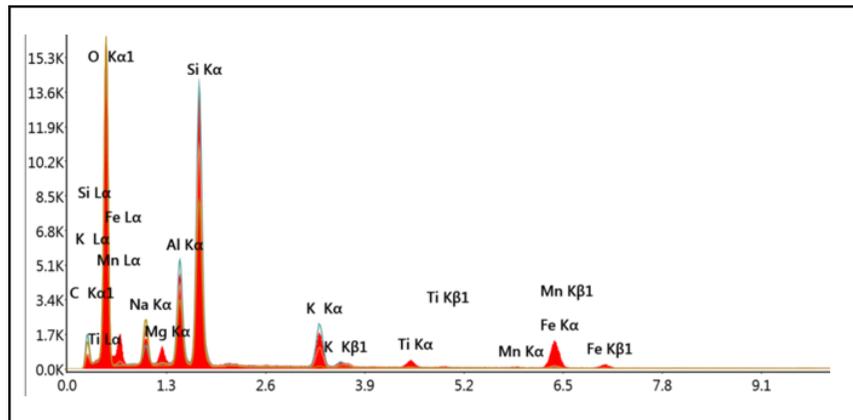
respectively), while in those from the other sites the distribution is slightly more homogeneous. In Ischia and Marmara, the tissues contain more large-size sediments compared to the others, whilst higher percentages of sand grains measuring less than 5 μm are observed in Gallipoli (22,2%) and Montenegro (18,7%).

4.4.2. *Sediment characterization*

For each population, the embedded sediments of two samples were examined by means of EDS analysis and the composition of 10 sediments per site was characterized (*Figure 21*). While the composition of embedded material in each population is, to a certain level, coherent with the lithology that characterizes their habitat (*Table 6*), abundance of Silicon is common to all. It is important, however, to consider that the EDS analysis is not able to discern crystals from amorphous materials.

For samples from Ischia and Gallipoli, analyses using the diffractometer were also carried out: as opposed to EDS, this instrument only detects crystallized materials. The resulting spectra (*Figure 22*) reveal a marked difference in the quality of foreign inorganic material incorporated by these populations: samples from Ischia contain Quartz crystals, as well as other Silicate minerals typical of the Island's lithotypes, those from Gallipoli only contain Quartz and Calcium Oxalate Hydrate.

Ischia									
#	% C	% O	% Si	% Na	% Mg	% Al	% K	% Ti	% Fe
1		61.8	18.2	4.3	1.8	6.9	2.6	0.7	3.8
2		68.5	17.7	3.1		7.2	3.5		
3		68.3	16.5	5.8	0.3	6.7	1.9	0.1	0.4
4	19.7	59.4	9.7	6.6		4.4	0.1		0.1
5		61.7	21.3	8.4	8.7				
6		64.5	35.5						
7	9.1	59.9	15.4	8.4		7.2	0.1		
8	47.9	28.0		0.4	0.3	0.2			0.2
9		57.0	14.4	0.7	10.0	7.5	4.7	1.7	3.9
10		75.2	24.2			0.6			



Marmara										
#	% C	% O	% Si	% Ca	% Na	% Mg	% Al	% K	% P	% Fe
1		67.7	29.3	1.2			0.7			0.1
2	44.3	46.2	0.2	4.0	1.7	0.7				
3	13.6	52.4	16.7	2.2	5.5	0.5	6.5	0.2		0.4
4	8.5	51.7	21.7	0.8	8.5		7.9	0.1		
5	9.7	61.9	25.2	1.1		0.4	0.5		1.2	
6	7.1	53.0	19.1	10.0	0.6	1.3	0.4	0.1	8.5	
7	37.3	41.3	11.6	6.3	0.8	0.3	0.7		1.7	
8	12.0	66.9	0.7	19.6			0.4		0.4	
9	11.1	59.0	14.8	2.1	0.8	0.5	5.9	3.9	1.9	
10	16.1	47.3	11.7	8.6	3.2	0.7	6.6	0.2	5.3	0.4

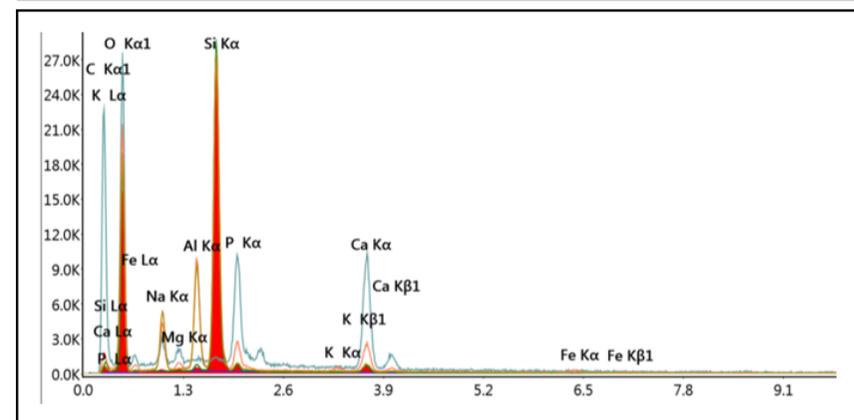


Figure 21 Results of EDX analysis on the embedded sediments of each population. The Tables report the composition of 10 particles in terms of Atomic weight percentage.

Montenegro											
#	% C	% O	% Si	% Ca	% S	% Na	% Mg	% Al	% K	% Ti	% Fe
1		81.9		8.6	8.6						
2		73.4	0.2	14.4	12.2						
3		32.5	67.3			0.2					
4	29.2	31.1	0.1	26.5	13.1						
5		85.1	0.5	13.9				0.5	0.1		
6		60.7	20.6			10.4		8.3			
7		59.3	21.9	0.2		9.9		8.7			
8	21.2	62.8	15.5	0.4							
9		43.5	0.8				2.9	2.9		5.0	44.9
10	14.9	63.8	14.2	3.1		2.6	0.8	0.5	0.1		0.1

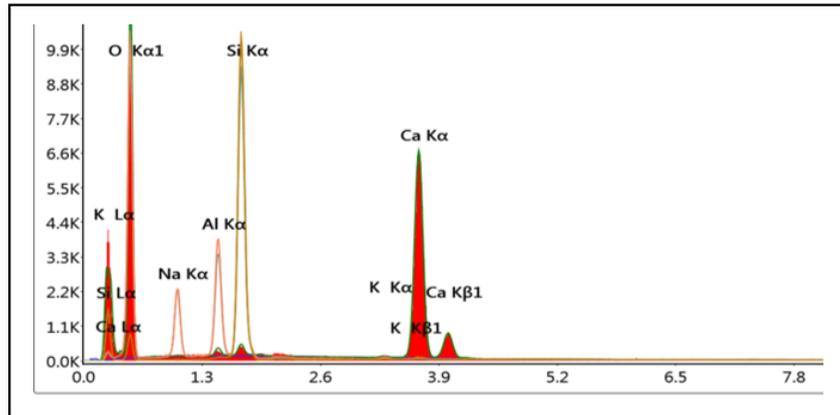
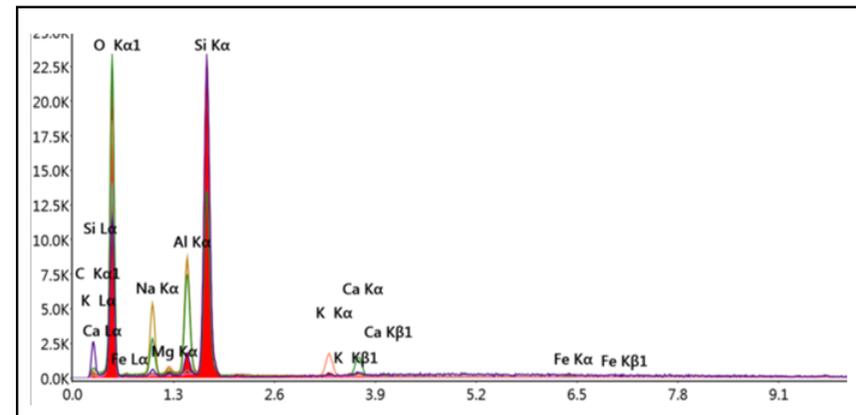


Figure 21 (continued)

Portofino									
#	% C	% O	% Si	% Ca	% Na	% Mg	% Al	% K	% Fe
1		70.8	25.2			0.7	2.3	0.5	0.6
2		66.8	32.2				1.0		
3		63.6	23.8			1.1	9.0	2.2	0.3
4		59.3	21.2	0.2	9.7	0.7	8.6	0.1	0.2
5		68.3	14.4	2.1	6.2	0.4	8.4		0.2
6		64.8	35.2						
7		63.1	36.9						
8		63.9	36.1						
9	20.1	57.4	13.0		0.6		5.3	3.7	
10		59.5	40.5						



Ustica								
#	% O	% Si	% Ca	% Na	% Mg	% Al	% K	% Fe
1	61.1	17.8	3.8	4.7	0.5	11.9	0.1	0.3
2	59.1	18.8	4.8	4.3		12.6	0.1	0.3
3	63.8	18.5	0.8	7.4		8.2	1.2	0.2
4	59.5	18.6	3.9	5.4		12.3	0.1	0.3
5	62.8	20.2		6.1	0.6	8.1	1.6	0.8
6	63.5	16.3	3.5	5.1				
7	60.3	18.8	3.1	5.9		11.5	0.3	0.2
8	58.3	17.4	8.2	3.3		11.9	0.4	0.6
9	48.2	16.7			16.5			18.6
10	71.0	26.3		0.7	0.5	1.1	0.2	0.2

Gallipoli											
#	% C	% O	% Si	% Ca	% Na	% Mg	% Al	% K	% Ti	% Fe	% Cr
1		79.5	5.0	10.9		1.3	2.9			0.4	
2	5.0	60.3	14.5	7.6	0.7	5.8	3.5			2.5	
3		41.9	58.1								
4	75.0	16.0	8.9		0.2						
5		66.8	20.3				7.8	5.2			
6	13.9	56.4	15.0		8.5		6.1				
7		63.4	21.9				8.4	6.3			
8		71.0	29.0								
9	43.4		0.4	1.0						47.5	7.73
10		62.3	16.0	8.2	0.9	6.8	3.2		0.2	2.5	

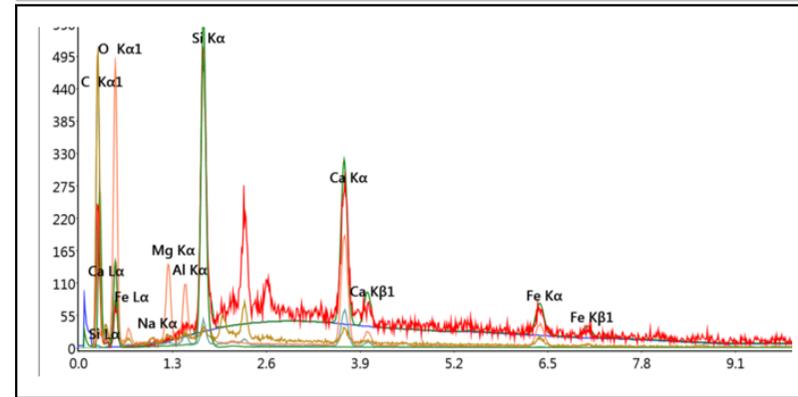
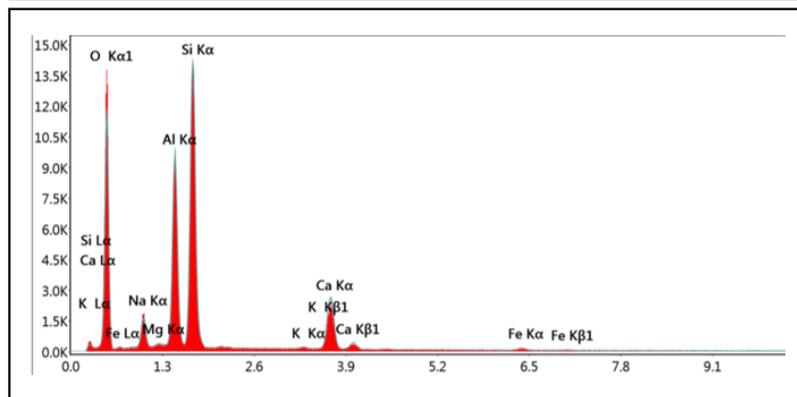


Figure 21 (continued)

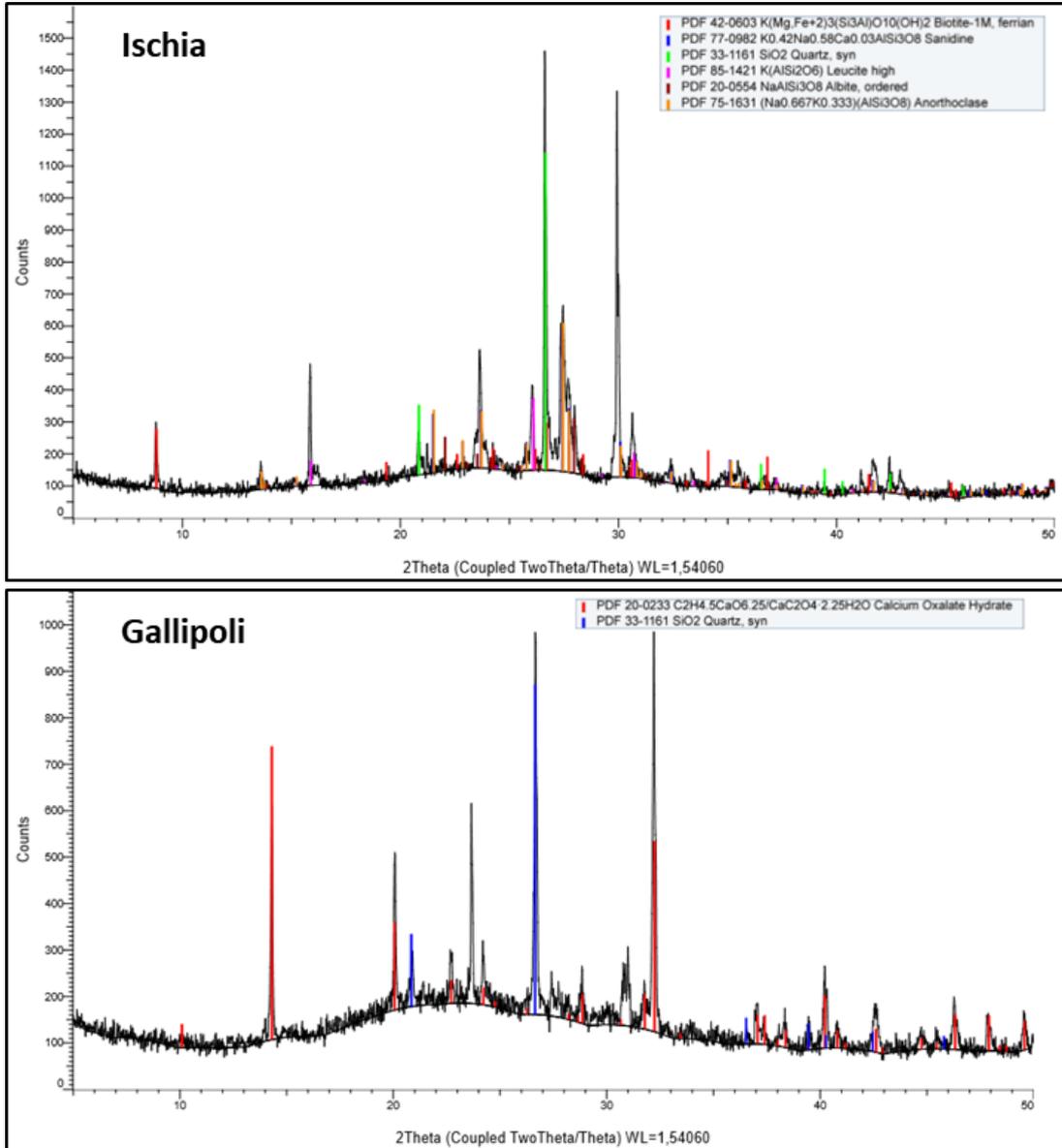
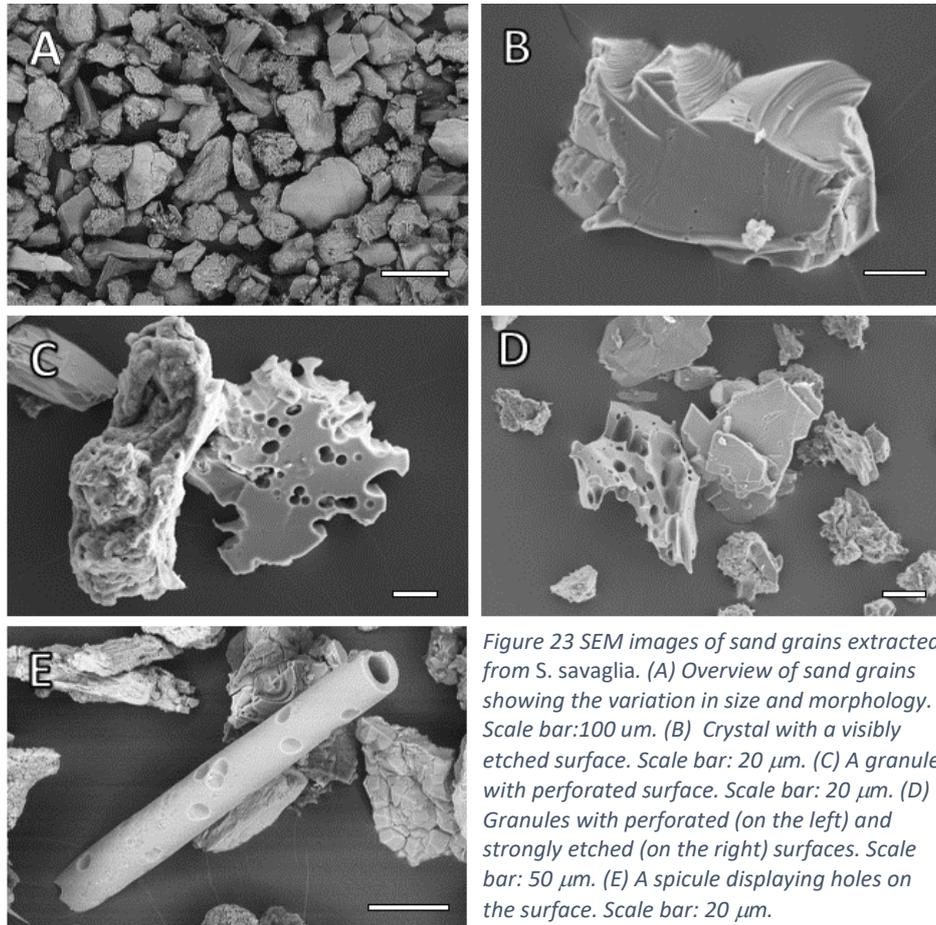


Figure 22 X-Ray spectra of embedded sediments from Ischia and Gallipoli and relative characterized minerals.

In *Figure 23*, in some pictures of incorporated sediments taken with a SEM, grains appear to be etched (*Figure 23B*), displaying small holes (*Figure 23C, D, F*) and clear corrosion patterns (*Figure 23C*). In *Figure 23E* appears a sponge spicule with holes, found in sediments extracted from samples from Ischia.



4.5. Collagen quantification

The amount of collagen in the tissues of *Savalia savaglia* was quantified for three samples of populations from Ischia, Montenegro and Gallipoli through the hydroxyproline assay: the results are expressed as μg of collagen per mg of wet weighted tissue. The analysis was integrated with the quantification of the total protein abundance by means of the Bradford assay, so that collagen to protein ratios in each sample could be compared. The results,

displayed in *Figure 24*, show that samples from Gallipoli have significantly larger amounts of collagen, both in relation to wet weight (ww) tissue and to total proteins.

In all the three populations, out of three sampled analysed two show consistent results, while a third is extremely different, hence the high standard deviation; since all nine samples were analysed simultaneously using the same protocol, a systematic error inherent to the measurement process is unlikely.

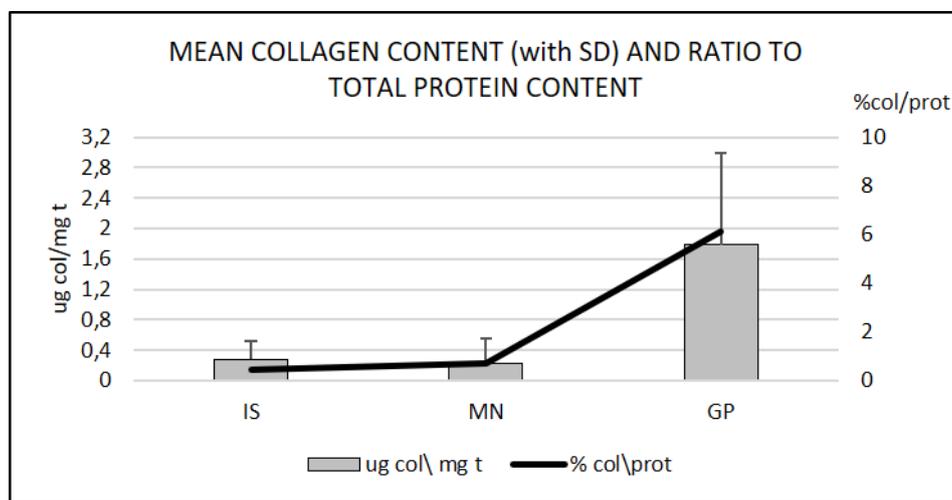


Figure 24 Collagen abundance in the tissue of *S. savaglia*, expressed as $\mu\text{g col/mg dw tissue}$ and as % ratio to total protein content.

4.6. Cnidome

The complete cnidome of populations from five sites (Gallipoli, Ischia, Portofino, Montenegro and Marmara) is described here. Cnidae with a capsule shorter than $7 \mu\text{m}$ were not considered, since the identification was impracticable (*Figure 25A*): these unidentified cnidae may also be still in formation, however it is impossible to verify this possibility using a light microscope. The column of samples from Marmara and Portofino was so encrusted with sand grains that it was hard to squash on the slide (*Figure 25B*) and this may have affected the results of the survey. Two dimensional classes of basitrichs were found: they are identified in the following tables and images as “basitrich 1” and “basitrich 2”.

The complete cnidome of *Savalia savaglia* is displayed in *Table 7*, and pictures taken at the light microscope can be seen in *Figure 26*.

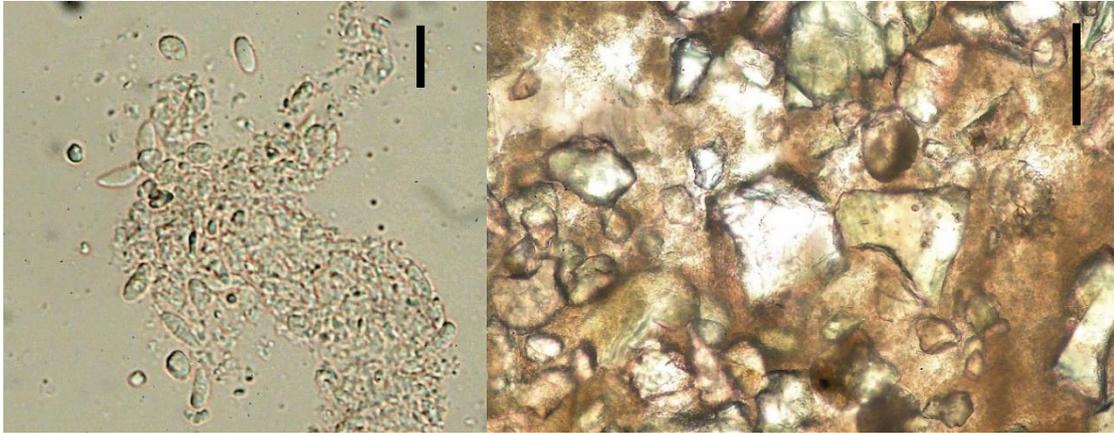


Figure 25 (A) Presumed nematocysts with size inferior to 7 μm , in the mesenteries of a polyp from Portofino. 100x objective, scale bar is 10 μm . (B) slide of the column of a polyp from Marmara, engulfed with abundant sand grains. 20x objective, scale bar is 20 μm .

Table 7 Cnidome characterization of each population. Ab, abundance class: 1, rare; 5 very common. n, number of measured capsules. FP, ratio of the number of polyps having a type of cnida to the total number examined. FS, ratio of the number of samples having a type of cnida to the total number examined.

ISCHIA						
Body part	Type	Ab	n	FP	FS	Range: length (mean, SD) x width (mean, SD) (m)
Tentacles	p-Mastigophores	1	10	3/6	2/3	10.0-17.0 (12.8, 1.8) x 5.0-5.5 (5.1, 0.2)
	Large Holotrichs*	1	3	2/6	2/3	20.0-23.0 x 8.0-10.0
	Spirocysts	5	180	6/6	3/3	7.0-28.0 (21.2, 2.3) x 3.0-5.0 (4.2, 0.5)
	Basitrichs 1	5	107	6/6	3/3	15.0-22.0 (18.7, 1.6) x 3.0-5.5 (4.2, 0.5)
Pharynx	p-Mastigophores	2	70	6/6	3/3	8.0-17.0 (13.4, 1.8) x 4.0-6.5 (5.1, 0.6)
	Spirocysts	1	43	5/6	3/3	18.0-25.0 (21.4, 1.9) x 4.0-6.0 (4.7, 0.4)
	Basitrichs 1	5	187	6/6	3/3	14.0-24.0 (19.0, 2.1) x 3.0-5.5 (4.2, 0.5)
Filaments	p-Mastigophores	5	219	6/6	3/3	10.0-20.0 (14.1, 1.7) x 4.0-6.0 (4.9, 0.5)
	Spirocysts	1	33	6/6	3/3	14.0-25.0 (20.1, 3.0) x 3.5-5.5 (4.5, 0.5)
	Basitrichs 1	2	43	5/6	3/3	9.0-25.0 (19.6, 3.7) x 2.0-6.0 (4.3, 0.9)
	Small Holotrichs	1	5	2/6	2/3	8.0-19.0 (8.8, 0.7) x 3.5-5.5 (4.2, 0.7)
Column	p-Mastigophores	1	17	5/6	3/3	10.0-18.0 (13.4, 1.9) x 3.0-5.5 (4.6, 0.6)
	Large Holotrichs	5	161	6/6	3/3	17.0-25.0 (20.4, 1.6) x 7.0-11.5 (9.8, 0.8)
	M. b-Mastigophores	5	110	6/6	3/3	14.0-22.0 (18.0, 1.8) x 9.0-14.0 (12.6, 0.9)
	Spirocysts	1	12	4/6	3/3	18.0-25.0 (21.3, 2.7) x 3.0-4.5 (4.0, 0.4)
MARMARA						
Body part	Type	Ab	n	FP	FS	Range: length (mean, SD) x width (mean, SD) (µm)
Tentacles	Atrichs	1	6	2/6	1/3	17-20 (18.3, 1.1) x 4-6 (4.8, 0.6)
	p-Mastigophores	1	21	4/6	3/3	11-20 (14.1, 2.4) x 2.5-6 (4.4, 0.7)
	Large Holotrichs*	1	3	2/6	1/3	19-20 x 8-9
	Spirocysts	5	142	6/6	3/3	13-28 (20.2, 2.9) x 2-6 (3.9, 0.7)
	Basitrichs 1	5	128	6/6	3/3	13-26 (18.8, 2.2) x 2.5-5.5 (3.8, 0.6)
Pharynx	Atrichs	1	4	2/6	1/3	16-20 x 4-5
	p-Mastigophores	2	64	6/6	3/3	11-21 (14.9, 1.8) x 3.5-5.5 (4.5, 0.5)
	Spirocysts	4	105	2/6	3/3	15-29 (21, 3.0) x 2.5-6 (4, 0.7)
	Basitrichs 1	5	125	6/6	3/3	10-22 (17, 2.4) x 2.5-5.5 (4.1, 0.6)
Filaments	Atrichs	1	5	2/6	1/3	12-19 (15.4, 1.5) x 4-5 (4.3, 0.4)
	p-Mastigophores	5	137	6/6	3/3	11-18 (14.8, 1.5) x 3.5-6 (4.5, 0.5)
	Large Holotrichs*	1	7	2/6	1/3	18-22 (20.7, 1.4) x 8-10.5 (9.8, 0.7)
	M. b-Mastigophores*	1	6	1/6	1/3	7-13 (9.8, 1.9) x 5-8 (6.7, 1.0)
	Spirocysts	1	28	4/6	3/3	14-27 (20, 3.2) x 3-5.5 (4.2, 0.6)
	Basitrichs 1	4	117	6/6	3/3	13-21 (16.5, 2.0) x 2.5-6 (4.2, 0.6)
Column	Atrichs*	1	5	1/6	1/3	18-22 (19.4, 1.5) x 3.5-5 (4.4, 0.6)
	p-Mastigophores	1	15	3/6	3/3	13-18 (15.4, 1.5) x 3.5-5 (4.3, 0.4)
	Large Holotrichs	5	162	6/6	3/3	13-27 (21, 2.0) x 6-12 (9.1, 1.1)
	M. b-Mastigophores	3	57	6/6	3/3	9-20 (14.4, 2.1) x 7-18 (11.2, 1.8)
	Spirocysts	1	26	6/6	3/3	17-25 (20, 1.9) x 2.5-4.5 (3.8, 0.6)
	Basitrichs 1	1	35	5/6	3/3	13-26 (17.7, 3.6) x 2-5 (3.9, 0.7)

Table 7 continued

GALLIPOLI						
Body part	Type	Ab	n	FP	FS	Range: length (mean, SD) x width (mean, SD) (μm)
Tentacles	Atrichs	1	8	1/6	1/3	20-32 (26.4, 4.3) x 4-6 (4.9, 0.6)
	p-Mastigophores	1	20	5/6	3/3	7-22 (14.6, 5.2) x 2-5 (3.4, 0.7)
	Spirocysts	5	141	6/6	3/3	12-32 (21.9, 3.4) x 2-5 (3.5, 0.8)
	Basitrichs 1	4	131	6/6	3/3	7-28 (18.4, 4.0) x 2-6 (3.8, 0.8)
Pharynx	Atrichs	1	4	1/6	1/3	28-34 x 5-6
	p-Mastigophores	2	36	6/6	3/3	7-20 (11.8, 4.3) x 2-5 (3.1, 0.9)
	Large Holotrichs*	1	7	2/6	1/3	9-20 (16, 4.2) x 6-11 (9, 1.7)
	Spirocysts	5	117	6/6	3/3	13-20 (21.3, 3.5) x 2-6 (3.6, 0.7)
	Basitrichs 1	5	136	6/6	3/3	8-29 (18.3, 3.3) x 2-6 (3.9, 0.8)
Filaments	p-Mastigophores	4	132	6/6	3/3	7-20 (14.6, 2.3) x 2-6 (3.9, 0.8)
	Large Holotrichs	1	7	2/6	2/3	9-13 (10.4, 1.4) x 3-6 (4.7, 0.9)
	Spirocysts	2	44	6/6	3/3	8-26 (19.3, 2.8) x 2-5 (3.6, 0.6)
	Basitrichs 1	3	99	6/6	3/3	8-24 (16.7, 3.4) x 2-5 (3.7, 0.7)
	Basitrichs 2	1	17	3/6	2/3	33-47 (40.3, 4.4) x 4-8 (6.1, 1.1)
Column	p-Mastigophores	2	23	4/6	2/3	8-18 (12.6, 2.5) x 2-5 (3.7, 0.7)
	Large Holotrichs	5	158	6/6	3/3	13-30 (20.8, 2.7) x 6-12 (8.5, 1.1)
	M. b-Mastigophores	1	11	3/6	2/3	10-19 (13.6, 3.1) x 9-17 (11.4, 2.3)
	Spirocysts	1	15	3/6	2/3	15-20 (17.7, 1.7) x 2-4 (2.9, 0.5)
	Basitrichs 1	3	93	6/6	3/3	9-26 (17.1, 3.8) x 2-5.5 (3.2, 1.0)
PORTOFINO						
Body part	Type	Ab	n	FP	FS	Range: length (mean, SD) x width (mean, SD) (m)
Tentacles	p-Mastigophores	2	29	6/6	3/3	14.0-21.0 (16.7, 1.7) x 4.0-6.0 (4.8, 0.5)
	Large Holotrichs	1	12	4/6	2/3	15.0-21.0 (17.7, 1.6) x 8.0-10.0 (9.2, 0.8)
	Spirocysts	5	141	6/6	3/3	15.0-28.0 (21.6, 2.7) x 2.0-5.5 (4.1, 0.6)
	Basitrichs 1	5	118	6/6	3/3	14.0-25.0 (18.6, 2.6) x 3.0-6.5 (4.4, 0.7)
Pharynx	Atrichs	1	5	2/6	2/3	13.0-18.0 (15.8, 2.0) x 3.5-4.0 (3.9, 0.2)
	p-Mastigophores	3	82	6/6	3/3	11.0-22.0 (15.5, 1.8) x 3.5-6.0 (4.7, 0.6)
	Large Holotrichs*	1	14	1/6	1/3	17.0-23.0 (20.2, 1.7) x 8.0-11.0 (9.5, 0.8)
	M. b-Mastigophores*	1	2	1/6	1/3	9.0-13.0 x 6.0-6.5
	Spirocysts	3	79	6/6	3/3	12.0-28.0 (20.5, 2.9) x 3.0-5.0 (4.6, 0.5)
	Basitrichs 1	2	111	6/6	3/3	11.0-26.0 (18.5, 2.3) x 2.5-6.0 (4.1, 0.6)
	Basitrichs 2*	1	7	1/6	1/3	35.0-49.0 (40.6, 5.6) x 5.0-7.0 (5.9, 0.7)
Filaments	Atrichs	1	6	2/6	2/3	15.0-23.0 (19.2, 2.6) x 4.0-5.0 (4.5, 0.3)
	p-Mastigophores	5	158	6/6	3/3	12.0-20.0 (15.4, 1.6) x 3.0-6.0 (4.5, 0.5)
	Spirocysts	1	31	6/6	3/3	13.0-25.0 (20.4, 2.7) x 4.0-5.5 (4.6, 0.5)
	Basitrichs 1	2	70	6/6	3/3	12.0-19.0 (16.7, 1.9) x 2.0-5.0 (4.1, 0.6)
	Basitrichs 2	2	31	2/6	3/3	28.0-49.0 (40.0, 4.5) x 5.0-7.5 (6.2, 0.5)
	Small Holotrichs	1	1	1/6	1/3	12.0 x 8.0
Column	p-Mastigophores	1	21	5/6	3/3	11.0-17.0 (14.8, 1.3) x 3.5-5.0 (4.3, 0.5)
	Large Holotrichs	5	118	6/6	3/3	13.0-23.0 (19.8, 1.4) x 8.0-12.0 (9.6, 0.9)
	M. b-Mastigophores	5	102	6/6	3/3	8.0-21.0 (14.9, 2.0) x 6.0-15.0 (11.4, 1.2)
	Spirocysts	1	28	4/6	2/3	17.0-25.0 (20.8, 1.9) x 3.0-5.0 (4.0, 0.6)
	Basitrichs 1	1	31	6/6	3/3	11.0-23.0 (17.1, 2.4) x 2.0-5.0 (2.8, 0.7)

Table 7 continued

MONTENEGRO						
Body part	Type	Ab	n	FP	FS	Range: length (mean, SD) x width (mean, SD) (µm)
Tentacles	Atrichs	1	23	4/6	3/3	13-36 (25.2, 5.0) x 2.5-6.5 (5.3, 0.8)
	p-Mastigophores	1	19	4/6	3/3	10-21 (14.7, 3.0) x 2-5 (4.1, 0.7)
	Large Holotrichs	1	7	2/6	1/3	17-23 (19.9, 1.9) x 6-9 (7.4, 0.9)
	M. b-Mastigophores	1	5	3/6	2/3	8-12 (10.2, 1.3) x 6-11.5 (9.3, 1.8)
	Spirocysts	5	130	6/6	3/3	14-29 (21, 2.8) x 2.5-5.5 (3.9, 0.7)
	Basitrichs 1	3	116	6/6	3/3	8-31 (18.5, 3.2) x 2-5.5 (4, 0.7)
Pharynx	Atrichs	1	18	4/6	3/3	15-24 (18.2, 2.5) x 4-6 (4.8, 0.6)
	p-Mastigophores	2	52	6/6	3/3	10-23 (14.8, 2.3) x 2-5 (4.3, 0.6)
	Spirocysts	4	83	5/6	3/3	17-27 (21.3, 2.5) x 2.5-5 (4, 0.6)
	Basitrichs 1	5	143	6/6	3/3	9-28 (18.4, 3.2) x 2-6 (3.9, 0.8)
Filaments	Atrichs	2	33	5/6	3/3	11-35 (19.2, 4.2) x 3-6.5 (4.4, 0.7)
	p-Mastigophores	4	125	6/6	3/3	11-23 (15.8, 2.2) x 3-7.0 (4.3, 0.6)
	Large Holotrichs	1	12	4/6	3/3	16-25 (21.2, 2.3) x 6-14 (8.9, 1.9)
	M. b-Mastigophores	1	16	5/6	3/3	8-16 (10.6, 2.4) x 5-12 (8, 1.9)
	Spirocysts	1	3	4/6	2/3	12-21 x 2-5
	Basitrichs 1	3	43	6/6	3/3	16-28 (21.5, 3.6) x 2-5 (3.8, 0.9)
	Basitrichs 2	3	62	6/6	3/3	32-54 (41, 3.7) x 4.5-7 (5.7, 0.6)
Column	Atrichs*	1	2	1/6	1/3	23-25 x 4.5-5.5
	p-Mastigophores	1	18	4/6	2/3	10-21 (16.3, 3.1) x 3-6 (4.4, 0.7)
	Large Holotrichs	5	145	6/6	3/3	13-27 (21.6, 2.7) x 4.5-12 (8.1, 1.2)
	M. b-Mastigophores	4	97	6/6	3/3	8-2 (15, 3.2) x 5-16 (10.9, 2.0)
	Spirocysts	1	13	4/6	3/3	16-25 (20.5, 2.7) x 3-4.5 (3.7, 0.5)
	Basitrichs 1	2	25	4/6	2/3	11-28 (18.6, 4.7) x 3-6 (4.2, 0.7)

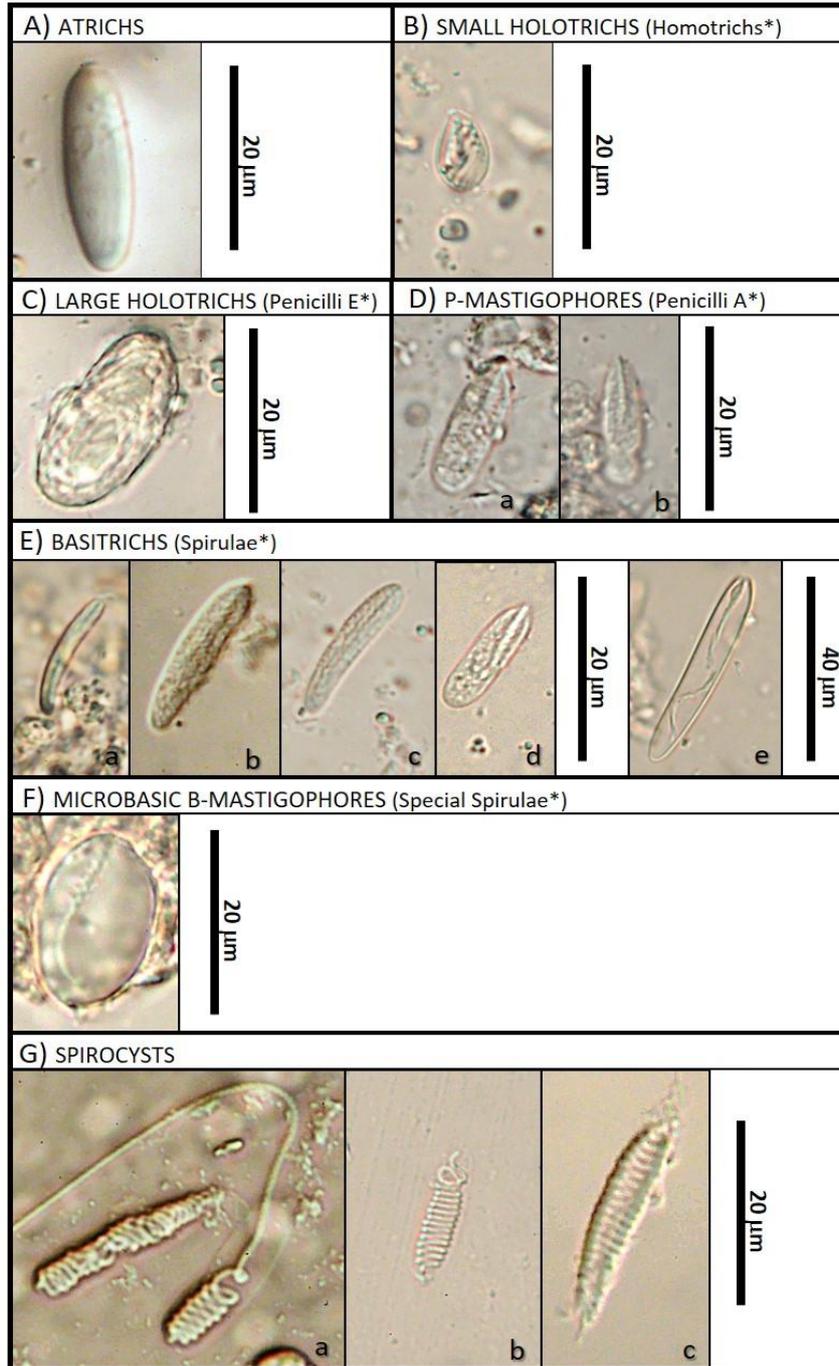


Figure 26 Images at the light microscope of *S. savaglia cnidae*. (A) atrich from tentacles of a polyp from Montenegro. (B) small holotrich in the filaments of a polyp from Montenegro. (C) large holotrich observed in the column of a polyp from Marmara. (D) p-mastigophore from the pharynx (a) and from the filaments (b) of samples from Portofino. (E) Basitrichs 1 observed in the column of samples in Gallipoli (a), in filaments from Portofino (b), in tentacles (c) and filaments (d) from Ischia; Basitrich 2 found in filaments from Montenegro (e). (E) Microbasic b-mastigophore in the column of a polyp from Montenegro. (G) Partially everted spirocyst (a) found in the pharynx of a polyp from Montenegro, other spirocysts from tentacles in a Marmara sample (b) and from those of a Montenegro sample (c). *, corresponding category name after den Hartog (1993).

A summary description of the cnidome of the species, based on all the data here presented, is shown in *Table 8* and *Image 27*.

Table 8 General cnidome characterization of *Savalia savaglia*. Ab, abundance class: 1, rare; 5 very common. n, number of measured capsules. FP, ratio of the number of polyps having a type of cnida to the total number examined. FS, ratio of the number of samples having a type of cnida; Fsi, number of populations having a type of cnida.

General Table							
Body part	Type	Ab	n	FP	FS	Fsi	Range: length (mean, SD) x width (mean, SD) (m)
Tentacles	p-Mastigophores	2	99	18/30	11/15	5/5	7-22 (14.9, 3.4) x 2-6 (4.3, 0.8)
	Large Holotrichs	1	25	6/30	3/15	1/5	15-23 (19, 2.1) x 6-10.5 (8.7, 1.2)
	Spirocysts	5	734	30/30	15/15	5/5	12-32 (21.2, 2.9) x 2-6 (3.9, 0.7)
	Basitrichs 1	4	600	30/30	15/15	5/5	7-31 (18.6, 2.9) x 2-6.5 (4, 0.7)
	M. b-Mastig.	1	5	3/30	2/15	1/5	8-12 (10.2, 1.3) x 6-11.5 (9.3, 0.8)
	Atrichs	2	37	7/30	5/15	3/5	13-36 (24.4, 5.2) x 2.5-6.5 (5.1, 0.8)
Pharynx	p-Mastigophores	3	304	30/30	15/15	5/5	7-23 (14.3, 2.6) x 2-6.5 (4.5, 0.9)
	Spirocysts	3	427	28/30	15/15	5/5	12-30 (21.1, 3) x 2-6 (4, 0.7)
	Basitrichs 1	5	702	30/30	15/15	5/5	8-29 (18.3, 2.8) x 2-6 (4, 0.7)
	Atrichs	1	31	9/30	7/15	4/5	13-34 (19.3, 4.9) x 3.5-6 (4.7, 0.7)
Filaments	p-Mastigophores	5	772	30/30	15/15	5/5	7-23 (14.9, 2) x 2-7 (4.5, 0.7)
	L. Holotrichs	1	29	6/30	5/15	2/5	9-25 (18.2, 5.1) x 3-14 (8, 2.5)
	Spirocysts	2	139	26/30	14/15	5/5	8-28 (17.5, 3.3) x 2-5.5 (4.2, 0.7)
	Basitrichs 1	3	372	29/30	15/15	5/5	2-28 (17.5, 3.3) x 2-6 (4, 0.7)
	Basitrichs 2	3	110	11/30	6/15	3/5	32-54 (40.6, 4.1) x 4-8 (5.9, 0.7)
	M. b-Mastig.	1	22	5/30	3/15	1/5	7-16 (10.4, 2.3) x 5-12 (7.7, 1.8)
	Atrichs	1	44	9/30	6/15	3/5	11-35 (18.8, 4.1) x 3-6.5 (4.4, 0.6)
Column	Small Holotrichs	1	12	5/30	5/15	3/5	8-12 (9.3, 1.2) x 3.5-8 (5.3, 1.2)
	p-Mastigophores	2	94	21/60	13/15	5/5	8-21 (14.4, 2.6) x 2-6 (4.2, 0.7)
	Large Holotrichs	5	744	30/30	15/15	5/5	13-30 (20.8, 2.3) x 4.5-12 (9, 1.2)
	Spirocysts	2	94	21/30	13/15	5/5	15-25 (20.1, 2.4) x 2-5 (3.7, 0.7)
	Basitrichs 1	2	184	21/30	11/15	5/5	9-28 (17.4, 3.1) x 1-6 (3.4, 1)
	M. b-Mastig.	5	377	27/30	14/15	5/5	8-22 (15.7, 2.8) x 5-18 (11.6, 1.7)
Atrichs	1	7	2/30	2/15	2/5	18-25 (20.7, 2.5) x 3.5-5.5 (4.6, 0.6)	

Atrich (26A): rather large, oblong capsule with a thick and apparently smooth tubule, which prior to discharge appears either coiled in a spring along the capsule or loosely arranged in loops at the top of the capsule. They can be found in all or only some tissues, in limited abundance, and generally they are larger in the tentacles.

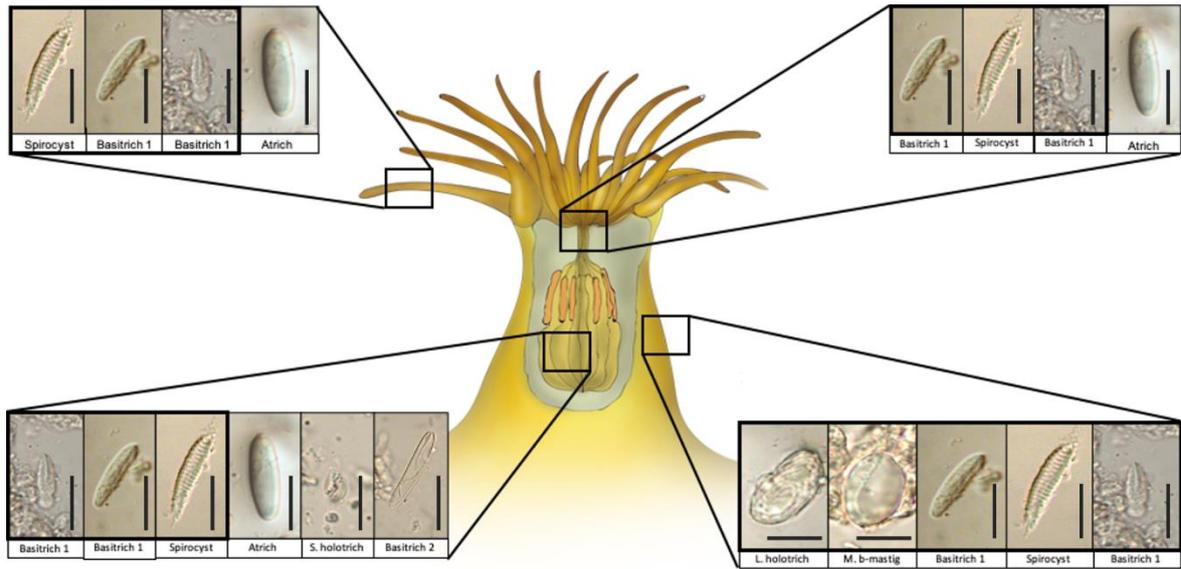


Figure 27 Section of a polyp of *S. savaglia* with the set of cnidae found in each body part. All scale bars are 10 μ m except for the Basitrich 2, which is 5 μ m. Section drawing by Di Camillo.

Small holotrich (26B): round-to-ovoid capsule with a thick, strongly coiled tapering tubule. It is rarely found and is present only in the mesenteric filaments, although given that its average length is just above the minimum size for identification, it may be more common than estimated.

Large Holotrich (26C): nematocyst with large, oblong-oval capsules and a rather thick tubule that, undischarged, is loosely and obliquely coiled. The shaft of the tubule is undetectable when unevverted. This category is predominant in the column, where it shows a wide size range, while in the other tissues, if present, it's quite rare, generally smaller and with narrower size ranges.

p-Mastigophore (26D): oval capsule and tubule with clearly visible, enlarged shaft; a v-notch can be sometimes, but not always, detected, while the distal thread is undetectable when undischarged. This category is observed in all tissues but is predominant in the filaments and quite rare in the column. Size means and ranges are consistent in all body parts.

Basitrich (26E): nematocyst with long, slender capsule with a long, mildly enlarged shaft and a long and strongly coiled thread. Two clearly separated dimensional classes were detected: a smaller one (a-c) common in all body parts and predominant in the pharynx, and a larger one (e) only detected in the mesenteric filaments. Basitrichs with less elongated capsules and rather shorter threads were occasionally observed in the mesenteries (d).

Microbasic b-mastigophore (26F): round-to ovoid capsule with a rather short tapering tubule. This is the only nematocyst in which the tubule's spines could be detected when undischarged. When ejected, the capsule and straight tubule become completely transparent and spines disappear, probably dissolving in contact with water. This type is very common in the column and may occasionally be present in the other body parts, where its size tends to be smaller.

Spirocyst (26G): elongated, slender capsule containing a long, spirally coiled isodiametrical thread with no visible shaft. When ejected (a), the tubule is loosened and tends to separate from the capsule, which becomes completely transparent. Spirocysts are present in all the tissues, with comparable sizes, and are strongly predominant in the tentacles.

5. Discussion

5.1. Distribution map

As mentioned before, most of the occurrence data were collected from the North-western Mediterranean, while records collected from the Levantine basin, although they suggest the presence of poorly investigated hot spots for *Savalia savaglia*, are fewer in number. The reasons for this disparity are manifold and related to both environmental features and the current knowledge on the marine fauna. The Eastern Mediterranean has been always considered to have a lower species diversity compared to the Western basin, because of its oligotrophic conditions and its complex geological history (Di Camillo *et al.*, 2018). It has been suggested, however, that this gradient could reflect a lack of data concerning the biodiversity in the Levantine basin, since the vast majority of studies are focused on the Western Mediterranean. Its great depths certainly pose logistic problems for investigations on the benthic layer: even abiotic features such as substrate are poorly surveyed. Furthermore, the involvement of diving centres operators from all the countries allowed to observe, based on the type of diving experiences offered, that generally recreational Scuba diving in the Levantine basin is more focused on sites of archaeological interest, shipwrecks or war relics, rather than the local flora and fauna. Naturally, the lack of interest for marine biodiversity could indeed be caused by its paucity.

In contrast, in the Western Mediterranean more amateurs and recreational divers develop and cultivate an interest in the marine biota, as attested by the high success of Citizen Science projects such as Reef Check Mediterranean. The Reef Check web-GIS is the largest database of underwater observations performed by trained volunteers with a standard protocol. The protocol, however, is mainly applied in the Western Mediterranean: the dataset retrieved

from the Reef Check web-GIS for *S. savaglia* only counts observations from Italy (86,6% of the total) and Croatia (13,4%). Given that the Reef Check dataset alone represents over 35% of all the occurrence records, it is clear that our biological sampling is strongly biased.

The survey answers were also obtained mainly from divers and diving centres operators from the North-western Mediterranean: only 4 records out of 80 were obtained from the Levantine basin. Being most of our sources dependent to SCUBA diving observations, the high bathymetry of the Eastern Mediterranean has been a limiting factor.

The scarcity of records obtained from the Web is due to the fact that, in most cases, authors of contents displaying colonies of *S. savaglia* either could not be contacted, could not provide basic information on their observation or did not want to.

The poster and survey also provided biased data: the first circulated mostly on Italian, French, Spanish and Greek blogs and fan pages, while most pages related to underwater activities outside these countries focused on spearfishing or wreck diving. One third of the survey answers were obtained from Italian divers. The linguistic barrier probably played an important role: the survey was in English and the accompanying mail was in Italian when sent to Italian people, and in English when addressed to foreign recipients.

Monitoring marine ecosystems is expensive and time-consuming: despite technology innovation and developing biodiversity information facilities, collating together information on species occurrence is still complicated (Di Camillo *et al.*, 2018). Citizen Science projects are a very promising source of scientific data, especially when they are based on robust, effective and easy to apply protocols and collected records are appropriately validated. The downside of the CS approach is that the retrieved information is limited in terms of spatial extent by the bathymetric limits for recreational SCUBA diving, and in terms of precision

by the lack of affordable and reliable GPS devices to trace underwater position (Sini *et al.*, 2017; Ponti, Turicchia and Costantini, 2019).

The main limits of retrieving species distribution data from scientific literature depend on the tendency of most authors to not share raw data and adopt clear and standardized coordinate systems (Di Camillo *et al.*, 2018). Of course, this matter is amplified when collecting data from the World Wide Web, since data providers are in fact not aware that they are providing information potentially useful for scientific research and they rarely share the precise position of their observations.

While bearing in mind the bias of our distribution dataset, it is unlikely that *S. savaglia* could be present in the South-eastern Mediterranean, at least at depths within the reach of SCUBA divers: the water temperature should be too high. At higher depths, substrate would probably represent a limiting factor, since it appears that the Levantine basin is mostly characterized by soft substrates (Bianchi *et al.*, 2012).

Comparing the distribution of *S. savaglia* with environmental parameters, two areas with distinctive characteristics strike our attention: the Sea of Marmara and the Bay of Kotor, in Montenegro. In the Sea of Marmara, physical characteristics are strongly influenced by the peculiar hydrology of the basin, with two water fluxes interchanging (Coll *et al.*, 2010; Artuz *et al.*, 2019; Topçu *et al.*, 2019). Dense populations of *S. savaglia* have been reported by multiple sources, associated with even more abundant colonies of gorgonians. Although the benthic environment in this area is generally characterized by stable temperature and salinity, episodes of water mixing can strongly alter local conditions; furthermore, the amount of dissolved oxygen is significantly lower than in the Mediterranean Sea, sometimes reaching hypoxic levels (below 2 mg/m³; Levin *et al.*, 2009). These extreme conditions do not seem

to make the area unsuitable for the growth of *S. savaglia* in general, however Topçu *et al.* (2019) described an event of mass mortality in the Sea of Marmara involving *S. savaglia* as well as the associated gorgonians, linking it to an episodic excessive sedimentation of anthropogenic origin. The authors suggest that a reduction of already low levels of dissolved oxygen may have contributed to mortality, impairing the immune system of these benthic suspension feeders and enabling the overgrowth of opportunistic microorganisms that covered the colonies.

In the Bay of Kotor, the shallower colonies of *S. savaglia* grow, at 10-12 m depth. Such low depths are usually unsuitable for this species, since it cannot tolerate temperatures higher than 22°C, but this area features slightly cooler waters than the open Adriatic Sea, thanks to several freshwater springs and riverine inputs. The freshwater input also affects salinity at the surface, triggering strong variations, whilst at 10-15 m depth salinity is already more stable, ranging from about 34 to 38 psu (Lucic *et al.*, 2012).

Savalia savaglia, according to the distribution dataset collected during this project, appears to be able to adapt to a wide range of environmental conditions, being mostly limited by high temperatures and the type of substrate. Its rarity is probably due to high anthropogenic disturbances, and especially the uncontrolled collection of branches, if not entire colonies, that was carried out in the past decades and still partially continues today (Barrajon Domenech *et al.*, 2008).

The strong association with gorgonians, especially with *Paramuricea clavata*, is another aspect worth considering. Despite recent reports of colonies growing directly on rocks (Ocaña and Brito, 2004; Ocaña Vicente *et al.*, 2007), which should be further investigated, the vast majority of the known *S. savaglia* colonies parasitized and completely engulfed

gorgonian colonies. Knowing this distinctive behaviour, the discovery of areas hosting colonies of *S. savaglia* without gorgonian forests led to some speculations on their origin. Studies on *P. clavata* suggest that individual colonies should reach ages up to 50-100 years (Coma, Pola and Zabala, 2001; Linares *et al.*, 2007). As other gorgonian species, *P. clavata* has low natural mortality rates, but also low recruitment rates: these biological traits make gorgonian forests particularly sensitive on a local scale to anthropogenic pressures, mostly represented by mechanical stress (recreational diving, fishing lines and nets, anchoring), and on a regional scale to mass mortality events triggered by climate change-related storms and thermal anomalies (Ponti, Turicchia and Costantini, 2019). As a result of these disturbances, many gorgonian forests in the Mediterranean are fragmented and considered in strong regression, but an effective protection is limited by a lack of knowledge of their distribution patterns now and even more in the past. Due to their ecological importance and their low resilience, sea fans are worthy of greater attention, and small scale studies on their population structure should be encouraged (Di Camillo *et al.*, 2018).

Given that *S. savaglia* is a parasitic species with a life span of several hundreds of years and it is strongly associated with gorgonians and in particular with *P. clavata*, having a life cycle of several tens of years, the distribution patterns of this zoanthid may serve as an indicator of the distribution of gorgonian forests in the past. Isolated or sparse colonies of *S. savaglia* in areas lacking its potential hosts may suggest that, in fact, gorgonians may have been present and then disappeared owing to the appearance of some stressors or in relation to a natural long-term life cycle. Our database includes some records having these features (*Figure 16*), but much more data should be collected to verify this hypothesis and fully exploit its implications in reconstructing past distribution patterns of gorgonian forests.

A clear understanding of distribution and population dynamics of these sessile invertebrates is essential to elaborate better conservation strategies. At present, *Savalia savaglia* is not effectively protected: less than 20% of its habitats are included in Marine Protected Areas and some of the largest populations, in Croatia and especially in the Sea of Marmara, are left out of the MPA network (*Figure 29*).

Numerous national, regional and international actions have been put in place to enhance the conservation of Mediterranean biodiversity, but their concern is only with marine mammals, sea turtles, some fish and a minority of ‘popular’ invertebrates and plants (Bianchi *et al.*, 2012). Instead of giving priority to popular or commercially exploitable species, protection strategies should follow ecological considerations: ecosystem engineers such as *S. savaglia* and other sessile invertebrates have an essential ecological role in enhancing local biodiversity and in mitigating the effects of climate change and anthropogenic pressures and deserve much more consideration (Cerrano *et al.*, 2010).

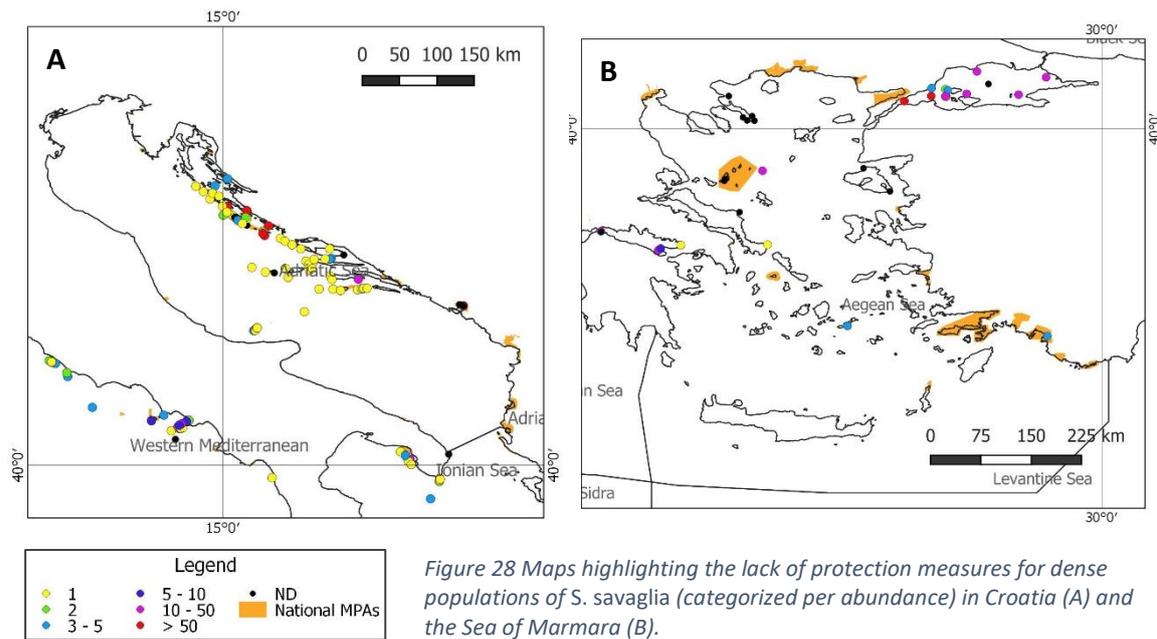


Figure 28 Maps highlighting the lack of protection measures for dense populations of *S. savaglia* (categorized per abundance) in Croatia (A) and the Sea of Marmara (B).

In general, the level of protection in the Mediterranean Sea is unsatisfactory. In 2016, MedPAN reported that 1231 MPAs and OECMs (Other Effective area-based Conservation Measures) in the Mediterranean cover 7,14% of its total surface: the 186 National MPAs only account for 1,6% and the percentage drops to 0,04% if we consider no-go, no-take or no-fishing zones. For the majority of sites, little is known about the management measures in place or about their effectiveness in protecting local biodiversity. In fact, almost 10% of European waters are covered mostly due to the Natura 2000 at sea network, which rarely affords strict restrictive measures. Over 72.77% of the protected surface is located in the Western Mediterranean, leaving the Central-eastern Mediterranean coasts essentially unprotected despite being considered “areas of conservation concern” (Di Camillo *et al.*, 2018).

The updated distribution of *Savalia savaglia* wants to be a useful tool to plan future monitoring activities, since in some locations its presence is reported but essential data on

population depth and density and associated diversity is still scarce; it should also serve as a warning flag of the lack of consistent protection measures in dense populated areas.

5.2. Distribution models

The AUC ranges between 0 and 1, with values above 0.9 indicating excellent predictions, from 0.7 to 0.9 indicating good predictions, between 0.5 and 0.7 indicating poor predictions and below 0.5 indicating predictions no better than random (Hosmer and Lemeshow, 2000). Based on this criterion, MaxEnt and RF models scored ‘excellent’, while LR scored ‘good’. Looking at the parameters of sensitivity and specificity, Random Forest performed better in the former, while MaxEnt scored higher in the latter: as mentioned in Section 3.2, however, when evaluating presence-only models, specificity is not a reliable performance, because it is measured on background data and not real-absence data. All this considered, Random Forest appears to be the model that best fits our dataset.

It is clear, however, that a useful model should fit the data well but other considerations, such as interpretability and scientific justification, are also important. Looking at the maps, all models identify as suitable habitats for *S. savaglia* areas that could hardly host this organism, like the Gulf of Gabès and the northern African coasts in general, characterized by high and strongly variable water temperatures. LR classifies the Italian coasts of the Adriatic Sea as potential areas of occurrence of *S. savaglia*, when this sector of the basin is notably unsuitable for similar organisms because of low depths, wide temperature ranges, and soft substrates.

Neither of the models seems to have correctly interpreted the effect of substrate, as the predicted distribution should be restricted to areas characterized by rocky substrates and all models include vast areas with a soft substrate. This wrong interpretation is mainly due to

the scarce resolution at which the models performed. Each 7 km² cell size of the substrate layer reported only the substrate type that occupies the largest surface, meaning that patchy rocky areas surrounded by large areas with soft substrates, or rocky walls with high slope and low horizontal extension, completely disappeared from the layer. Although the availability of high-quality environmental datasets is increasing day by day, the resolution is still a difficult issue when applying modelling to marine ecosystems (Reiss *et al.*, 2015). Even if high-resolution environmental layers did exist, however, most commercial computers lack the necessary analytic power to work with such heavy data. In short, a technological improvement is still required before optimal predictions from SDMs can be expected, at least at such a large scale.

In general, based on what is known of the ecology of *S. savaglia* and the diversity of specific regions of the Mediterranean, Random Forest is confirmed as the model giving the best prediction for the distribution of this species. A great weakness in this model is that it does not provide information on the quantitative contribution of each covariate to the prediction: as a consequence, it is hard to verify if the model interpreted well the influence of the environmental variables on the distribution of *S. savaglia*. This problem characterizes many SDMs and is the greatest obstacle to a clear understanding of their learning processes and to an informed interpretation of the outputs (Elith and Graham, 2009; Phillips *et al.*, 2017). The response curves of environmental variables may give at least an idea of how each variable affected the prediction of the RF model: they show trends either coherent with what is known of the ecology of *S. savaglia*, or in any case logical in the context of a species distribution. All response curves are available in the Supplementary material SC.

LR shows the worst results judging by both performance measures and distribution patterns. The explanation probably lies in the core function of this model: binary logistic regression is an extension of linear regression where the dependent variable is categorical and not continuous – in other words, the outcome can have only two possible values, “Presence” or “Absence”. The model predicts the effects of covariates on the dependent variable and estimates the probability that the outcome is “Presence” against the probability that it is “absence”. To obtain a good prediction from this type of model, a presence-absence dataset is needed (Phillips *et al.*, 2009). The response curves of our LR model clearly show how the model tried to fit its predictions into two discrete responses, rather than into a percentage scale of probability: they all display a linear trend or a sigmoid curve. Adapting LR to presence-only biological data is rather common, but the performance of the model is extremely variable, depending on the quality of the biological sampling, the number and distribution of pseudo-absence points, the type of response enhanced by environmental data. The matter of biological sampling bias is central to SDM in marine ecosystems (Phillips *et al.*, 2009). Biological sampling bias or survey bias is the tendency of the occurrence data, used as training and testing data, to exhibit strong spatial bias in survey effort: some sites are more likely to be sampled than others, so that they only give a partial vision of the potential suitable habitats of the target species. Usually, in marine ecosystems biological data are biased towards coastal and shallow areas (Martin *et al.*, 2014), especially if they are of political, social and economic importance (Reiss *et al.*, 2015). The impact of survey bias is limited if both presence and absence records present the same biased distribution. In presence-only datasets, mitigating the effects of this bias is harder. The model needs information on the range of environmental conditions present in the sampling area, including those of points where the target species was not recorded: this information can be provided

by background points, or pseudo-absence data. The explicit function of pseudo-absence data is, in fact, to provide a sample of the set of conditions available in a region and not to pretend that the species is absent in that specific point. If a spatially biased sample proportionately covered the full range of environments in the region, then it would cause no problem in a model based on environmental data. However, this is usually not the case (Phillips *et al.*, 2009).

A Habitat Suitability model for *S. savaglia* was already performed by Giusti, Innocenti and Canese (2014), but at a much lower spatial scale: the study areas, located at the northern border of the Messinian Strait, covered an overall surface of 6,5 km². The authors applied the Ecological Niche Factor Analysis (ENFA), based on the idea that the ecological niche of a species is a hypervolume in the multidimensional space, each dimension defined by an environmental variable. The considered predictors, acquired at high resolution by means of multibeam echo sounder and ROV transects, were all Eco-Geographical Variables: slope, aspect, curvature, plan curvature and profile curvature were calculated from the bathymetry. This model approach provides good performance in the prediction, allowing to take into consideration important factors acting at a local scale, such as hydrodynamic conditions: however, it cannot be applied at a regional scale, where the coarse resolution of available data leads to the flattening of environmental variability.

Our model can be considered as a first step in understanding the distribution patterns of *S. savaglia* at a regional scale, and a useful tool to assess which areas deserve priority both in field monitoring and in Suitable Habitat modelling at local scale.

Further research henceforth will focus firstly on collecting a presence-absence dataset with the aim to correct the biological sampling bias of presence-only models. The performance of

our SDM could be then ameliorated by merging the outputs of different types of presence-absence models. Another interesting approach would be to build a co-occurrence model of *S. savaglia* with *Paramuricea clavata*, or gorgonians in general, seeing that they are strongly associated (Bell, 1891; Zibrowius, 1985): at present, there are only few methods available to include species interactions in SDM, although their role in defining the ecological niche of species is considered to be fundamental (Reiss *et al.*, 2015). Moreover, we plan on applying environmental layers for future climate scenarios to estimate the potential effects of climate change on the distribution of *S. savaglia*; some future layers for 2040-2050 and 2090-2100 are already available from the Bio-Oracle database (Assis *et al.*, 2018). Predictive modelling provides valuable insights for sustainable long-term management of marine ecosystems. Finally, it is also planned to estimate the past distribution of gorgonian forests applying SDM to historical data, in order to ascertain our hypothesis that isolated or sparse colonies of *S. savaglia* far from gorgonian forests may act as indicators that gorgonian forests were once also present and at some point they disappeared.

5.3. Embedded sediments

All the examined populations contained abundant siliceous materials, even though some are situated in a geologic context with a prevalence of rocks of carbonate nature (for instance Marmara, Montenegro and Gallipoli). This may suggest that a certain selection of incorporated sediments towards siliceous particles occurs in *Savalia savaglia*, as it was observed for the sponge *Chondrosia reniformis* (Bavestrello *et al.*, 1995; Cattaneo-Vietti *et al.*, 2004; Cerrano *et al.*, 2007; Giovine *et al.*, 2013). *S. savaglia* also appears to select sediments based on their dimension, favouring particles sized 10 to 50 μm and it is a

behaviour common to several zoanthid species (Herberts, 1972). Other factors, however, may play a role in this mechanism and there might be temporal variability; this matter should be further investigated with monthly samplings of both *S. savaglia* tissues and *in situ* suspended sediments, via sediment traps, so that a direct comparison can be made. How *S. savaglia* incorporates foreign material is still uncertain: sediment may enter through transepithelial assimilation, as seen for *Palythoa* sp. by Haywick and Mueller (1997). In terms of abundance of embedded sediments, variability is extremely high: it is hard to trace a pattern, since, again, many factors may affect this process, either environmental - such as the presence of obstacles to direct input of sediments, meteoric events, wave motion, water circulation – ecological – the surrounding benthic assemblage, the population density, disturbances from predators or epibionts – or biological – the age and sex of the colony, the reproductive stage, its state of health.

In general, however, at least in terms of sediment composition, colonies from different populations and sites can be relatively well characterized; it may be interesting to examine, seasonal variations of sediment composition likely affected by flood events and resuspension processes, as demonstrated for *Chondrosia reniformis* (Cerrano *et al.*, 1999).

Regarding the purpose of this process, the possibilities are various: some animals incorporate foreign material to make themselves less palatable to their predators (e.g. some sponge-feeding nudibranchs retain and utilize their spicules for self-defence, according to Fuhrman *et al.*, 1979), others do so to strengthen their structure against currents and mechanical disturbances (Haywick and Mueller, 1997). In this study we tested the hypothesis that *S. savaglia* incorporates and etches Siliceous particles to increase the synthesis of collagen (see

Section 1.6.1), hypothesis that found confirmation for *Chondrosia reniformis* (Bavestrello *et al.*, 1995; Cattaneo-Vietti *et al.*, 2004; Cerrano *et al.*, 2007).

5.4. Correlation between collagen abundance and embedded sediments

The amount of collagen in the tissues of *Savalia savaglia* was quantified in samples from three populations, Ischia, Gallipoli and Montenegro, and the results were compared with the data related to the incorporated sand and spicules (*Figure 30*).

Sand abundance does not appear to be correlated with collagen content, seeing that colonies from Gallipoli are by far the poorest in sand (13121 particles/mg dw tissue against 44892 in Montenegro and 54648 in Ischia) and the richest in collagen in terms of both ww tissue (1,782 µg/mg ww tissue against 0,279 Ischia, and 0,219 in Montenegro) and total proteins (6,11% of total proteins against 0,45% in Ischia and 0,69% in Montenegro). Looking at the amount of spicules, samples from Ischia have very few (meanly 596 per mg dw tissue), those from Gallipoli are the richest (14630 per mg dw tissue) and specimens from Montenegro have a medium amount (6387 spicules/mg dw tissue). The high number of spicules found in Gallipoli may suggest that they have a role in promoting the synthesis of collagen, but if that were the case, the trend of collagen abundance in the three sites should reflect that of the amount of spicules: instead, collagen content in Ischia and Montenegro are comparable.

The spicule in *Figure 23E*, consequently, was probably etched before *S. savaglia* incorporated it, perhaps by a sponge. Granulometry as well appears to be irrelevant in this matter, being rather consistent in all three samples.

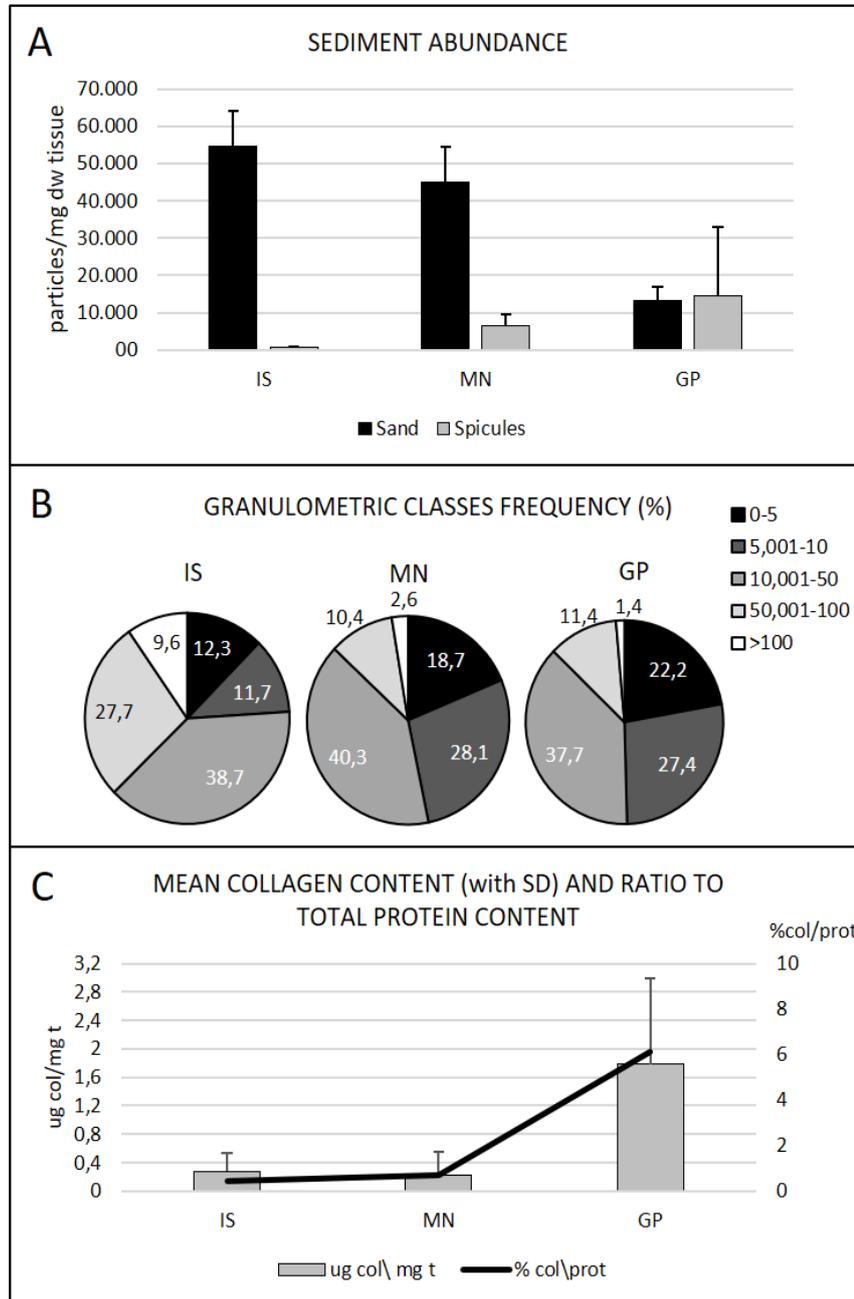


Figure 29 (A) Average sand and spicule abundance and (B) granulometry, and (C) collagen abundance in samples from Ischia, Montenegro and Portofino.

Conversely, the composition of incorporated foreign material seems to be the key to corroborate our hypothesis: the spectra obtained by means of EDS and X-Ray analysis reveal

that, whereas samples from Ischia and Montenegro embedded sediments of various composition, Gallipoli contains a considerable amount of quartz crystals and calcium oxalate hydrate (*Figures 21, 22*). These results, integrated with the SEM images of strongly etched mineral particles, suggest that collagen production in samples from Gallipoli is enhanced by the partial dissolution of assimilated quartz crystals.

Calcium oxalate is one of the metabolic end products of ascorbic acid (the main reactant in proline hydroxylation during collagen synthesis), spontaneously generated when the latter is released in an alkaline environment such as sea water. Bavestrello et al. (1995) reported that ascorbic acid is able to partially dissolve the surface of quartz, highly increasing the concentration of soluble silica in the surrounding medium. Krasko *et al.* (2000) demonstrated that high silica concentration stimulates the collagen gene expression in the sponge *Suberites domuncula* Olivi, 1792 and this mechanism was later confirmed in *Chondrosia reniformis*. Looking at the results of this study, a similar process may also occur in *S. savaglia*; the amount of both embedded sediments and collagen in its tissues, however, is much smaller than what is observed in these sponge species, especially since *S. savaglia* can rely on its skeleton for body support, while *C. reniformis* cannot secrete spicules and has to build up a thick collagenous ectosome to gain stiffness. *In vivo* experiments should be performed to better understand this process in *Savalia savaglia*.

It is plausible that other factors influence the levels of collagen production in *S. savaglia* specimens, as clearly demonstrated by the observed inter-population variability. Biological features, such as sex of the colony, reproductive stage and particularly age, should play a key role. Increasing the number of samples and doing replicates of the analyses would allow

to better assess this variability and these data, correlated with other biological data, may suggest which features influence the production of collagen the most.

5.5. Cnidome

5.5.1. Inter-population variability

The cnidome variability between colonies and populations of *Savalia savaglia* is little known: only two papers described the complete cnidome of *S. savaglia* specimens, collected in Ría de Arousa (Atlantic coast of Spain) (Altuna, Sinniger and Aldrey, 2010), Ceuta and Banyuls Sur Mer (Ocaña and Brito, 2004). These two studies already show some minor differences: atrichs and a class of big basitrichs (the latter only present in the mesenteries) only appear in the Galician specimens, and differences in size and abundance of some categories are also present (Altuna, Sinniger and Aldrey, 2010). In general, Ocaña and Brito (2004) found less variety of categories within each tissue. Our results, while coherent with those found in literature, also show high inter-population variability in terms of both nematocyst categories and capsule size. *Figure 31* shows the variability between the surveyed populations in terms of abundance class.

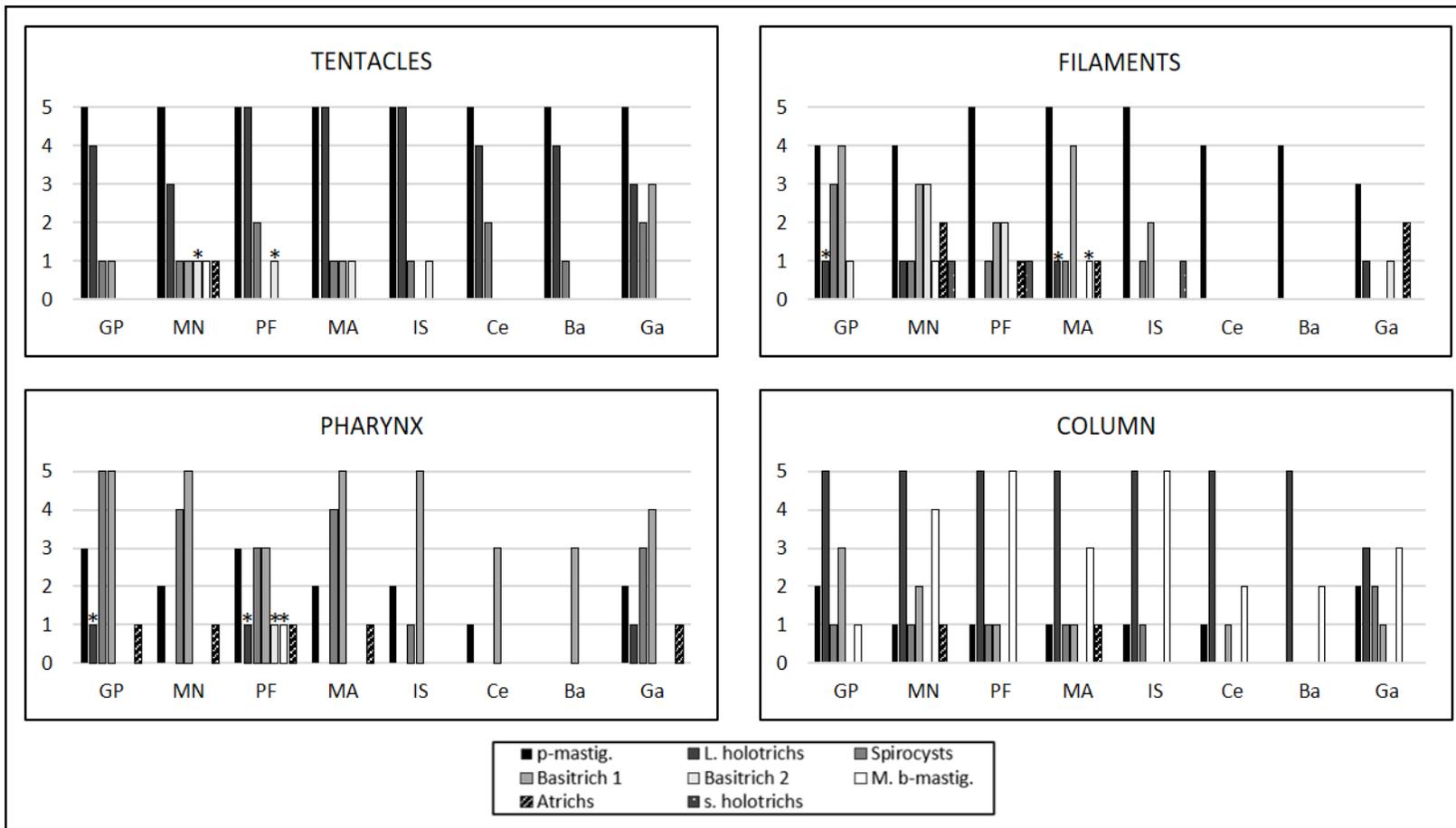


Figure 30 Comparison between the cnidome set of all the surveyed populations of *S. savaglia* in terms of abundance class of a cnidocyst type in each body part. Ce, Ceuta; Ba, Banyuls; Ga, Galicia. * indicates plausible contamination. Data on the cnidome of populations from Ceuta and Banyuls and from Galicia were retrieved from Ocana and Brito (2004) and Altuna, Sinniger and Aldrey (2010), respectively.

Atrichs: this category is not observed by Ocana and Brito (2004) in specimens from Ceuta and Banyuls but is present in the pharynx and mesenteries of the Galician specimens (Altuna, Sinniger and Aldrey, 2010). In this study, it results absent in the Ischia population, whereas in the other four populations it is usually observed in the tentacles, pharynx and mesenteric filaments, except for polyps from Portofino which only have it in the pharynx and filaments. The specimens from Montenegro have abundances and sizes comparable with what indicated by Altuna, Sinniger and Aldrey (2010), being larger in tentacles ($25.2 \pm 5 \times 5.3 \pm 0.8 \mu\text{m}$) than in filaments ($19.2 \pm 4.2 \times 4.4 \pm 0.7$) and pharynx ($18.2 \pm 2.5 \times 4.8 \pm 0.6$); in Marmara, Gallipoli and Portofino atrichs appear to be smaller and rarer.

Small holotrichs: this nematocyst type is only mentioned by Ocana and Brito (2004) in relation to the mesenteric filaments, but identification is uncertain and there are no indications on the size or abundance. As for the Galician specimens, they appear to be lacking this category. In this study, small holotrichs are very rarely observed in the mesenteric filaments of polyps from Portofino and Ischia and are not detected in the other sites.

Large holotrichs: these nematocysts are present in great number in polyps from Ceuta and Banyuls, but only in the column (one capsule is observed in the tentacles of a polyp from Ceuta). Altuna, Sinniger and Aldrey (2010) also indicate them in the pharynx and mesenteries, although in such small amounts that the authors suggest a possible contamination from the column. In all the samples studied here, large holotrichs are abundant in the column, while in other tissues are occasionally found in much lesser amounts. In many cases (tentacles in Ischia, pharynx in Portofino and Gallipoli, tentacles and filaments in Marmara) a tissutal contamination is suggested as well, based on the conservation status of

the surveyed polyp and on the frequency of observations in relation to both the total of polyps and the total of colony branches examined. In the tentacles of polyps from Portofino and Montenegro and in the mesenteric filaments of those from Montenegro and Gallipoli, the frequency of records is higher so the category is probably actually present. Seeing the scarce abundance of large holotrichs in these tissues, however, further analyses on more samples should be performed. Regarding the size of this category, in the column it is consistent in all surveyed specimens (17-21 x 8-10 μm on average). On the contrary, large holotrichs in the other tissues are slightly smaller according to Altuna, Sinniger and Aldrey (2010) (8.2 ± 0.2 x 4.1 ± 0.5 μm in the pharynx, 8.8 ± 1 x 3.4 ± 0.4 μm in the mesenteries), slightly larger according to Ocana and Brito (2004) (26 x 10 μm in the tentacles) and are more homogeneous in our samples, except for those from Gallipoli (16 ± 4.2 x 9 ± 1.7 μm in the pharynx, 10.4 ± 1.4 x 4.7 ± 0.9 μm).

p-Mastigophores: this category is present in all the tissues of polyps from Ceuta and Galicia, only in tentacles and mesenteries, where they are always predominant, in those from Banyuls. The capsule dimension in these samples is rather homogeneous (13-17 x 4-5 μm on average), although the p-mastigophores found by Ocana and Brito (2004) in the tentacles are more elongated (18-19 x 4-5 μm). The length range for this nematocyst type, however, is generally quite wide. All the populations examined in this study present p-mastigophores in every tissue, being strongly predominant in the filaments, and their size is homogeneous and comparable with the results offered by Ocana and Brito (2004) and Altuna, Sinniger and Aldrey (2010).

Basitrichs: Ocana and Brito (2004) observed this category in large number in the tentacles and pharynx of polyps from both Ceuta and Banyuls; it is absent in the mesenteries

and found only once in the column of a sample from Ceuta. Altuna, Sinniger and Aldrey (2010) report the presence of basitrichs in all tissues of their samples, being rather abundant in the tentacles and pharynx and rare in column and mesenteric filaments. In this survey, basitrichs are detected in all tissues of polyps from all the sites, except for Ischia where they are not present in the column; abundances in each body part are consistent with data reported by the other authors. Regarding the capsule size, this category shows the greatest variability even within the same tissue of a polyp; it is possible to clearly discern two dimensional classes, here named “basitrich 1” (maximum length always under 30 μm ; on average 17-20 x 3.5-5 μm) and “basitrich 2” (maximum length always exceeding 30 μm ; on average 40-41 x 5.5-6 μm). The latter is present only in the mesenteric filaments of polyps from the populations of Galicia, Gallipoli, Montenegro and Portofino, while lacks in samples from Ischia, Marmara, Ceuta and Banyuls. In contrast with Altuna, Sinniger and Aldrey (2010), who indicate basitrichs 2, but not basitrichs 1 in the filaments of the Galician specimens, in the populations from Gallipoli, Montenegro and Portofino they can both be detected, in large amounts no less.

Microbasic b-mastigophores: this category is detected in average amounts in the column of specimens from Ceuta and Banyuls, with a roundly shaped capsule. In the Galician population, these cnidae are only present in the column as well, but their capsule is more oblong. In this study, the specimens from Gallipoli, Marmara, Montenegro and Portofino present roundly shaped microbasic b-mastigophores, while these nematocysts in polyps from Ischia are more similar to those found in Galicia in terms of shape and size ($18 \pm 1.8 \times 12.6 \pm 0.9 \mu\text{m}$ those from Ischia, $16.8 \pm 1.2 \times 10.3 \pm 1 \mu\text{m}$ those from Galicia). This type of cnida,

albeit in much lower amounts and reduced size, is frequently found in the tentacles ($10.2 \pm 1.3 \times 9.3 \pm 1.8 \mu\text{m}$) and filaments ($10.6 \pm 2.4 \times 8.0 \pm 1.9 \mu\text{m}$) of polyps from Montenegro.

Spirocysts: as opposed to Ocana and Brito (2004), that place this typology only in the tentacles, and to Altuna, Sinniger and Aldrey (2010), who find it in tentacles and, in smaller quantities, in the pharynx of their samples, in our survey spirocysts are detected in all body parts for most of our samples, no population excluded. The cnida, however, is confirmed to be predominant in the tentacles in all the examined populations. Its size is rather homogeneous in all body parts and populations.

Although an inventory of the cnidome, with their distribution and size, is an essential component of most taxonomic descriptions, its diagnostic value at lower taxonomic levels is variable, seeming to differ with taxon. Complicating the characterization of species through their cnidome are the facts that not all members of a species may have the same types of cnidae, even at the same life-cycle stage, and size of nematocysts of a species may vary geographically and with size of individual. Even within an individual, size of nematocysts of a particular type from a single tissue varies (Fautin, 2009). It is likely that nematocyst complement and size reflect both phylogeny and biology and this may complicate the interpretation of data. Several studies investigated which factors may affect the typology and size of cnidae in specific organisms, as reviewed by Fautin (2009). Some of these factors are the size of the organism, its life stage, its dietary choices, adaptation to environmental conditions and ecology.

The results of this study show a strong variability in anatomical location, abundance and size of cnidae types among different populations of *Savalia savaglia*. As Ocaña and Brito (2004) point out commenting their own results, cnidome variations between colonies from different

localities could simply be attributed to the high morphological variability found in all zoanthid species. A comprehensive investigation in more location and with periodical samplings could assess whether ecological features (e.g. abundance of preys and their seasonal availability, presence of predators, competition for space) have a role in the cnidome development.

A general description of the cnidome of the species, however, is here presented as a preliminary assessment, hoping on one hand that it can facilitate its identification based on morphological features, and on the other hand that further studies can verify which factors determine the development of a specific cnidome in *S. savaglia* specimens.

The comparison between populations of *S. savaglia* offers interesting reflections on how environmental, biological and genetic features shape the cnidome of individuals of *S. savaglia*. Further observations on more populations, preferably performed on fresh polyps, should be made to better define consistent characteristics of the cnidome and to elaborate hypotheses on the factors influencing it.

5.5.2. *Hypotheses on the function of different categories*

The functional classification of nematocysts generally includes prey capture (penetrant cnidae) defense (volvent cnidae), and adherence (glutinants). Mariscal (1974) showed that nematocysts of some types must function in both offense and defense, especially seeing that some cnidarians (such as sea anemones) usually have a limited variety of nematocyst types. In suggesting the possible function of certain cnidae types, their anatomical location and response to specific stimuli must be taken into account as well as their structure, because according to Fautin (2009) it is uncertain whether morphologically similar nematocysts have similar functions in different species or even in different parts of one animal.

Having pointed out these concerns on the topic of nematocysts function, following Mariscal's argument for other anthozoan species, we can hypothesize the functional role of the nematocyst types present in *Savalia savaglia*. Spirocysts, being glutinant cnidae mostly present in the tentacles, may have a role in the capture of preys. Basitrichs, abundant in the tentacles and pharynx, and p-mastigophores, may then deliver venom to the preys with their penetrating tubules. Large holotrichs and microbasic b-mastigophores, located mainly in the column, are likely to have a defensive role. Further studies on the biology and response to stimuli of *S. savaglia* nematocysts is needed to ascertain these hypotheses.

6. Conclusions

Despite its known fundamental role in structuring benthic habitats on long temporal scales, and despite being included as threatened species in the Barcelona and Berne Conventions, *Savalia savaglia* is still very understudied, both in terms of ecology and distribution and in terms of its biology. This generalized lack of knowledge about this species prevents the introduction of effective measures to guarantee the conservation of *S. savaglia* itself as well as the habitat it creates. Species distribution maps and, in recent years, predictive models are more and more frequently used in Marine Spatial Planning and conservation management. Although species distribution models will never be able to replace actual monitoring programmes, they can help directing monitoring efforts to most suitable areas, thus saving time and money. Our model fits well in this role, showing at a regional scale what should be monitored first to increase our knowledge on *Savalia savaglia*.

This study also wanted to assess whether different populations of this species can be characterized based on morphological or biological features: our findings confirm this hypothesis, while also highlighting insights for future research. The populations of *S. savaglia* we considered show strong variability in their cnidome, not only between individuals of different populations, but also, to a lesser extent, between specimens of a single population. These results should encourage further investigations on which ecological features locally steer the development of a specific set of cnidae and why. Moreover, the foreign inorganic material embedded in the tissues of *S. savaglia*, although homogeneously selected based on granulometry by all the investigated populations, shows variability in its composition, with a clear link to the specific lithology of each sampling site. We analysed more deeply this biological mechanism in three populations of *S. savaglia*, finding that these

also differ in the amount of collagen: the abundance of collagen in the tissues of our samples is positively correlated with the abundance of crystalline forms of silica, suggesting a mechanism similar to that observed in the sponge *Chondrosia reniformis*. More analyses on different populations and *in vivo* experiments should be performed to better describe this biological feature.

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SUPPLEMENTARY MATERIAL

SA-Occurrence data collection

Table S1 Sources of occurrences from scientific literature

	Citation
Coppo, S., Diviaco, G., Tunesi, L., 2009. Environmental and conservation relevance of the Punta Manara coralligenous beds (Eastern Ligurian Sea). p. 75-81. In: Proceedings of the 1st Symposium on the Coralligenous and other calcareous bioconcretions of the Mediterranean Sea, Tabarka, 15-16 January 2009	Coppo et al., 2009
Kružić, P., 2007. Anthozoan fauna of Telašćica nature park (Adriatic Sea, Croatia). <i>Natura Croatia</i> , 16 (4), 233-266.	Kružić et al., 2007
Pardo, E., Aguilar, R., García, S., de la Torriente, A., Ubero, J., 2011. Documentación de arrecifes de corales de agua fría en el Mediterráneo occidental (Mar de Alborán). <i>Chronica naturae</i> , 1, 20-34.	Pardo et al., 1987
Gili, J.M., Pagès, F., Barangé, M., 1987. Zoantarios (Cnidaria, Anthozoa) de la costa y de la plataforma continental catalanas (Mediterráneo occidental). <i>Miscellanea Zoologica</i> , 11, 13-24.	Gili et al., 1987
Salomidi, M., Smith, C., Katsanevakis, S., Panayotidis, P., Papathanassiou, V, 2009. Some observations on the structure and distribution of gorgonian assemblages in the eastern Mediterranean Sea. In : Actes du 1er Symposium sur la Conservation du Coralligene et autres bio-concretions de Mediterranee, Tabarka, 16-17 Janvier 2009, pp. 242-245.	Salomidi et al., 2009
Pais, A., Trainito, E., Romor, M., Contis, M.G., 1992. Sulla presenza di <i>Gerardia savaglia</i> (Bertoloni, 1819) nelle acque dell'isola di Tavolara (Sardegna nord-orientale). <i>Oebalia</i> , suppl. XVII, 377-378.	Pais et al., , 1992
Salomidi, M., Zibrowius, H., Issaris, Y., Milionis, K., 2010. <i>Dendrophyllia</i> in Greek waters, Mediterranean Sea, with the first record of <i>D. ramea</i> (Cnidaria, Scleractinia) from the area. <i>Mediterranean Marine Science</i> , 11 (1), 189-194.	Salomidi et al., 2010
Artüz, M.İ., Artüz, M, Artüz, O.B., 1990. Mercan Türlerine Getirilen Yasaklar İle İlgili Görüşler. T.C. Çevre Bakanlığı Raporu K. K. G. M. Su Ürünleri Sirküleri Düzenlemeleri 1990.	Artüz et al., 1990
Bell, F.J., 1891. Contributions of our knowledge of Antipatharian corals. <i>Transactions of the Zoological Society of London</i> , 13, 141-142.	Bell et al., 1891
Cerrano, C., Bavestrello, G., Palma, M., Previati, M., Schiaparelli, S., 2007. Una popolazione di <i>Gerardia savaglia</i> (Bertoloni, 1819) nell'area marina protetta di Portofino. <i>Biologia Marina Mediterranea</i> , 14 (2), 156-157.	Cerrano et al., 2007
Cerrano, C., Previati, M., Palma, M., Arillo, A., 2006. Distribuzione di <i>Gerardia savaglia</i> (Bertoloni, 1819) (Cnidaria, Zoanthidea) nell'Area Marina Protetta di Portofino. <i>Biologia Marina Mediterranea</i> , 13, 164-165.	Cerrano et al.,2006

Cossu, A., Pascucci, V., Chessa, L.A., Andreucci, S., Deluca, M., et al., 2011. Caratterizzazione fisiografica, geomorfologica e bionomica della rias di Santa Teresa di Gallura (Sardegna nord orientale). <i>Biologia Marina Mediterranea</i> , 18 (1), 305-306	Cossu et al., 2011
Oceana, 2010b. Seamounts of The Balearic Islands. Proposal for a Marine Protected Area in the Mallorca Channel (Western Mediterranean). 62 pp.	Oceana, 2010
Zibrowius, H., 1985b. Comportement agressif du Zoanthaire <i>Gerardia savaglia</i> contre le Gorgonaire <i>Paramuricea clavata</i> (Cnidaria: Anthozoa). Rapports et Procès-verbaux des Reunions. Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée, 29, 351–353.	Zibrowius et al., 1985
Ocaña, O., Brito, A., 2004. A review of <i>Gerardiidae</i> (Anthozoa: Zoantharia) from Macaronesian Islands and the Mediterranean sea with the description of a new species. <i>Revista de la Academia Canaria de Ciencias</i> , 15, 159-189.	Ocaña et al., 2004
Öztürk, B., & Bourguet, J.P., 1990. Données préliminaires sur le corail noir de la Mer de Marmara (Turquie) <i>Gerardia savaglia</i> (Bertolini, 1819). <i>Istanbul University Journal of Aquatic Products</i> , 4 (2), 45-48.	Öztürk et al., 1990
Cristo, B. & Pais, A., 1997. Nuove segnalazioni di <i>Gerardia savaglia</i> (Bertolini, 1819) in Sardegna. <i>Civiltà del mare</i> , 1, 34-36.	Cristo et al., 1997
Cristo, B., 2003. Contributo alla conoscenza della distribuzione di <i>Gerardia savaglia</i> (Anthozoa: Zoantharia) lungo le coste della Sardegna. <i>Biologia Marina Mediterranea</i> , 10 (2), 544-546.	Cristo et al., 2003
Bussotti, S., Buia, M.C., Di Capua, I., Gambi, M.C., Loreti, M., et al., 1999. Preliminary biocenotic characterization of the protected area "Banco Santa Croce" (Gulf of Naples, Italy). <i>Biologia Marina Mediterranea</i> , 6 (1), 133-135.	Bussotti et al., 1999
Arena, P. & Li Greci, F.L., 1973. Indagine sulle condizioni faunistiche e sui rendimenti di pesca dei fondali batiali della Sicilia occidentale e della bordura settentrionale dei banchi della soglia Siculo-Tunisina. <i>Quaderni del Laboratorio di Tecnologia della Pesca</i> , 1 (5), 157-201.	Arena et al., 1973
Schmidt, H., 1972. Bionomische Studien an mediterranen Anthozoen: die Anthozoenfauna des Strombolicchio (Äolische Inseln). <i>Marine Biology</i> , 15 (3), 265-278.	Schmidt, 1972
Laubier L. & J. Theodor, 1967. Sur la présence à Banyuls-sur-mer du zoanthaire <i>Gerardia savaglia</i> (Bertolini). <i>Vie Milieu, (Sér. A)</i> 18 (1): 223-225.	Laubier et al., 1967
Enrichetti, F., Bava, S., Bavestrello, G., Betti, F., Lanteri, L., Bo, M., 2019. Artisanal fishing impact on deep coralligenous animal forests: A Mediterranean case study of marine vulnerability. <i>Ocean & Coastal Management</i> , 177: 112-126.	Enrichetti et al., 2019
De la Torriente A, González-Irusta JM, Aguilar R, Fernández-Salas LM, Punzón A, Serrano A. Benthic habitat modelling and mapping as a conservation tool for marine protected areas: A seamount in the western Mediterranean. <i>Aquatic Conserv: Mar Freshw Ecosyst</i> . 2019; 1-19.	De la Torriente et al., 2019
Aguilar R, Garcia S, Perry AL, Alvarez H, Blanco J, Chimienti G, Montesanto F, Mastrototaro F. Deep-Sea Habitats and communities in the Aeolian Islands (North Sicily). 2 nd Mediterranean Symposium on the conservation of Dark Habitats (Antalya, Turkey, 16 January 2019).	Aguilar et al., 2019
Macic V, Trainito E, Torchia G, Some peculiarities of the Hexacorallia assemblages along the montenegrin coast. <i>Proceedings of the 3rd Mediterranean Symposium on the conservation of the Coralligenous and other bio-concretions</i> (Antalya, Turkey, 15-16 January 2019)	Macic et al., 2019

Alvarez H, Perry AL, Blanco J, Garcia S, Aguilar R. 2019. Towards the creation of a marine protected area in the Aeolian Islands. Results of the 2018 Aeolian Expedition. Oceana, Madrid.	Alvarez et al., 2019
Project MedKey Habitats - Algérie : Île de Rachgoun Cartographie des habitats marins clés de Méditerranée et initiation de réseaux de surveillance	Rac/Spa, Algérie 2016
RAC/SPA - UNEP/MAP, 2011. Rapid assessment survey of coastal habitats to help prioritize the suitable new areas needing a status of protection for the development of a network of Marine and Coastal Protected Areas in Montenegro. By Badalamenti F., Garcia Charton J.A., Treviño-Otón J., Mačić V., and Cebrian D. Ed. RAC/SPA - MedMPAnet Project, Tunis: 52 p + Annexes.	Rac/Spa, Montenegro 2014
CAR/ASP - PNUE/PAM, 2012. Le Cap des Trois Fourches (Méditerranée, Maroc): caractérisation écologique et orientations de gestion. Par Bazairi H. Limam A., Benhoussa A., Mellouli M., El Khalidi K., Navarro-Barranco C., González A.R., Maestre M., García-Gómez J.C. et Espinosa. F. Ed. CAR/ASP - Projet MedMPAnet, Tunis: 122 p + Annexes.	Rac/Spa, Morocco 2012
Rouveyrol, P.N. (2019). Données d'occurrence Espèces issues de la base Natura 2000 : espèces d'intérêt communautaire et autres espèces remarquables renseignées dans les FSD. Version 1.1. UMS PatriNat (OFB-CNRS-MNHN), Paris.	Rouveyrol, PN, 2019
Vafidis, D., Koukouras, A. (1998). Antipatharia, Ceriantharia and Zoantharia (Hexacorallia Anthozoa) of the Aegean Sea with a check list of the Mediterranean and Black Sea species. Annales de l'Institut Oceanographique, Paris, 74 (2), 115-126.	Vafidis & Koukouras, 1998
Pardo, E. et al. (2011). Documentación de arrecifes de corales de agua fría en el Mediterráneo occidental (Mar de Alborán), <i>Chronica naturae</i> , (1), pp. 20–34.	Pardo et al., 2011
Salomidi, M. et al. (2010). Dendrophyllia in Greek waters, Mediterranean Sea, with the first record of <i>D. ramea</i> (Cnidaria, Scleractinia) from the area. <i>Mediterranean Marine Science</i> , 11- 1, 189-194.	Salomidi et al., 2010
Meinesz, A. (1990). Présence de Zoanthaire <i>Gerardia savaglia</i> dans la réserve naturelle des îles Lavezzi. <i>Travaux scientifiques du Parc Naturel Régional et des Réserves Naturelles de Corse</i> , 27: 29-35	Meinesz, A., 1990

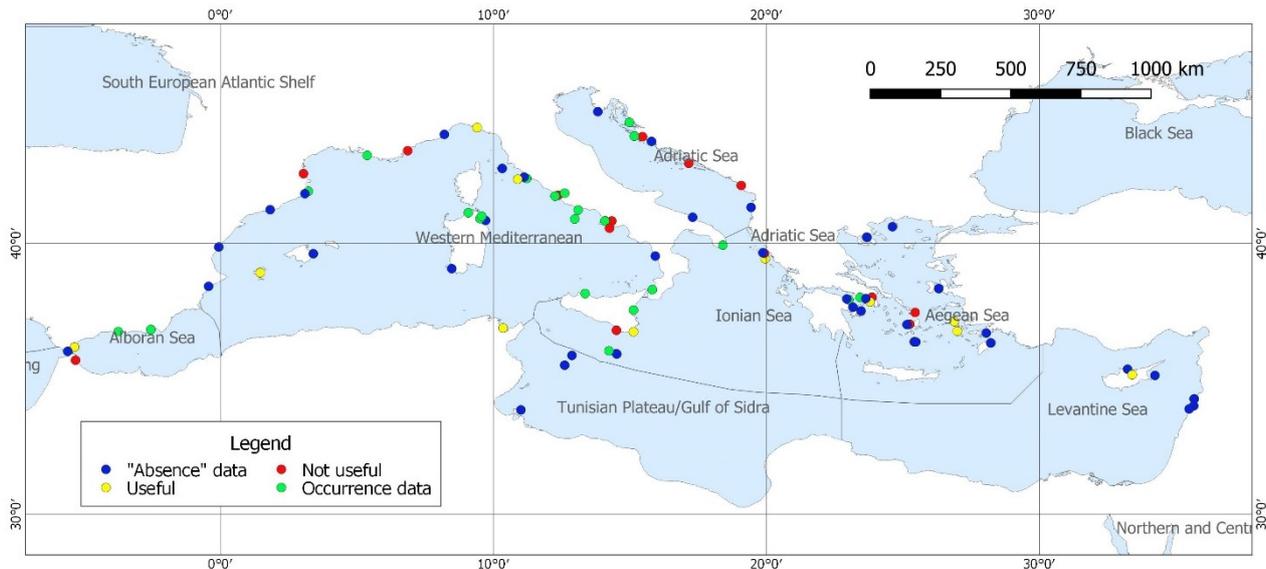


Figure S1 Map of the Diving Centres which answered the email. Answers are classified as such: "Absence" data, the owner said *S. savaglia* is not present in the diving sites they go to; Useful, the owner didn't give a presence or absence record, but helped by sharing the survey or by providing useful contacts; Not useful, the owner answered, but provided neither data nor other resources; Occurrence data, the owner provided records of *S. savaglia* directly via email or by filling the survey. Although some absence data were collected from Diving centres operators, it was impossible to validate these records, considering the risk of the species not being recognised; moreover, these absence data were too scarce compared to the collected presence data. For these reasons these records were not used to build a presence-absence SDM, but they may be validated and applied in distribution modelling in the next phase of this project.

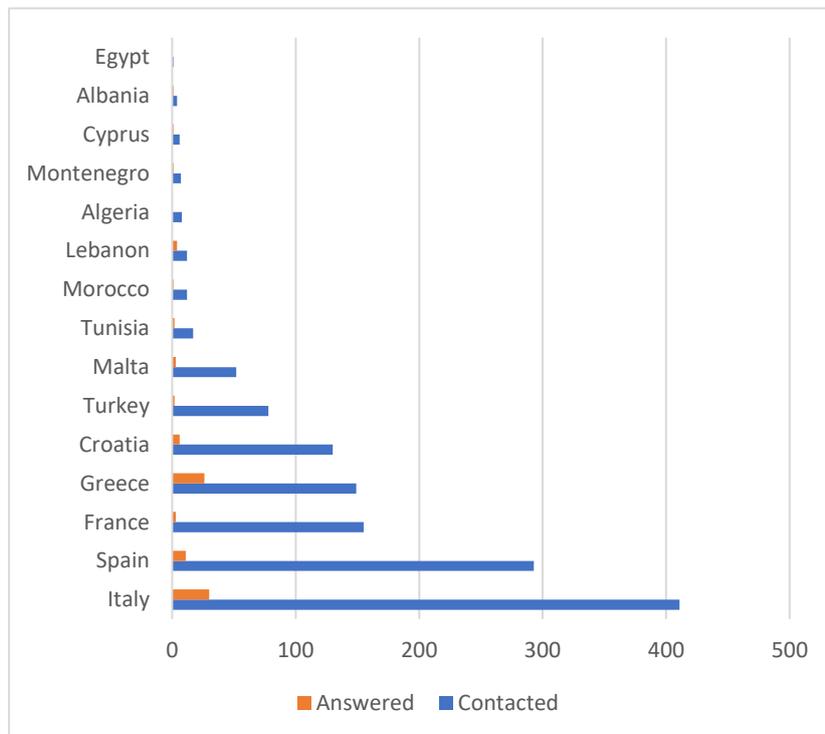


Figure S2 Number of Diving Centres operators contacted via mail or other means of communication and of those who answered for each country.

Have you seen this species?

Cecilia Varotti <cecilia.marbio@gmail.com>

A: Posidonia Ecosports <info@posidoniecosports.com>

Hello,

my name is Cecilia and I'm an italian scuba diver and marine biology researcher; I'm trying to collect information on the distribution of a marine species in the Mediterranean, and you might help me!

The species I'm working on is *Savalia savaglia*: its common name is falso coral negro. I attached a photograph.

Have you ever seen this species during your dives in Spain? Could you fill out this survey about it? Here's the link:

<https://forms.gle/eMAPqNktULNzBqYM7>

Also, if you have any additional information about it, please feel free to write me an email! Thank you very much for your help!!

Cecilia Varotti

2 allegati



Savalia M. Di Francesco.jpg
26K



savalia_savaglia-macro.jpg
11K

Figure S3 an example of email sent to Diving centres operators to collect data on the distribution. When addressed to Italians, the mail was written in Italian.

SA-Occurrence data collection: Google survey for the data collection

General Information

*Campo obbligatorio

1. Age *

2. Gender

Contrassegna solo un ovale.

M

F

3. Education level *

Contrassegna solo un ovale.

Primary school

Middle school

High school

Bachelor's degree

Master's degree

PhD

Researcher

Technical school

4. Certification Type (PADI, Fipsas, etc.) *

5. Certification Level *

Seleziona tutte le voci applicabili.

- I level (18 m)
- II level (30 m)
- Deep (40-42 m)
- Rescue diver
- Master
- Instructor
- Tech diver
- Rebreather

Have you seen this species?

6. Have you ever seen *Savalia (=Gerardia) savaglia* during your dives in the Mediterranean? *



Contrassegna solo un ovale.

- Yes *Passa alla domanda 7.*
- No

Sighting details

Please, fill in the following section with data that refer to one sighting only; if you have seen *Savalia savaglia* in more than one site, you can compile the form multiple times, one for each sighting. Thank you.

7. Where have you seen it? *

Please write down State, Region, locality and diving site; if possible, add GPS coordinates.

8. At which depth have you seen it? *

9. When have you first seen it? *

10. If you've returned to the same site other times, considering your last visit, how would you say the colony/colonies changed over time? *

Contrassegna solo un ovale.

- Increased number/size
- Reduced number/size
- There have been no changes
- I've only been there once
- I don't know

11. How many colonies are there? *

Contrassegna solo un ovale.

- 1 *Passa alla domanda 13.*
- 2
- 3 - 5
- 6 - 10
- 11 - 50
- > 50

Sighting details

12. What was their distribution like? *

Contrassegna solo un ovale.

- Sparse in a vast area (10 mt distance or more from one another)
- Less than 10 mt from one another
- I can't say

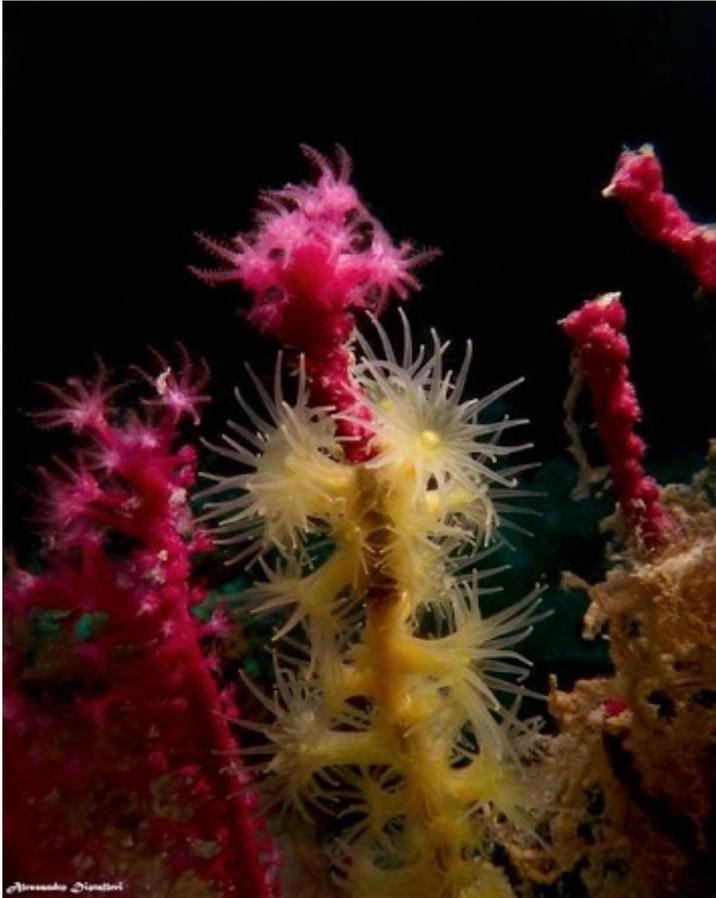
Sighting details

13. Was there one or more of these species of Gorgonians or Black Corals in the same area? *

Seleziona tutte le voci applicabili.

- Paramuricea clavata*
- Eunicella singularis*
- Eunicella verrucosa*
- Eunicella cavolini*
- Leptogorgia sarmentosa*
- Antipathella subpinnata*
- None of the above

14. Was Savalia growing on the skeleton of other animals? *



Contrassegna solo un ovale.

Yes

No

15. If the answer is yes, which species was it growing on?

16. If you have any other relevant information, please write it here:

17. If you have videos or photos of Savalia and would like to share them with me, please upload them here:

If your files exceed 1 GB, you will need to zip them.

File inviati:

Questi contenuti non sono creati né avallati da Google.

Google Moduli

SB – Maps of environmental variables

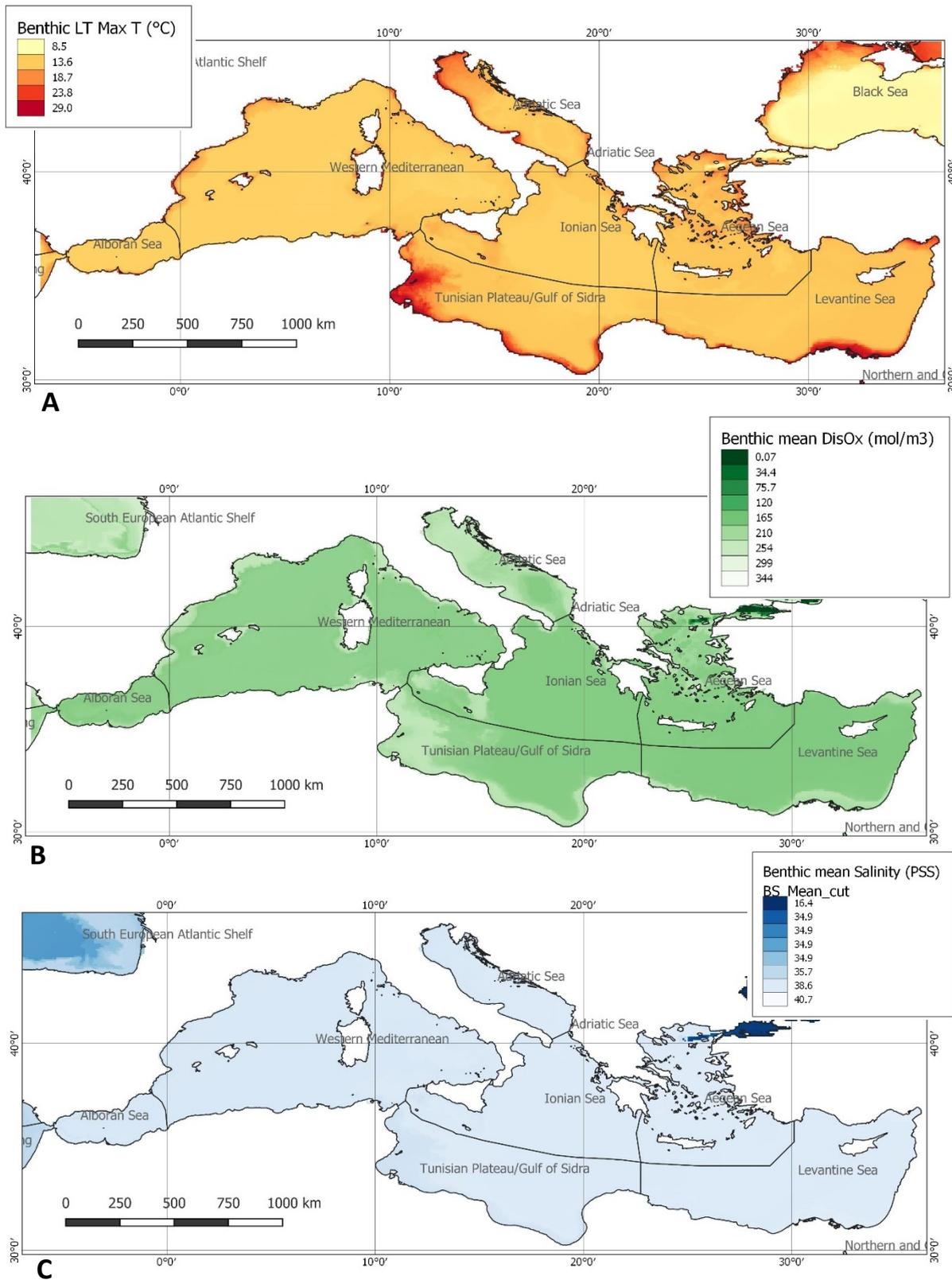
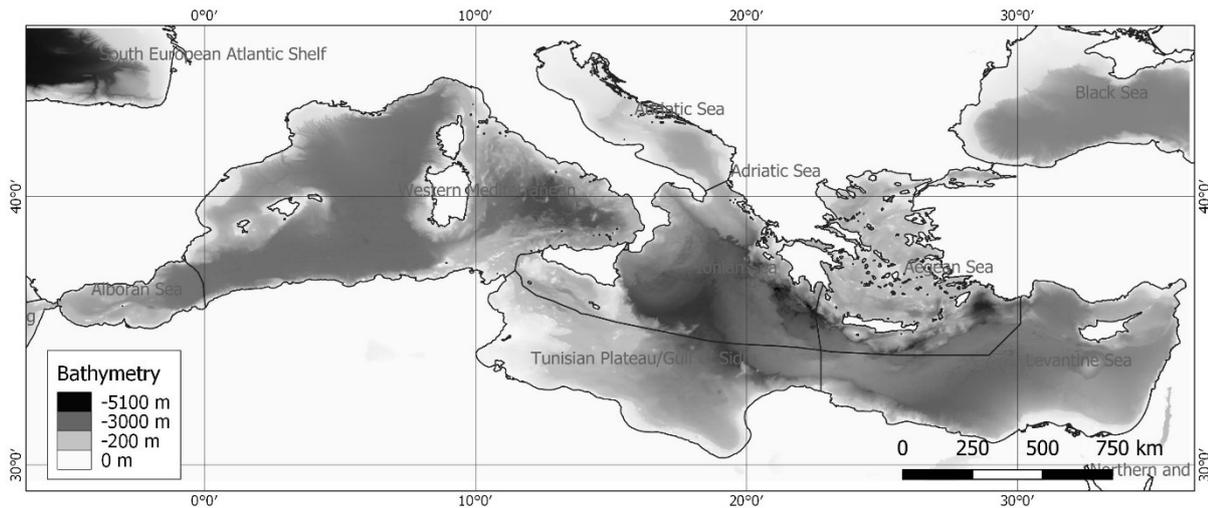
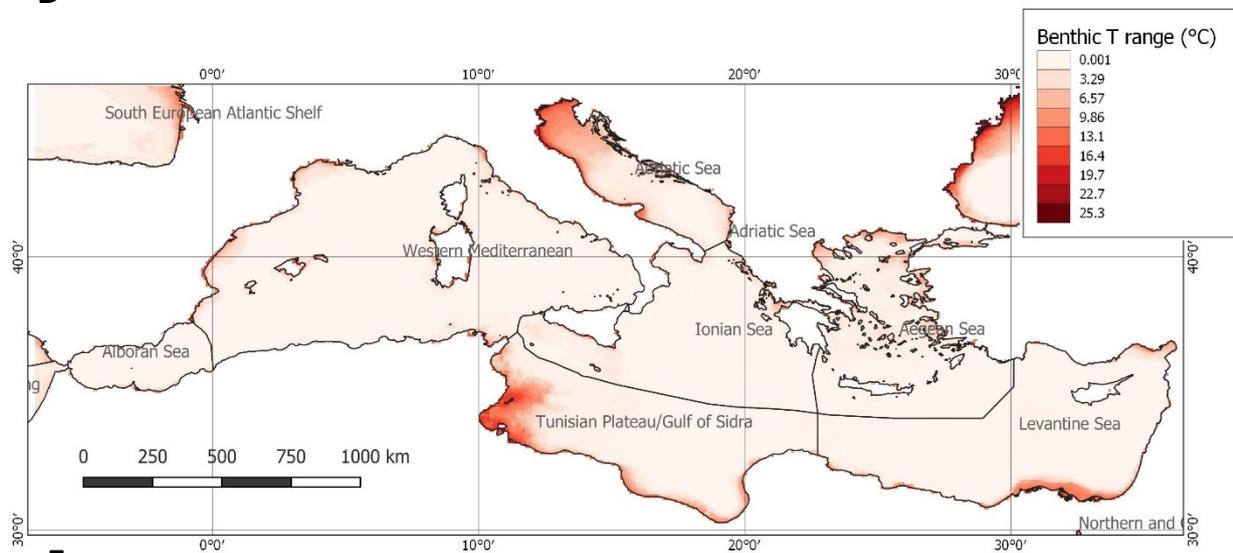


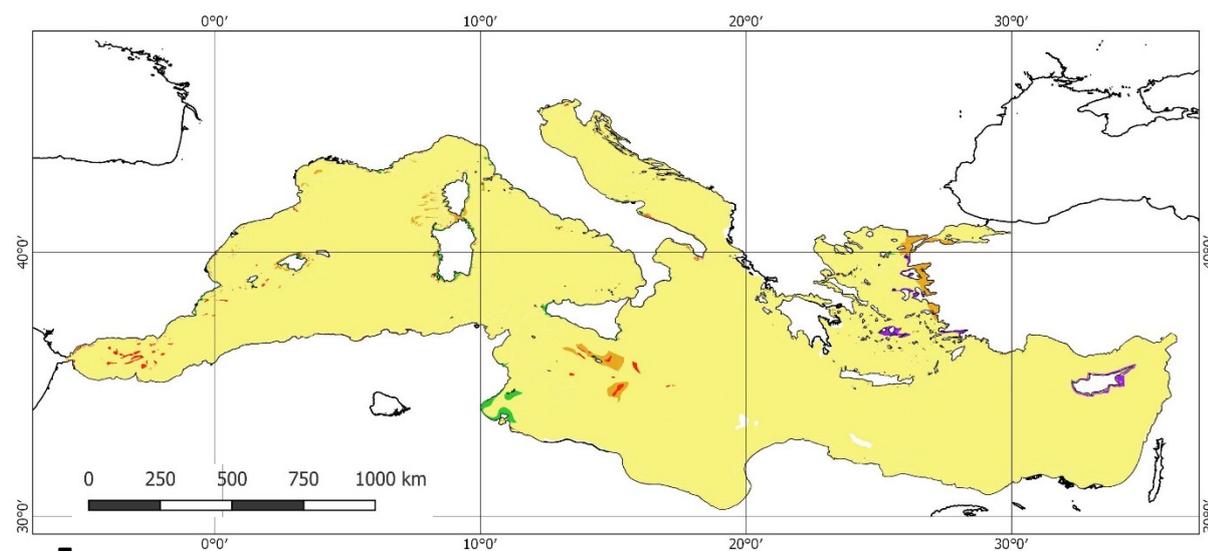
Figure S5 Maps of (A) the long term maximum benthic temperature (°C), (B) the long term minimum benthic dissolved oxygen (mmol/m³), (C) the mean benthic salinity (PSS), (D) the bathymetry (m), (E) the mean benthic temperature range (°C), (F) the substrate type in the Mediterranean Sea. Layers A to C and E were taken from the Bio-Oracle database, layer D from the GEBCO dataset and layer F from EmodNET.



D



E



F

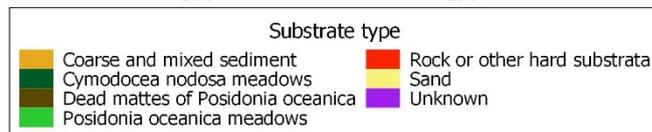
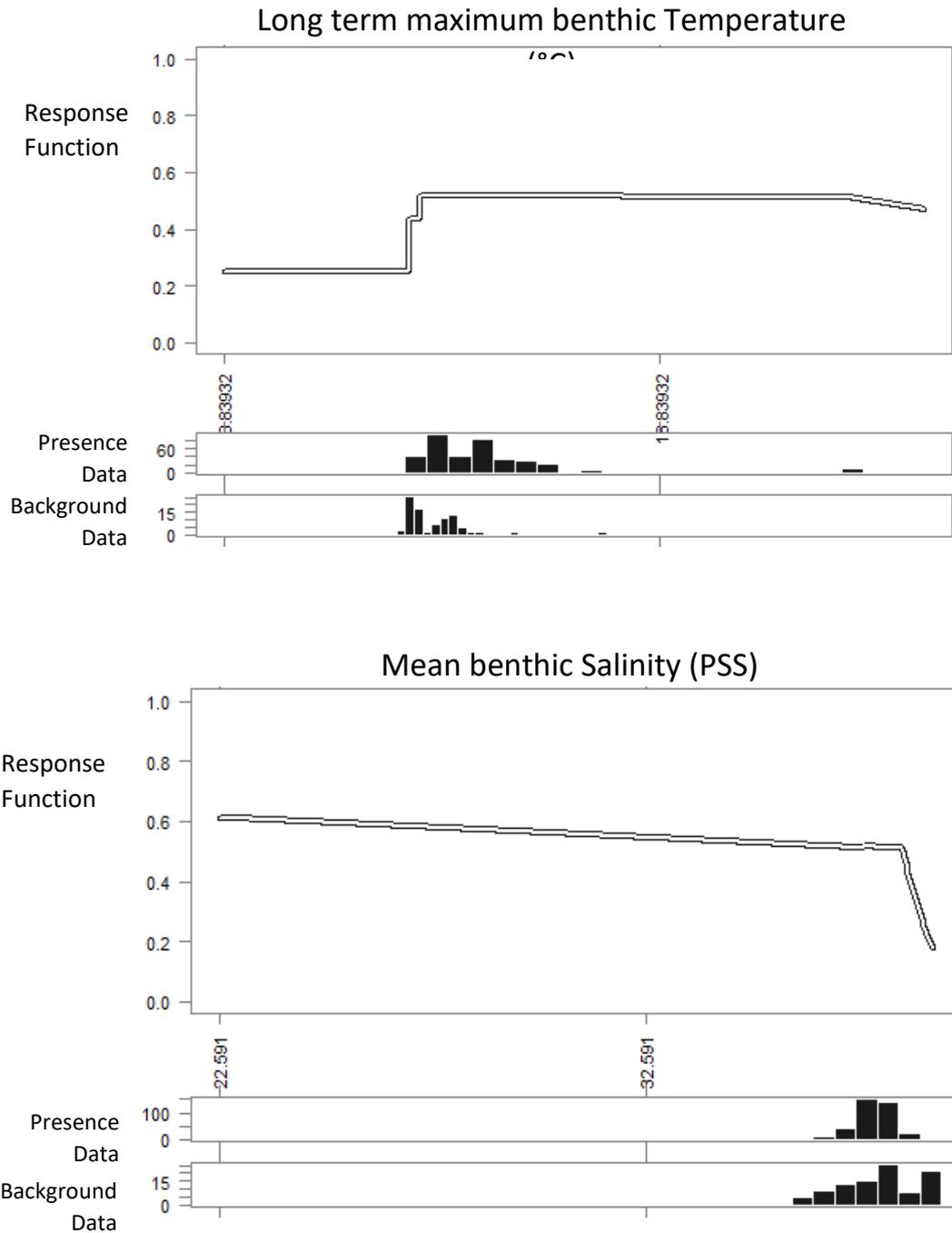


Figure S5 continued.

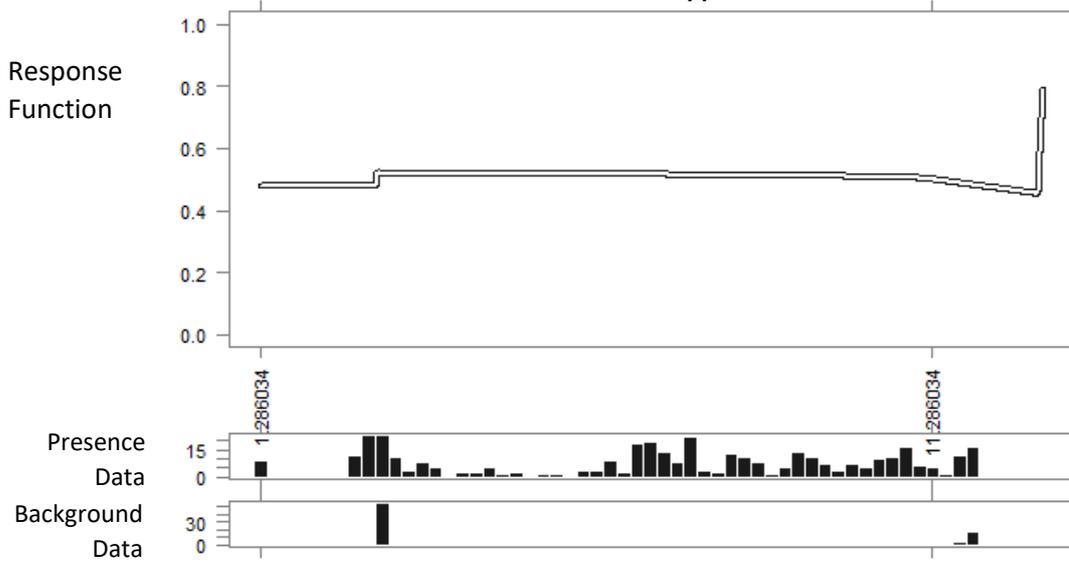
SC – Models response curves and modelling script

The following graphs show the response curves of each predictor in our three models: These plots show the relationship between the probability of occurrence for a species and each of the environmental variables. For each plot, the response is modelled for one environmental variable while the other environmental variables are held constant at their mean.

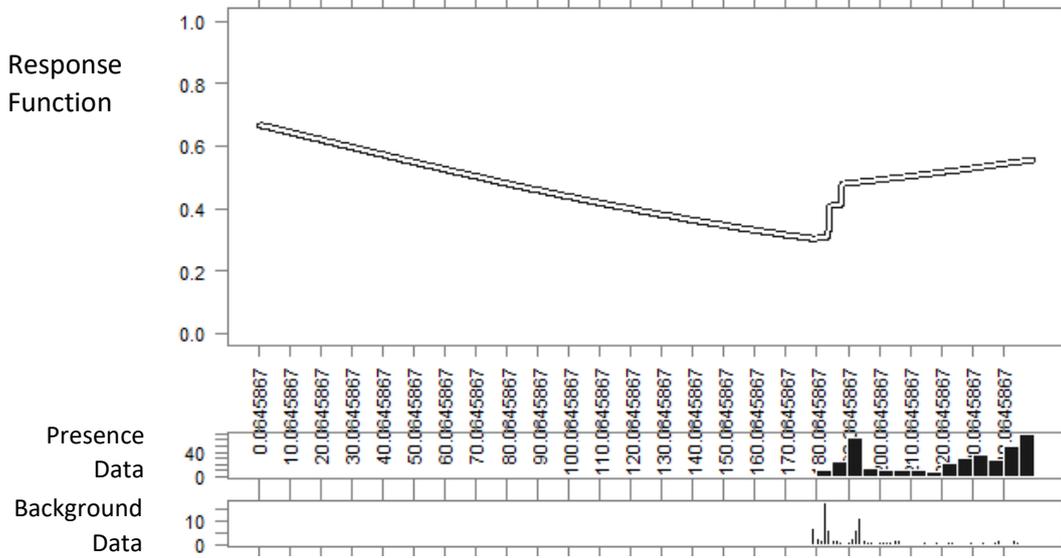
MaxEnt



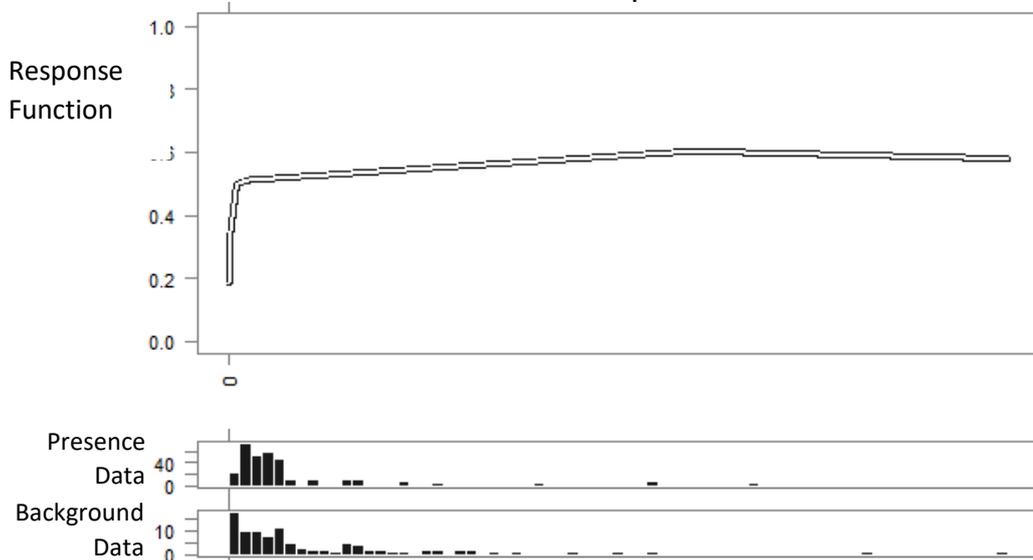
Substrate type



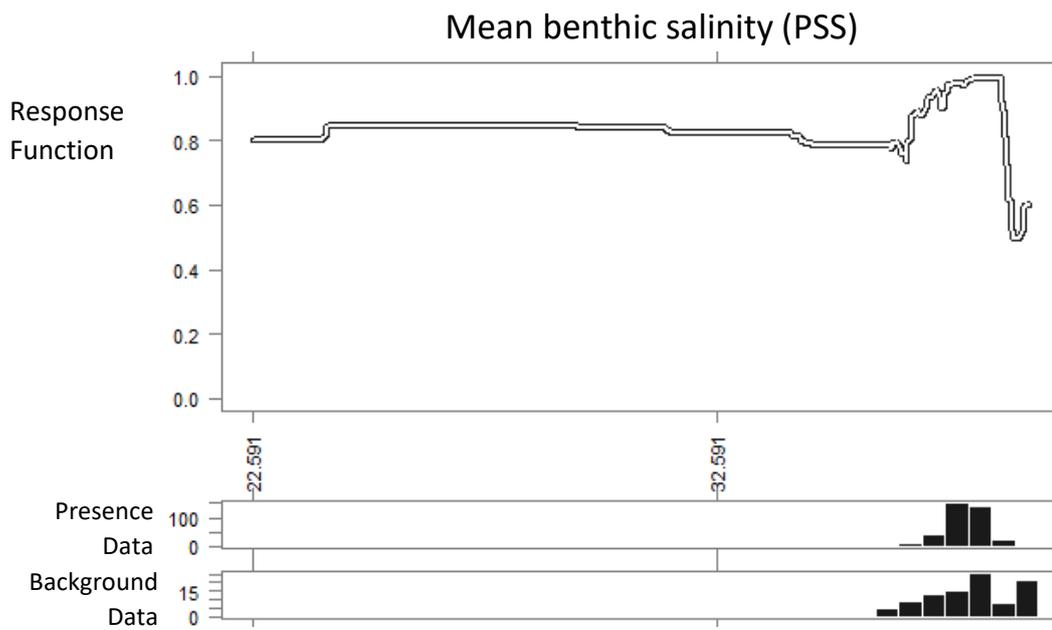
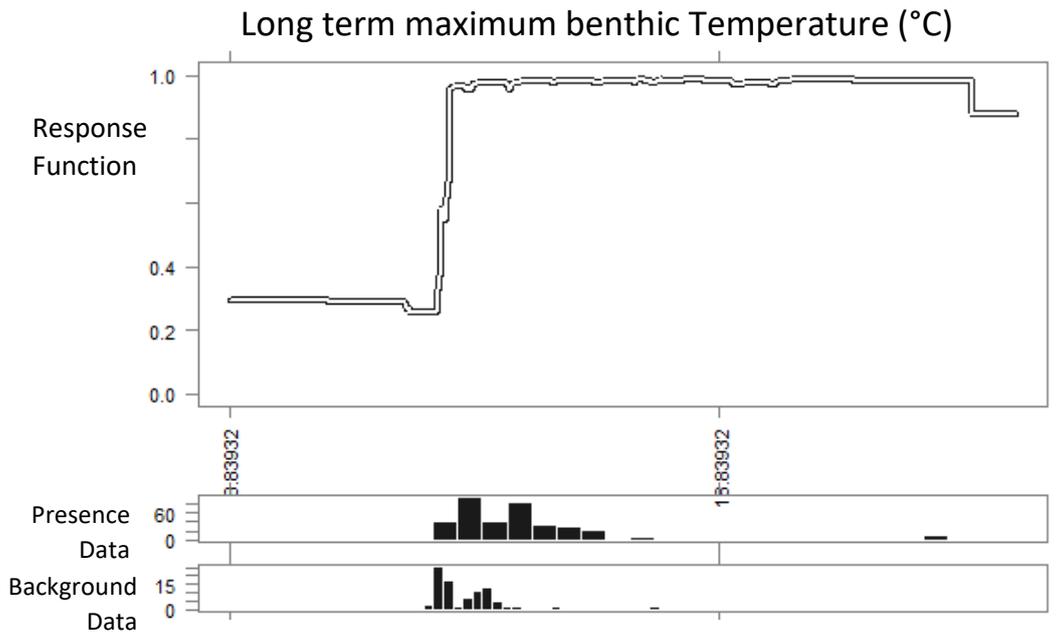
Long term minimum dissolved Oxygen (mmol/m³)

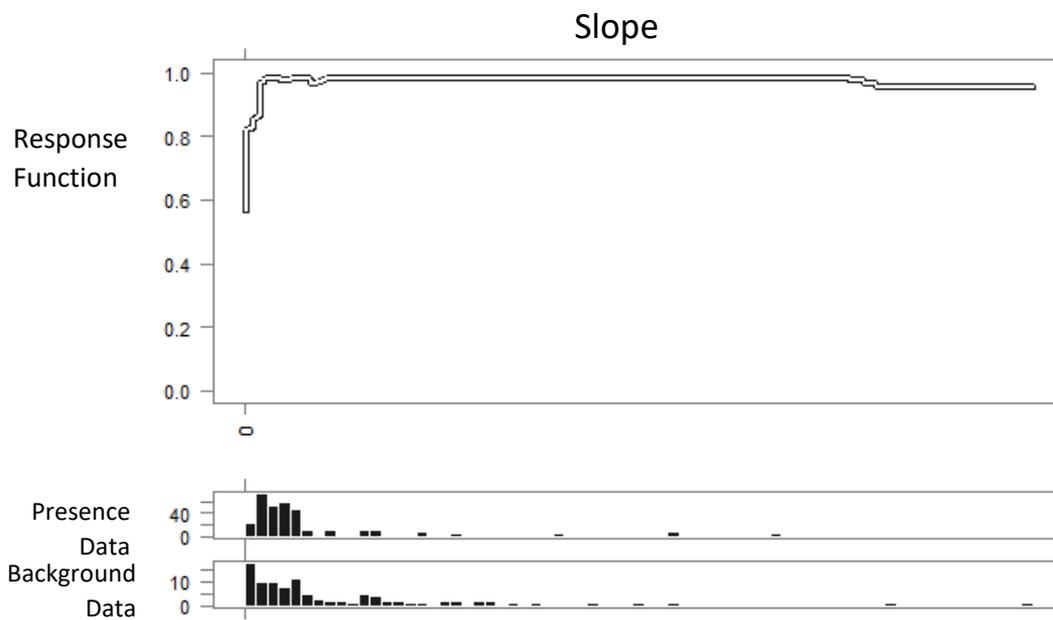
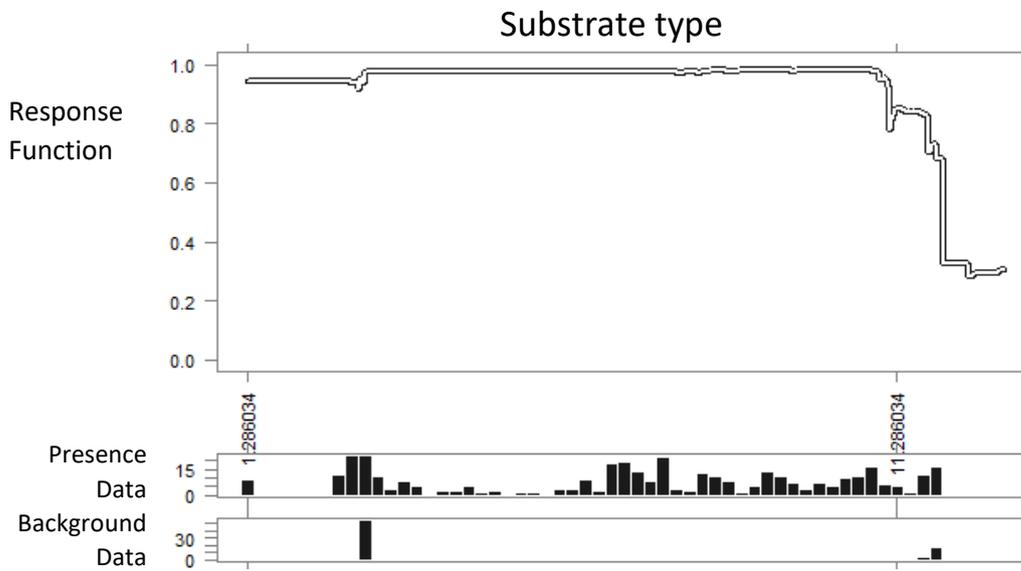
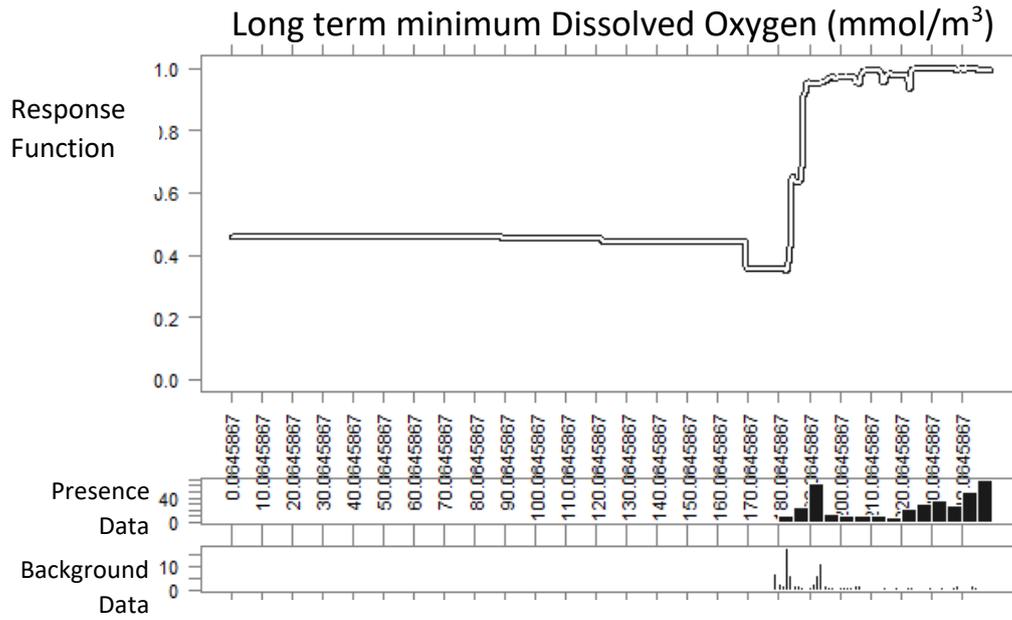


Slope

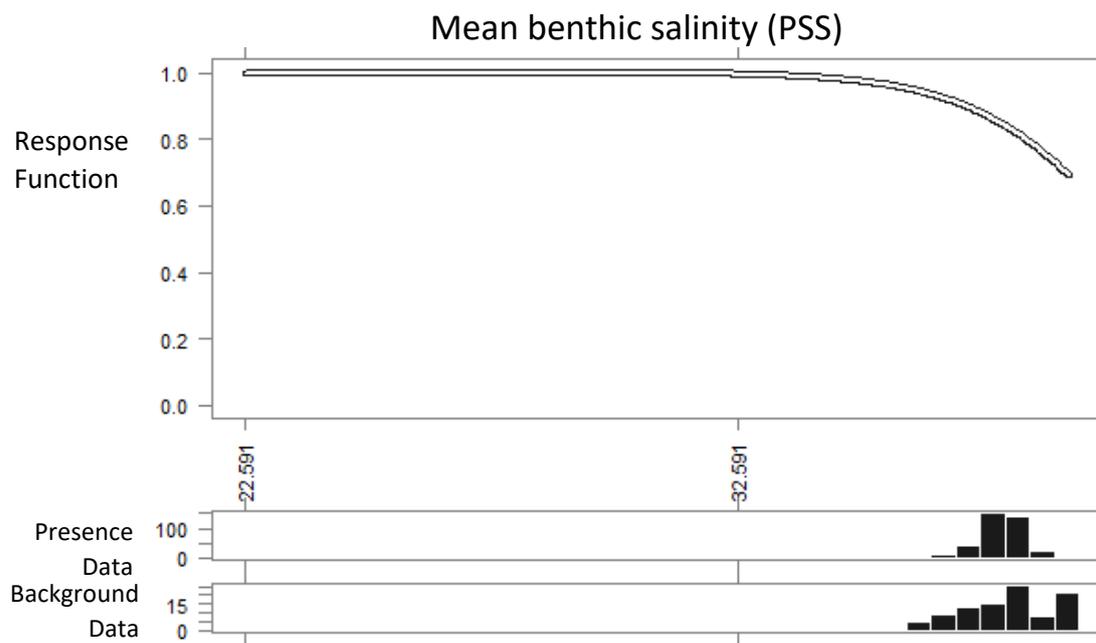
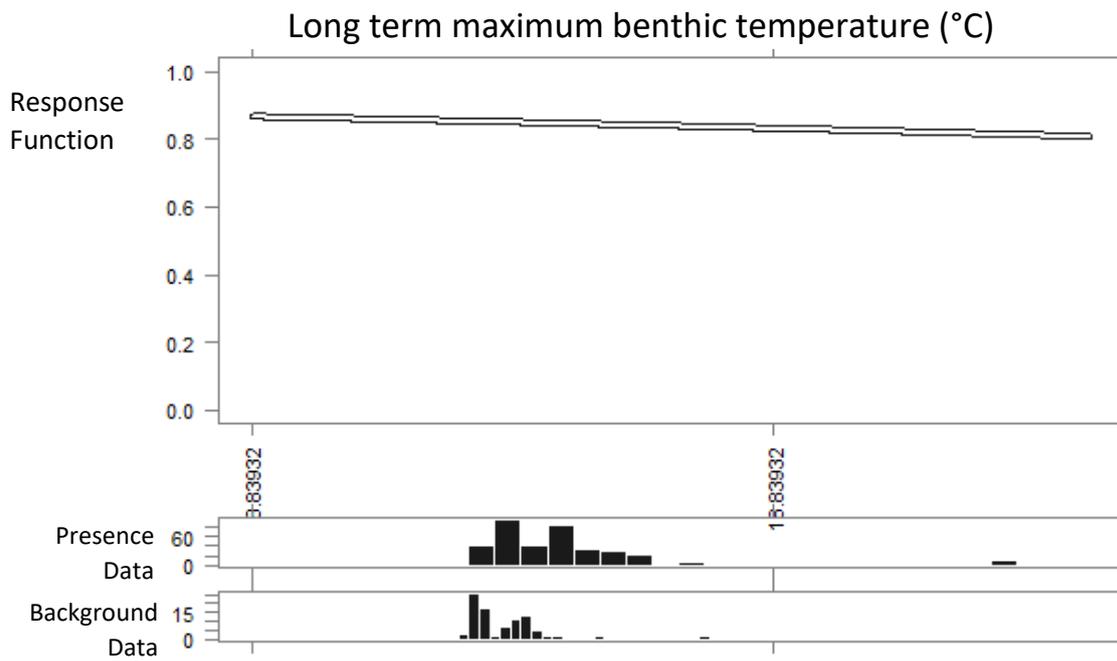


Random Forest

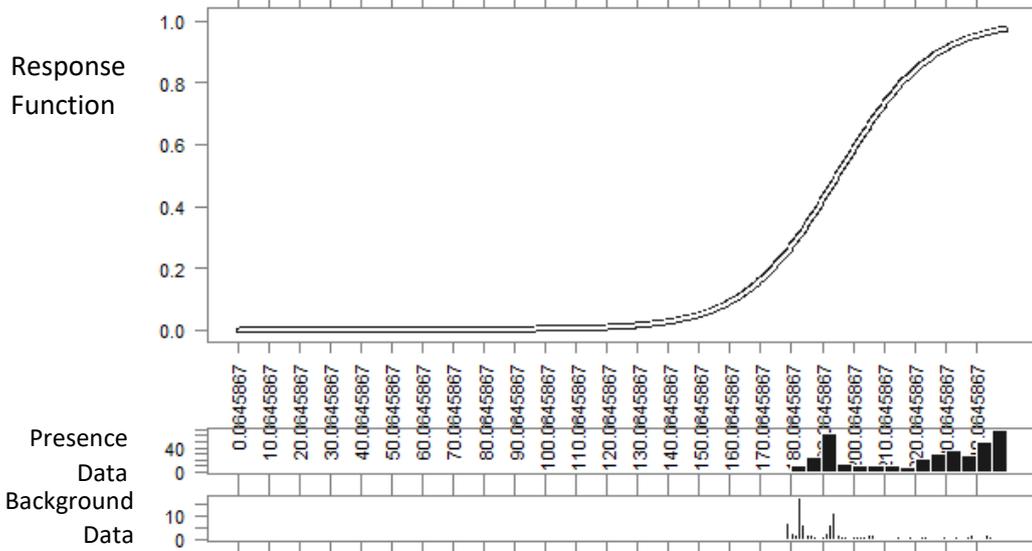




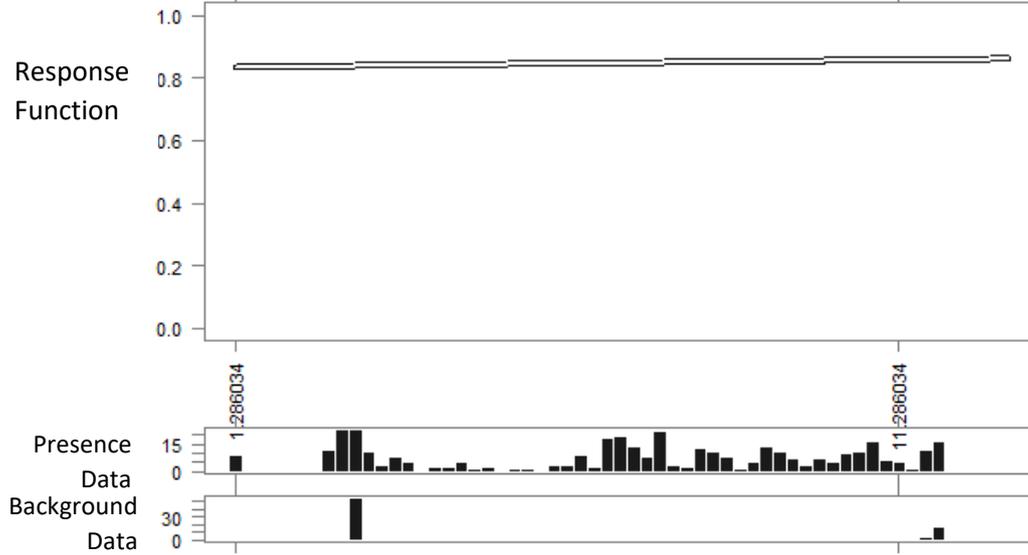
Logistic Regression



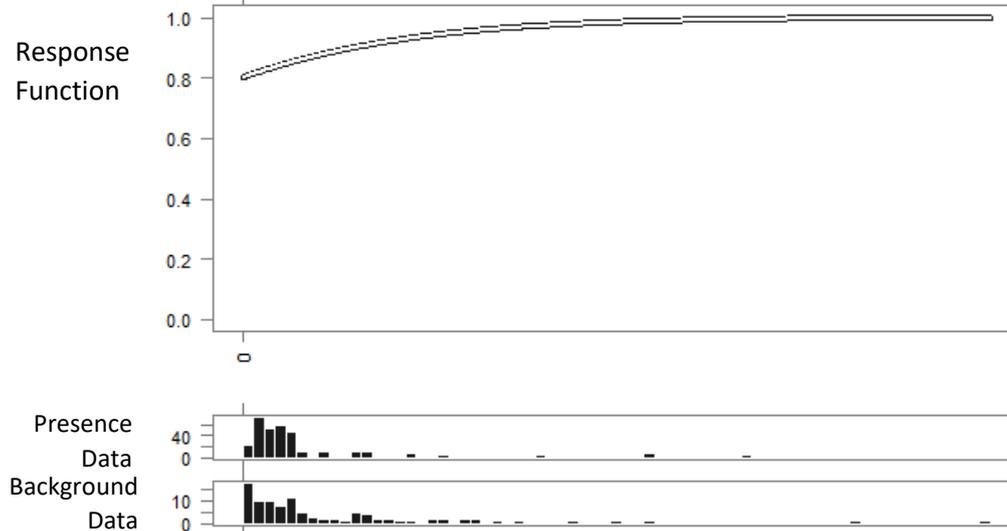
Long term minimum benthic dissolved Oxygen (mmol/m³)



Substrate type



Slope



SC – Models response curves and modelling script

```
---
pdf_document:
  html_document:
    df_print: paged
    latex_engine: xelatex
    word_document: default
author: "Varotti & Pulido Mantas"
date: "22/6/2020"
output:
  html_document: default
  pdf_document: default
  word_document: default
title: "Ssavaglia_project"
---

##1. Set up the working environment
####1.1 Load packages
Modelling in R requires several packages. Specifically, the "dismo"
package, which contains *maxent* function that calls *maxent.jar* in R, the
*raster* package, which provides functions for analyzing gridded data, the
*rgeos* package, which provides functions for analyzing spatial data. As
well as *zoon* package that will manage all the Modelling workflow.

#####Thread 1
```{r setup1,message=FALSE,warning=FALSE}
library("raster")
library("dismo")
library("rgeos")
library("rgdal")
```

#####Thread 2
```{r setup2,message=FALSE}
#install.packages("rJava")
library("rJava")
#####Setting working directory
setwd("C:/Users/cvaro/Desktop/UNI/TESSIIIIII/Distribuzione/Modelling/")
```

#####Thread 3
settings for the R-markdown document
```{r setup_knit}
library("knitr")
knitr::opts_knit$set(root.dir = 'C:/Users/cvaro/Desktop/UNI/TESSIIIIII/
Distribuzione/Modelling/')
opts_chunk$set(tidy.opts=list(width.cutoff=60),tidy=TRUE)
```

####1.2 Set up the Maxent path
In order for Maxent to work properly in R, the *maxent.jar* file needs to
be accessible by *dismo* package.

#####Thread 4
```{r maxent,message=FALSE}
download maxent.jar 3.3.3k, and place the file in the desired folder
utils::download.file(url="https://raw.githubusercontent.com/mrmaxent/
Maxent/master/ArchivedReleases/3.3.3k/maxent.jar",
```

```

 destfile=paste0(system.file("java",
package="dismo"),"/maxent.jar"),
 mode="wb") ## wb for binary file, otherwise maxent.jar
can not execute

also note that both R and Java need to be the same bit (either 32 or 64)
to be compatible to run

to increase memory size of the JVM and prevent memory issues with
Maxent.jar
options(java.parameters = c("-Xss2560k", "-Xmx2g"))
```



```

##2. Prepare data input
####2.1 Load environmental layers
In our example, we use bioclimatic variables (downloaded from Bio-
oracle.org) as input environmental layers. We stack our environmental
layers so that they can be processed simultaneously.

#####Thread 5
```{r load_rasters, message=FALSE, warning=FALSE}
# prepare folders for data input and output

#install.packages('sdmpredictors')
library(sdmpredictors)
datasets <- list_datasets(terrestrial = FALSE, marine = TRUE)
#View(datasets)
layers <- list_layers(datasets)
View(layers)

#Select the variables to feed the model.
layercodes <- c("BO2_dissoxltmin_bdmax", "BO2_templtmax_bdmax",
"BO2_salinitymean_bdmax", "MS_biogeo06_bathy_slope_5m")
#
clim <- load_layers(layercodes, equalarea = TRUE)
#View(clim)

clim_raster <- raster::stack(clim)
#View(clim_raster)

europe <- raster::crop(clim_raster, extent(-5e5,40e5, 36e5, 55e5))
#plot(europe)

#Substrate layer added already with same resolution, if not run the masked
lines down on this chunk.
subst <- raster("C:/Users/cvaro/Desktop/UNI/TESSIIIIIIII/Distribuzione/
Modelling/Subst-final.tif")
crs(subst)
res(subst)
View(subst)
#Define same projection for substrate & bioclim layers
Subst_proj <- projectRaster(subst, crs = crs(europe))

Subst_crop <- raster::crop(Subst_proj, extent(-5e5,40e5, 36e5, 55e5))
plot(Subst_crop)

```


```

```

#BUT we have a problem with the resolution?
res(clim)
res(Subst_crop)

#Resample to same grid:

Subst_res = resample(Subst_crop, europe, "bilinear")
res(Subst_res)

#####
#writeRaster(Subst_res, filename = "Subst-res.tif",
format="GTiff",overwrite=TRUE)
#####

#Still problems with Bioclim data since still have BlackSea. Remove data
which falls outside one of the rasters:

ex = extent(europe)
Subst_crop = crop(Subst_res, ex)
plot(Subst_crop)

Subst_f <- asFactor(Subst_crop)
plot(Subst_f)
europe_F = mask(europe, Subst_f)
plot(europe_F)

#Tutto a posto with resolution and extension!

biogeoclim <- stack(europe_F, Subst_f)
names(biogeoclim) <- c("minLTdissOx_B", "Tmax_bent", "mean_BS", "slope",
"Substrate")
plot(biogeoclim)

#If we want to save variable layers at this point, run the code:

#writeRaster(biogeoclim[[1]], filename = "minLTdissOx_B.asc",
format="ascii")
#writeRaster(biogeoclim[[2]], filename = "Tmax_bent.asc", format="ascii")
#writeRaster(biogeoclim[[3]], filename = "mean_BS.asc", format="ascii")
#writeRaster(biogeoclim[[4]], filename = "slope.asc", format="ascii")
#writeRaster(biogeoclim[[6]], filename = "Substrate.asc", format="ascii")

Printing data source citation:
print(layer_citations(layercodes))

...

####2.2 Explore correlation between environmental layers.

```{r stats1, message=FALSE, warning=FALSE}

#####
# A little bit of statistics study correlation between bioclimatic
variables#
#####

```

```

##Correlation Matrix
pearson_correlation_matrix(biogeoclim)
biogeo.cor <- pearson_correlation_matrix(biogeoclim)

##2 types of Correlogram
library(corrplot)
corrplot(biogeo.cor, type="upper")

#####

col <- colorRampPalette(c("#BB4444", "#EE9988", "#FFFFFF", "#77AADD",
"#4477AA"))
corrplot(biogeo.cor, method="color", col=col(200),
         type="upper", order="hclust",
         addCoef.col = "black", # Add coefficient of correlation
         tl.col="black", tl.srt=45, #Text label color and rotation
         # Combine with significance
         r.cor = biogeo.cor, sig.level = 0.01, insig = "blank",
         # hide correlation coefficient on the principal diagonal
         diag=FALSE
         )
...

####2.3 Occurrence data
#####2.3.1 Import occurrence data

In this case, we are using a .CSV file of S. savaglia occurrence data. If
some error occurs during the import by code, we will import it manually in
order to check that all variables are in the correct format. To do so we
will go to the upper pannel, File/Import Dataset / From text (import
readr).

#####Thread 6
```{r prepare, message=TRUE, warning=FALSE}
##Add manually occurrence data (CSV);
library(readr)
occ_full <- read_delim("C:/Users/cvaro/Desktop/UNI/TESSIIIIIII/
Distribuzione/Modelling/Occ_Savalia.csv",
 ";", escape_double = FALSE, col_types = cols(lat = col_number(), long
= col_number()), trim_ws = TRUE)

View(occ_full)

attach(occ_full)
```

#####2.3.2 Clean occurrence data
Since some of our records do not have appropriate coordinates and some have
missing locational data, we need to remove them from our dataset. To do
this, we creatd a new dataset named "occ_clean", which is a subset of the
"occ_raw" dataset where records with missing latitude and/or longitude are
removed. This particular piece of code also returns the number of records
that are removed from the dataset. Additionally, we remove duplicate
records and create a subset of the cleaned data with the duplicates
removed.

```

```

#####Thread 7
```{r clean_data1}
remove erroneous coordinates, where either the latitude or longitude is
missing
occ_clean <- subset(occ_full, (!is.na(lat))&(!is.na(long))) # "!" means the
opposite logic value
cat(nrow(occ_full)-nrow(occ_clean), "records are removed")

remove duplicated data based on latitude and longitude
dups <- duplicated(occ_clean[c("lat", "long")])
occ_unique <- occ_clean[!dups,]
cat(nrow(occ_clean)-nrow(occ_unique), "records are removed")
View(occ_unique)
#Define the dataset as spatial projected dataset
library(sp)

points <- SpatialPoints(occ_unique[,c("long", "lat")], lonlatproj)

proj4string(points) <- CRS("+init=epsg:4326")

crs(points)

points <- spTransform(points, equalareaproj)

crs(points)
crs(biogeoclim)

occfile <- tempfile(fileext = ".csv")

write.csv(cbind(coordinates(points), value=1), occfile)

plot the final occurrence data on the environmental layer
plot(biogeoclim$Substrate,col = topo.colors(12)) +
 plot(points,
 pch = 15,
 col = "darkred",
 cex = 0.5,
 add = TRUE)
the 'add=T' tells R to put the incoming data on the existing layer
```

#####2.4 Take a look on occ-env data.
```{r env-occ data exploration}
library(ade4)
#Extract environmental info for Savalia presence
envdata <- extract(x=biogeoclim, y=cbind(points$long, points$lat))

envdata
envdata <- na.omit(envdata)
pcal <- dudi.pca(envdata,scannf = F, nf = 2)
pcal
round(pcal$eig/sum(pcal$eig)*100, 2)

```

```

Plot the PCA to look for potential outliers in the environmental data.
plot(pcal$li[,1:2])

s.corcircle(pcal$co)
From the correlation circle, we want to select variables on different
axes and not in the same/overlapping environmental space. If there are
multiple variables in the same quadrant of the circle, we should choose the
one with a longer arrow, as that means it varies more over the
environmental space, which is preferred. However, it is also important to
know which environmental features are more relevant to your study species,
and to choose according to the ecology of each species.
A note on arrow directions: same direction = highly correlated,
orthogonal (this means at a 90 degree angle) = unrelated, opposite
directions = negatively correlated.

...

##3. Modelling
```{r modelling1}

##### Zoon:
#install.packages('zoon')
library(zoon)
library(SDMTools)
mod1<-workflow(occurrence = LocalOccurrenceData(occfile,
occurrenceType="presence",
  columns = c("long", "lat", "value")),
  covariate = LocalRaster(stack(biogeoclim)),
  process = BackgroundAndCrossvalid(k = 2),
  model = list(MaxEnt, RandomForest, LogisticRegression),
  output = Chain(PrintMap(points=TRUE), ResponseCurve))

#process = OneHundredBackground,process, Randomly creates 100 pseudoabsence
or background datapoints.
#process = BackgroundAndCrossvalid(k = 2), providing an argument to the
module BackgroundAndCrossvalid. We are setting k (the number of cross
validation folds) to 2.

#output = Performance Measures, which calculates a number of measures of
the effectiveness of our model: AUC, kappa, sensitivity, specificity etc.
#output = Appify, PrintMap, ResponseCurve

Occurrence(mod1)
Covariate(mod1)
Process(mod1)
Model(mod1)[[1]]
Model(mod1)[[2]]
Model(mod1)[[3]]

#####
#####

```

```
#Collating the output into one plot we can see the very different forms of
the models and can see that the XXX model has a higher AUC (implying it
predicts the data better).
```

```
citation('zoon')
```

```
...
```