Biodiversity and phylogeography of Northeast Atlantic and Mediterranean sponges
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CHAPTER 1

GENERAL INTRODUCTION
MARINE BIODIVERSITY AND MOLECULAR TOOLS

Uncovering and understanding the main biodiversity patterns is crucial to support sound conservation policies in the increasingly threatened marine realm. The loss of marine biodiversity threatens to impair ecosystem functioning and consequently the services it provides to a growing human population (climate regulation, food, and pharmaceuticals, to name just a few). The term biodiversity usually refers to species richness but it encompasses several interconnected aspects of biological diversity ranging from intraspecific genetic diversity to ecological communities and ecosystems, all of which require special (and integrated) conservation attention (Sala & Knowlton, 2006; Worm et al., 2006).

The introduction of molecular tools into the biological sciences added a new dimension to the study of marine biodiversity (Féral, 2002). Indeed, genetic appraisals enable us to uncover the cryptic marine diversity, characterize the genetic structure, assess the connectivity between populations, and unveil which factors, ecological and historical, shape the genetic structure (Knowlton, 1993, 2000; Grosberg & Cunningham, 2001; Hellberg et al., 2002). In the late 1980’s, phylogeography emerged as a discipline that approaches historical biogeography from a molecular perspective. This discipline, which combines information derived from phylogenetics and population genetics, focuses on the principles and processes responsible for the geographic distribution of genealogical lineages within and among (closely related) species (Avise, 2000).

These molecular avenues have major implications for marine conservation and management strategies, because they brought to light the concept of evolutionary history to complement species diversity and endemism in the establishment of significant units for conservation, and the design of marine protected areas (Moritz, 1994; Avise, 1998; Crandall et al, 2000; Palumbi, 2003).

SPONGES

The phylum Porifera (from Latin porus + ferō = pore-bearer) constitutes a group of aquatic animals that is widely distributed geographically and bathymetrically in both marine and freshwater ecosystems. They are a dominant invertebrate group in hard-bottom benthic communities throughout the temperate, tropical, and polar zone from intertidal to abyssal depths (Sarà & Vacelet, 1973). Sponges are regarded amongst the most primitive extant animal groups. Fossil sterols,
Identical to those produced by current demosponges, represent the oldest evidence of metazoan presence in the fossil record suggesting the existence of Porifera in the late Neoproterozoic at 635 Myr ago (Love et al., 2009).

Sponges are sedentary filter-feeders and their body, which lacks true tissues or organs, is organised around a system of incumbent and excurrent water-bearing canals. The beating of flagellated cells (choanocytes) keeps a water flow running through this system bringing oxygen and food particles (detritus, plankton, and bacteria) to the sponge. Sponges play important ecological roles in bioerosion, reef formation, substrate consolidation, benthic-pelagic coupling, and habitat provision that have major implications for ecosystem functioning (reviewed in Bell, 2008). In addition, as sessile organisms, sponges developed a range of chemical defence strategies against predators, spatial competitors, or as antifouling (e.g. Becerro et al., 1997, 2003). These secondary metabolites with antimicrobial, analgesic, antiviral, and anticancer activities have placed sponges among the most prolific and promising producers of medical compounds and increasingly attract the interest of pharmaceutical companies (Munro et al., 1994, 1999).

Sponge taxonomy and systematics is mainly based on internal morphological features such as the type, shape, size, and arrangement of the skeletal structures. The skeleton is composed of calcareous or siliceous spicules and/or collagen fibrils and spongin fibres. Additional characteristics such as cytological or chemical features can be informative but these are used less frequently (Van Soest & Braekman, 1999; Erpenbeck & Van Soest, 2007). Despite the remarkable efforts that culminated in the recent revision of the classification system in *Systema Porifera* (Hooper & Van Soest, 2002) there are still many unsolved questions regarding the phylogenetic relationships at various systematic levels (see reviews in Reiswig, 2006; Boury-Esnault, 2006; Manuel, 2006; Erpenbeck & Wörheide, 2007). Recent platforms such as the Sponge Genetree Server (Erpenbeck et al., 2008; SGS, http://www.spongegenetrees.org/) and the Porifera Tree of Life Project (PorTOL, http://www.portol.org/) will certainly help to answer those questions and hence contribute for a better understanding of the evolution of this phylum.

After a long-lasting debate on the mono- or paraphyletic status of the phylum (e.g. Borchiellini et al., 2001; Medina et al., 2001) and its position in the Tree of Life (e.g. Dunn et al., 2008), a recent phylogenomic study has confirmed that the Porifera constitutes a monophyletic group at the base of the metazoans
(Philippe et al., 2009). The phylum comprises three extant (Demospongiae, Calcarea, and Hexactinellida) and one extinct (Archaeocyatha) classes, 25 orders, 127 families, and 682 genera (Hooper et al., 2002). Approximately 8300 species are currently recognised (WPD, Van Soest et al., 2009) but the true diversity of this group is estimated to comprise probably twice this number (Hooper & Lévi, 1994). Particularly little is known about the sponge fauna of several geographic regions (e.g. west coast of Africa, northwest and southeast Pacific, Red Sea) and the fauna that is associated with remote ecosystems (e.g. deep-sea, caves, and seamounts). Moreover, recently quite a number of morphologically cryptic species have been uncovered. International projects such as the Sponge Barcoding Project (SBP, http://www.spongebarcoding.org/), and the World Porifera Database (WPD, http://www.marinespecies.org/porifera/) are in this context the initiatives that will contribute most to the discovery and cataloguing of the diversity of the phylum.

THE ATLANTO-MEDITERRANEAN REGION AND ITS SPONGE FAUNA

The Northeast Atlantic and Mediterranean encompass an important marine area that covers a wide range of subtropical, temperate, and subarctic climatic conditions. It also comprises a variety of coastal, oceanic, shallow and deep-sea, as well as island and seamount ecosystems that harbour a high biological diversity. This region’s geological and climatological history (e.g. Messinian Salinity Crisis of the Miocene or the Pleistocene glaciations) as well as its marine biodiversity are one of the best documented worldwide, which renders this area an excellent model to address biodiversity and biogeographic questions from below to above the community level (see some reviews in Patarnello et al., 2007; Maggs et al., 2008).

The Atlanto-Mediterranean is also the region for which the sponge fauna has been most comprehensively studied. This results from a long-lasting European taxonomic tradition from the very beginning of sponge systematics, owing to researchers such as Schmidt, Bowerbank, Topsent, Stephens, and Burton, through to our days with the contributions of Boury-Esnault, Vacelet, Lévi, Van Soest, Pansini, Sarà, Uriz, Cristobo, and the remarkable explorations of the late 19th and early 20th centuries (Challenger, Ingolf, Prince Albert I of Monaco). Although dispersed, the available information on species distributions,
along with the biological characteristics of sponges, makes this taxonomic group an excellent model group for phylogeographic studies.

Despite a remarkable variety of both sexual and asexual reproductive strategies, sponges are thought to have limited dispersal abilities. Sponges can be gonochoristic or hermaphroditic and regarding the embryonic development they can be viviparous/brooders or oviparous/spawners (Maldonado & Riesgo, 2008). The vast majority of the species have indirect development with production of lecitotrophic larvae with a short planktonic life, limited swimming capacity, and phylopatric behaviour (Maldonado & Bergquist, 2002; Maldonado, 2006; Mariani et al., 2006). Asexual reproduction, by means of fragmentation, budding, and gemmulation does occur (Fell, 1993), but clonality has been shown to play a structuring role only at small spatial scales, in the order of a few meters (Zilberberg et al, 2006b; Calderón et al., 2007; Blanquer et al., 2009). With such characteristics one would expect sponge species to have relatively restricted distribution ranges and highly structured populations. Furthermore, given an apparent geographical distribution limited by water temperature, we would also expect to observe an imprint of past climate on both the metacommunity as well as on intraspecific diversity. Despite their potential as model organisms, studies on sponge phylogeography and population genetics in this area are until now restricted to two species, viz. the poecilosclerid Crambe crambe (Duran et al., 2004a, b, c; Calderón et al., 2007), and the halichondrid Scopalina lophyropoda (Blanquer, 2007; Blanquer et al., 2009). The available data, although limited, already support some of these predictions. Highly structured populations as a result of restricted gene flow was found to occur in both species, and the supposedly widely distributed S. lophyropoda turned out to comprise a number of cryptic species, each with a much more restricted distribution (Blanquer and Uriz, 2007).

**SPONGE PHYLOGEOGRAPHY AND POPULATION GENETICS**

As in most groups, allozyme electrophoresis was the first method employed at the population and interspecific level in sponges. In Porifera, this method that relies on the differential electrophoretic mobility of alternative alleles of a particular enzyme locus, had its peak of use in the late 1980’s, and mainly addressed population differentiation and uncovered cryptic species complexes (see review in Solé-Cava & Boury-Esnault, 1999). Although having been
progressively replaced by other markers they still prove valuable in addressing questions such as the relative contributions of sexual and asexual reproduction to the genetic structure of sponge populations (e.g. Zilberberg et al., 2006a, b).

Several attributes of the mitochondrial genome such as a small effective population size, lack of significant recombination due to its uniparental inheritance, fast mutation rate, and technical amenability have made mtDNA markers the primary choice for phylogeographic studies (Avise, 1987, 2000). In sponges, mtDNA genes have proven to be amongst the slowest evolving of all metazoans (Duran et al., 2004a; Wörheide, 2006) exhibiting rates only comparable with those of Cnidaria (Van Oppen et al., 1999; Shearer et al., 2002; France & Hoover, 2002). Indeed, in only a few cases mtDNA markers such as the cytochrome oxidase subunit I (COI) exhibited enough variation to address questions at the population level in Porifera (López-Legentil and Pawlik, 2009; Xavier et al., submitted; Chapter 5). Several hypotheses have been put forward to explain the slow mtDNA rate of evolution in lower metazoans, including generation time, metabolic rate, exposure to mutagens, rate of cell division, and presence of a mitochondrial mismatch repair systems (Van Oppen et al., 1999; Wörheide, 2006; Hellberg, 2006) but this subject remains contentious (e.g. Lanfear et al., 2007).

Nuclear ribosomal genes (rDNA) are arranged in transcription units that comprise the 18S coding region, the internal transcribed spacer 1 (ITS-1), the 5.8S coding region, the internal transcribed spacer 2 (ITS-2), and the 28S coding region. These units are separated by the intergenic spacer (IGS) and an external transcribed spacer (ETS). The various rates of evolution among the different regions of the rDNA make these markers extremely versatile to answer questions at different taxonomic levels. They occur in several hundreds of copies and although homogenization by a process of concerted evolution seems to be the rule, several exceptions have been reported (Hillis & Dixon, 1991; Weider et al., 2005). Due to a faster evolutionary rate, the internal transcribed spacers (ITS-1 and ITS-2) have been successfully employed in population level studies in Porifera (e.g. Wörheide et al., 2000; Duran et al., 2004b). However, the level of intragenomic polymorphism has been shown to vary extensively depending on the studied species (Wörheide et al., 2004; Redmond & McCormack, 2009) having reached a staggering uncorrected genetic distance of 29% in *Axinella aruensis* (Alvarez et al., 2007).

Nuclear introns, i.e. portions of a gene that are not translated into a protein,
are relatively free from the functional constraints of coding regions (exons) and therefore tend to exhibit higher rates of evolution than exons (Hare, 2001). These markers have proved their utility in population-level studies in several groups (e.g. Jarman et al., 2002) and were also successfully employed in two sponge species, viz. the calcareous *Pericharax heteroraphis* (Bentlage & Wörheide, 2007) and *Leucetta chagosensis* (Wörheide et al., 2008).

Microsatellites or simple sequence repeats (SSRs) are short (1-6 bp) tandemly repeated motifs occurring mostly in non-coding genomic regions. Their abundance, ubiquity, and high polymorphism have rendered them an extremely valuable (and popular) tool in population genetics as they allow fine-scale studies on population structure and connectivity and to unveil processes such as asexual reproduction. However, the high microbial content of many sponges hinders the technical development of these markers and for this reason microsatellites have been developed for only seven species: *Crambe crambe* (Duran et al., 2002), *Halichondria panicea* (Knowlton et al., 2003), *Scopalina lophyropoda* (Blanquer et al., 2005), *Hymeniacidon sinapium* (Hoshino & Fujita, 2006), *Spongia agaricina* (Noyer et al., 2009), *Spongia officinalis* (Dailianis & Tsigenopoulos, 2009), and *Ephydatia fluviatilis* (Gigliarelli et al., unpublished).

**THESIS OUTLINE**

In this thesis I aimed to i) uncover the biodiversity patterns of the Atlanto-Mediterranean demosponge fauna, by examining taxonomic diversity at the metacommunity level, looking at cryptic diversity and investigating genetic diversity at the intraspecific level; and ii) unveil how that diversity is distributed in space over this region and which ecological and historical processes have shaped that distribution. To attain these goals I assembled information on species distributions, generated molecular data, and used a diverse array of phylogenetic, phylogeographic, and statistical tools.

In Chapter 2, I assess the diversity patterns and biogeographical affinities of the northeast Atlantic and Mediterranean demosponge assemblages at a metacommunity scale based on the distributional data of 745 species throughout 28 areas. Diversity patterns were assessed and correlated to contemporary and historical values of sea surface temperature, and zoogeographical affinities between ecoregions were examined through ordination and classification methods.
CHAPTER 3 is a survey and description of the demosponge assemblages associated with one of the most understudied marine ecosystems – the seamounts. This study results from a sampling trip to the Ormonde and Gettysburg seamounts (Gorringe Bank) located off the southwest coast of Portugal, and represents an example of the many surveys that have been performed in the course of this project. Collected material was identified and assemblages compared to those of nearby areas to understand the zoogeographical affinities of the seamount fauna.

In CHAPTER 4, I uncover the cryptic diversity of an ecologically important and allegedly cosmopolitan species and establish the *Cliona aff. celata* complex, based on mitochondrial and nuclear ribosomal molecular data. I further provide an overview of all cases of cryptic speciation reported to date in Porifera and discuss the potential causes, and caution for the consequences of, undetected cryptsis in this phylum.

In CHAPTER 5, I assess the genetic population structure of the poecilosclerid sponge *Phorbas fictitus*, based on sequences of the mtDNA COI gene, at two spatial scales: a regional scale comparing mainland (Iberian) and insular (Macaronesian) populations, and a local (Archipelagic) scale focusing on different island populations of the Azores archipelago.

In CHAPTER 6, I study the genetic structure and elucidate the pylogeographical history of a haplosclerid sponge - *Petrosia ficiformis* - throughout its Atlanto-Mediterranean distribution range. Molecular data of two mitochondrial gene fragments and one newly developed nuclear ribosomal gene fragment were analysed using multiple approaches such as phylogenetic inference and nested clade phylogeographic analysis.