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# **ABSTRACTS: NINTH WORLD SPONGE CONFERENCE 2013**

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**ORAL ABSTRACTS**

**Insights into population dynamics of the intertidal *Phyllospongia* sp. in central Great Barrier Reef**

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Sponges are important structural and functional components of benthic habitats. Despite the significant ecological roles that sponges play in diverse aquatic environments, information fundamental to their management and conservation is surprisingly scarce. The physical environment plays a significant role in shaping populations of marine species, however, fundamental biological and ecological processes including growth, reproduction, larval behaviours and survival are critical to population maintenance and are key contributors to the establishment of population demography. Here, we will focus on *Phyllospongia* sp., a foliose dictyoceratid sponge (Thorectidae, Phyllosponginae) that occurs on the intertidal coastal reef flats of the Palm Islands group in the central Great Barrier Reef (GBR). Intertidal populations of *Phyllospongia* sp. within the Palm Islands group are abundant and conspicuous, with a distribution that is constrained to a narrow shallow depth range (intertidal reef flat). These sponge populations were monitored using fixed quadrats for two years unveiling patterns of reproduction, growth, survival and recruitment. Manipulative laboratory based experiments were also undertaken to establish larval settlement behaviours. We will discuss how these processes may have contributed to the distribution of this species and propose why intertidal populations of *Phyllospongia* sp. face a great risk of local extinction in the face of climatic and environmental changes.

**Developmental gene expression indicates homology of poriferan and eumetazoan body plans**

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Elucidation of macroevolutionary transitions between diverse metazoan body plans remains a major challenge for evolutionary biology. Even the fundamental aspects of these transitions, reflected by homologies of body axes and germ layers, are notoriously difficult to resolve between bilaterian and non-bilaterian clades. We addressed homologies between sponge and eumetazoan body plans by analysis of developmental gene expression in a Calcaronean sponge, *Sycon ciliatum*. Using a combination of candidate gene approach and RNAseq screen for genes differentially expressed along the adult body axis, we have identified and analyzed expression of potentially informative marker genes. Wnt ligands,

which are expressed in the posterior end of bilaterians and oral end of cnidarians, are expressed in staggered domains in the posterior end of the larva and around the osculum at the apical adult end of *Sycon*. Tgf-beta ligands, associated with dorsal/ventral patterning in bilaterians, and expressed orally in cnidarians, are expressed around the osculum of *Sycon*. Nanos, which is a conserved bilaterian posterior marker, and expressed around the cnidarian oral end, is expressed in posterior cells of *Sycon* embryos. The eumetazoan endomesoderm markers Brachyury and GATA are expressed in the choanoderm of *Sycon*. Our results demonstrate similarity in developmental gene usage in larval and adult body patterning between sponges and eumetazoans, especially cnidarians. We postulate that the basic body plans of sponges and cnidarians are homologous, with choanoderm being an evolutionary precursor of gastrodermis, and the osculum corresponding to the mouth of the polyp.

### **Genomes of calcareous sponges reveal surprisingly complex developmental toolkits**

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Analysis of the developmental toolkit of the siliceous sponge *Amphimedon queenslandica* revealed representatives of majority of eumetazoan developmental gene classes, such as transcription factors and components of the signaling pathways. Large eumetazoan families of developmental regulators are almost universally represented by less family members in *Amphimedon*, neatly in line with expectations that the developmental toolkits of morphologically simple basal metazoans are significantly less complex than these of complex eumetazoans. Sponge monophyly has been questioned by some analyses, which suggested that siliceous sponges were the first to split from the animal tree of life, followed by calcisponges and homoscleromorphs. Correct or not, this scenario highlights the need to investigate genomes of representatives of all three sponge lineages.

We have used next generation sequencing, mainly Illumina, to sequence the genome of the calcareous *Sycon ciliatum* and annotate it with extensive transcriptome dataset. We have first focused on selected components of the developmental toolkit: signaling pathways (wnt, tgf-beta and hedgehog) and transcription factors (t-box, sox, fox, homeobox and bhlh families). Many of these gene families are significantly larger in *Sycon* than in *Amphimedon*, and in several the gene numbers within *Sycon* subfamilies exceed these in eumetazoans. Phylogenetic analyses indicate that gene family expansions leading to complexity in *Sycon* are independent of these leading to complexity in the eumetazoans.

To gain insight into evolutionary history of this complexity, we have next sequenced genome and transcriptome of *Leucosolenia complicata*, a distantly

related calcaronean with a different body plan. With some minor differences, the two genomes appear to contain equally complex developmental toolkits, suggesting the expansions of the families predates or coincides with the emergence of calcaroneans. We are currently sequencing genomes of two *Clathrina* species, representing Calcineans, a sister group to the Calcaroneans.

### **Tissue homeostasis in sponges: defining 'growth states' via cell proliferation and cell loss**

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Sponge biomass is continuously controlled through the balance of cell proliferation and cell loss, also known as tissue homeostasis. These essential processes in sponge physiology vary according to different growth states, such as regenerative wound healing, somatic growth and steady state. In the latter, rapid cell proliferation is matched with high levels of cell loss resulting in cell turnover rather than net increase in biomass. This 'steady state' was first observed in the tropical reef sponge *Halisarca caerulea*. In this study we aim to define growth states in sponges by investigating cell turnover during different growth states over various time scales, from hours to days to weeks. For steady state conditions, sponge species from various ecosystems and habitats were studied, including Caribbean reef (*Halisarca caerulea*, *Scopalina ruetzleri* and *Chondrilla caribensis*), Caribbean mangrove (*Mycale microsigmatosa*) and the Mediterranean (*Chondrosia reniformis*). *H. caerulea* was used as a model species for regenerative wound healing and somatic growth. Cell turnover was investigated using a combination of morphological studies with immunohistochemical techniques on histological thin sections (light microscopy and TEM). Cell proliferation was determined via the incorporation of the thymidine-analogue BrdU (5-bromo-2-deoxyuridine) into newly synthesized DNA. To study cell loss, shedding of old cells was quantified by collecting expelled cells in the form of detritus, whereas apoptosis was identified using antibodies against active caspase-3. Growth states were identified by distinctive patterns of cell proliferation and cell loss; during steady state, a high percentage (20-50%) of choanocyte proliferation was balanced by large amounts of cell shedding and subsequent detritus production, and minimal apoptotic activity. During regenerative growth, *H. caerulea* showed <1% of total cell proliferation and instead migration of cells towards the wound was observed. Cell loss via shedding was also reduced in regenerative tissue. The identification of cell turnover patterns involved in sponge growth states provides insight into

fundamental physiological functioning of sponge tissue and may assist in the recognition and optimization of sponge growth in vivo and in controlled culture.

### **For the Record: An update on the sponge fauna of the Sahul Shelf Province**

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The Sahul Shelf Province is a marine bioregion extending across northern Australia and the Gulf of Papua. It includes three ecoregions (Bonaparte Coast, Arnhem Land to Gulf of Carpentaria, Arafura Sea). A total of 224 valid species have been recorded for this bioregion according to the World Porifera Database (WPD), but this represents only 20 % of the total number of morphospecies (or Operational Taxonomic Units, OTUs) known for the area. During two recent multidisciplinary surveys in the Joseph Bonaparte Gulf approximately 700 sponge specimens were collected using an epibenthic sled from 65 sites, between 22-197 m depth. The specimens were assigned to 283 species and OTUs, representing three classes, 53 families and 117 genera. The majority of species belong to the class Demospongiae, at least four species to Calcarea and one species to Hexactinellida (collected at 102 m). Within the Demospongiae a total of 13 orders were represented; the number of species/OTUs within these orders vary from two chondrosids to 55 poecilosclerids. Only 20% of all OTUs could be assigned to a named species. At least 48 genera and six species represent new records for the Province, and 75% of all dendroceratid species and 41% of lithistid species represented new regional records. The most common species were *Xestospongia testudinaria*, *Scleritoderma* sp. 1, *lanthella falbelliformis*, *Oceanapia* sp. 1, *Echinodictyum mesenterinum*, and *Microscleroderma* sp. 1. The majority of species (64.2%) were collected only at one station. Some species commonly found in shallow waters (above 13 m) were also found in deeper regions of the shelf (e.g. *Reniochalina stalagmites* collected at 197 m) indicating that sponges might have larger depth ranges than previously thought. The number of species/OTUs (224) is approximately the same as that recorded for other areas within the Sahul Shelf (e.g. Darwin and Cobourg Peninsula (274 spp) and Wessel Islands (315 spp)) which have been sampled with larger efforts (87 and 133 sites respectively) and are considered biodiversity 'hotspots'. Species composition among ecoregions of the Sahul Shelf are likely to be similar, and comparisons with OTUs previously recorded are currently in progress

**Sponge-Seagrass interactions: Investigating the interaction between turtle grass (*Thalassia testudinum*) and *Halichondria melanadocia***

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Seagrasses are an important foundational species in nearshore ecosystems worldwide. They serve as nurseries, feeding grounds, stabilize sediments, and store carbon. Because of their ecological importance, understanding controls on seagrass productivity is critical. Bahamian nearshore waters host extensive beds of turtle grass (*Thalassia testudinum*; hereinafter seagrass) that support diverse invertebrate communities, including many species of sponge. In this study, we investigated the interaction between seagrass and *Halichondria melanadocia* (hereinafter sponge), which commonly grows around the base of seagrass shoots in Bahamian waters. Seagrasses in Bahamian waters are often nutrient limited and many species of sponge are known to increase nutrient availability through transformation of nitrogen into forms usable by primary producers. We hypothesized that the presence of sponge will impact seagrass productivity through one of two mechanisms: shading of seagrass (decreased productivity) or increased nutrient availability as a result of sponge presence (increased productivity). We quantified the percent cover of seagrass and the abundance of sponge at six sites surrounding Abaco Island, Bahamas. Seagrass shoots growing in close proximity with and without sponges growing were analyzed for nutrient content. Finally, we determined the effect of sponge presence on the growth of seagrass. Seagrass may facilitate sponge abundance by providing substrate for the sponge, as the density of sponge increased with increasing seagrass cover. There is no evidence that sponges provide nutrients to the seagrass as there was no significant difference in the morphology or nutrient content within paired seagrass samples. Our results suggest that *H. melanadocia* and *T. testudinum* have a commensal relationship where the seagrass is unaffected by the sponge while the sponge benefits from settlement substrate provided the seagrass.

**Haplotype versus morphotype of cosmopolitan sponges (Porifera: Demospongiae) in Indonesian Archipelago**

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The Indonesian archipelago is located in the Indo-West Pacific (IWP) centre of marine biodiversity and has various unique marine habitats distributed around thousands of islands. The Indonesian sponge (Porifera) fauna is still insufficiently studied, which poses major obstacles for marine area management, as sponges are important indicators for marine habitat monitoring. Situated between the Pacific- and the Indian Ocean, the archipelago is characterized by sometimes-strong ocean currents, especially around the only tropical pathway for the inter-ocean exchanges. These strong currents are likely to influence the marine larval dispersal among reefs, as it has been shown for various marine taxa. Although sponges are relatively simple animals, they have complex characteristics. However, they are difficult to identify due to their low numbers of defining taxonomic characters. Many cryptic sponge species have been revealed in morphologically similar specimens through molecular methods. Here we studied four widespread and common Indonesian demosponge species, each from a different order: *Clathria reinwardti* (Poecilosclerida), *Stylissa massa* (Halichondrida), *Aaptos suberitoides* (Hadromerida) and *Hyrtios erectus* (Dictyoceratida), aiming to a better understand the sponge diversity of Indonesian waters. We examined haplotype diversity using mitochondrial- and nuclear DNA sequence markers as well as morphological characters such as spicules morphometrics, skeletal architecture and outer morphology features. From the molecular studies we found that each species constitutes of several haplotypes although with similar morphotypes, which showed that they might be candidates for speciation. This might have implications for marine area management, as it will influence the understanding of sponge diversity of Indonesian waters

**The impact of petroleum exploration on deep sea sponges: A physiological, cellular and molecular approach**

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Anthropogenic sediment stress on the deep sea has increased substantially in recent decades, driven by oil and gas exploration expanding to the continental shelf and beyond. Large-scale physical disturbance of the seafloor induced by exploration drilling of the hydrocarbon industry releases extensive sediment plumes into the water column, and induces large deposition events of drilling wastes that could impact nearby benthic communities. In Norway, more than 250,000 tonnes of drilling wastes (i.e. drill cuttings and drilling muds) are released into the ocean annually. However, the impact of these plumes and sedimentation events on deep sea organisms has lacked rigorous scientific investigation. The documented sensitivity of sponges to suspended sediments and sedimentation events has increased concerns regarding the impact of petroleum exploration on mass aggregations of sponges commonly encountered on the continental shelf of Norway.

Using the sponge *Geodia barretti*, a common sponge on the Norwegian continental shelf; we investigated the sensitivity of the deep sea sponge to drilling wastes. A series of short (12 hour) and longer-term (6 and 14 day) laboratory experiments were undertaken to test the effects of environmentally realistic concentrations of commonly used drilling muds (bentonite and barite) and natural sediments on the physiological (pumping and respiration), cellular (lysosomal membrane stability, DNA damage, lipid peroxidation and glutathione) and molecular (metabolomics) responses of *G.barretti*. This experimental study provides the first documented evidence that deep sea demosponges are sensitive to elevated suspended sediment concentrations, resulting in decreased physiological functioning, reduced cellular viability and shifts in metabolomic profiles.

Exposure of *G.barretti* to the three different suspended sediment types initiated different physiological, cellular and molecular responses. Short-term exposure to all sediment types initiated a shutdown in pumping activity, lasting the duration of the exposure period. However, changes in cellular and metabolomic biomarkers of *G.barretti* were only initiated during exposure to barite, and not to natural sediments or bentonite exposures. Furthermore, longer-term exposure of *G.barretti* to suspended barite further elevated cellular and molecular effects, while exposure to natural sediments did not significantly initiate cellular or molecular responses. The results demonstrate the sensitivity of deep sea sponges to increased anthropogenic sediment stress.



**Sponge Gardens, Sponge Metabolism and Benthic Pelagic Coupling:  
Where does all the carbon go?**

Chris Battershill, Conrad Pilditch  
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Across an average sponge garden, resident sponges can process 100ml seawater s<sup>-1</sup>m<sup>-2</sup>, frequently filtering upto 95% of potential food particles present, to a size of 50µm. In a series of feeding experiments approximately 20 µg C.l-1 POC, 120 µg C.l-1 DOC and 360 µg C.l-1 ultraplankton were retained (Bannister et al, 2013). This equates to 8,600 l seawater per m<sup>2</sup> of an average 'sponge garden' per day being processed by resident sponges accounting for over 4.3t C per day per km<sup>2</sup> of reef flat. Sponge covered reef walls are likely to process considerably more. Sponge metabolism can also be extremely high with, for example, 20kg of sponges consuming all oxygen in a cubic meter of water in 30minutes. However, despite high levels of apparent feeding activity, the consumption of carbon isn't reflected in growth, indeed most sponge communities remain highly stable in population density and individual size over time. Where does the carbon go? Is it burnt in metabolism? What is the contribution to boundary layer CO<sub>2</sub> and how do microbial symbionts interact with sponge metabolism?

We examine benthic carbon flux associated with sponge communities in order to assess the importance of sponge gardens to benthic - pelagic coupling and raise hypotheses as to the role of sponges in contributing to benthic carbon budgets.

**Sponge-dominated reefs as a potential future trajectory for coral reefs**

James Bell  
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Sponges represent one of the dominant fauna on many coral reefs and there is increasing evidence from experimental studies and field observations that sponges may be one group to benefit from declines in coral as a result of environmental degradation, ocean acidification and rising sea surface temperatures. While the coral reef scientific community is largely focused on changes to algal-dominated states, sponge reefs already exist and I will discuss my work and the wider evidence to support my view that further increases in sponge abundance will occur in the future. I believe this is particularly possible in the Indo-Pacific where shifts to algal dominated systems in response to declining environmental quality appear less widespread than in the Caribbean. I will discuss the impacts of changing environmental conditions on population dynamics of individual sponge species as well as assemblage level effects. I will also consider the potential for sponges to benefit from environmental change and degradation, and consider subsequent reef ecosystem impacts. Of particular interest are giant barrel sponges, *Xestospongia* spp., given their ecological

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significance, longevity and widespread distribution. I will discuss recent insights into the population biology of *Xestospongia* spp. inferred from genetic data and population studies, and their apparent resilience to disturbance events despite low dispersal rates and heavily reliance on self-recruitment. I will also discuss potential trajectories for Indo-Pacific reefs where sponges are the dominant fauna and how this might influence overall reef ecosystem functioning. This includes recent observations on transitions to more sponge-dominated states at Palmyra Atoll in the central Pacific, in the Wakatobi Marine National Park, Indonesia and at Bahia in Brazil. These systems provide examples of what a more sponge-dominated reef future might look like, and will help us to better understand how reefs can be managed in the future.

### **Stability of a sponge community of a Mediterranean rocky cliff during a 25 year period**

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During the last decades, Mediterranean shallow water benthic communities have experienced significant changes in taxa composition and distribution. They were due to mass mortality events, occurred from 1999 to 2006, to emergence of species once considered rare and depletion of others, previously considered abundant. These variations can be related to current climatic changes that, in their turn, have influenced the diffusive capacity of some species, by modifying their spatial and bathymetric distribution range. To evaluate these changes on long-living species with a limited larval dispersal capacity, as most sponges are, a quantitative knowledge of the hard bottom community structure through a pluri-decennial span of time is required.

Aim of this work is the study of a sponge community settled on a rocky cliff of the Portofino Promontory (Ligurian Sea) from 15 to 20 m depth. The evolution of the community was studied by comparing the photographs of 16 rectangular frames (50 x 70 cm) taken in in the same season (November 1987 and 2012) after a time lapse of 25 years. In both periods, the benthic assemblage was quantitatively dominated by macroalgae, sponges and the zoanthid *Parazoanthus axinellae*.

The assemblage was deeply changed in the 25 year span, mainly due to variations in algal covering. The three species more diffused in 1987 (*Peysonnellia squamaria*, *Flabellia petiolata* and *Halimeda tuna*) reduced up to 95% their original covering and were replaced by a turf of filamentous algae. On the contrary, the sponge community remained almost unaltered, both from a quantitative and qualitative point of view. The main components of this

community were *Agelas oroides*, three species of *Axinella* (*A. damicornis*, *A. verrucosa* and *A. polypoides*), a group of encrusting red or orange species (mainly *Crambe crambe* and *Spirastrella cunctatrix*). All these species showed differences lower than 10% in their covering. Also the number of individuals of the same species remained virtually unvaried.

These data indicate that the considered assemblage shows low competitive patterns, a strong resilience of sponges in the space occupation and a very low consumer pressure, determining a high stability of the sponge community during a very long time.

**Wnt pathway components in the sponge *Halisarca dujardini*  
(Demospongiae)**

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In Eumetazoans, Wnt pathway is involved in multiple processes during development and regeneration, including cell-fate determination, proliferation and death, polarity, adhesion, and motility. In sponges, Wnt pathway is implicated in patterning of body axis during embryonic development and adulthood, as well as in ostia formation. We are thus particularly interested in function of the Wnt pathway during regeneration in sponges. We expect it might play roles in patterning of aquiferous system, in regulation of regeneration process and/or as guide for cells de- and transdifferentiation. We have used Illumina technology to sequence transcriptome and to generate preliminary draft assembly of the genome of *Halisarca dujardini*, a demosponge whose development and regeneration is well studied at morphological level. We identified multiple Wnt pathway components, including nine Wnt genes, in addition to dishevelled, beta-catenin and frizzled. We are now using combination of semi-quantitative RT-PCR and in situ hybridization to assess expression of these genes in different parts of the body (especially the osculum and the periphery) and during regeneration in the wound tissue. We expect our results will bring insights into molecular mechanisms of axis patterning in demosponges, and by comparison with other metazoans, into evolutionary history of symmetry establishment mechanisms.

**Chemical diversity of marine bacteria *Salinispora arenicola* and “*Salinispora pacifica*” associated with the host sponges *Dercitus xanthus* and *Cinahreya australiensis***

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**Background:** Understanding the pattern of bacterial secondary metabolite production can provide important information about ecological interactions for evolutionary responses related to environmental changes. Marine sponges are known to harbour diverse populations of microbes that produce a vast array of bioactive natural products (Taylor et al. 2007). Host specificity ? the association of organisms with only a few or many host species ? is recognized in eukaryotic ecology as a key determinant of diversity. However, its implications for microbial diversity have received little attention and as a result relatively little is known regarding the large scale metabolite production by these microbial species, particularly when they are associated with other organisms and their hosts.

**Objectives:** To investigate the (bio) chemical profiles of *Dercitus xanthus* and *Cinahreya australiensis* sponge-associated marine bacteria *S. arenicola* and ?*S. pacifica*?, collected from the Great Barrier Reef (GBR), off the north east coast of Australia.

**Methods:** Microbial extracts were prepared by solvent (ethyl acetate) extraction of bacterial cell mass, followed by solvent evaporation and reconstitution. Ultra High Performance Liquid Chromatography-Quadrupole Time of Flight Mass Spectrometry (UHPLC-QToF-MS) was used to acquire metabolic profiles with high mass accuracy and sensitivity. Multivariate analysis techniques: Principal Component Analysis (PCA), Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Hierarchical Clustering Analysis (HCA) have been used for the analysis of data in order to explore the underlying (bio) chemical diversity.

**Results:** Sponge-dependent metabolic diversity was evident in two bacterial species: *S. arenicola* and ?*S. pacifica*?, at their species and sub-species levels. In addition, we found a clear variation of bacterial metabolite production associated with the geographic location of collection.

**Discussion:** This study identifies clear patterns of species-, intra-species- and location-dependent secondary metabolite production in sponge-associated bacteria, thereby providing the basis for future studies of sponge-microbe interactions and relationships to predict the role of secondary metabolite production and how they are related to ecology and evolution.

**Reference:**

Taylor MW, Radax R, Steger D, Wagner M (2007). *Microbiol Mol Biol Rev* 71:295-347.

**Population connectivity in a reef-forming glass sponge, *Aphrocallistes vastus*: a Next Generation Sequencing ddRAD approach.**

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Reef-forming glass sponges, as ecosystem engineers, provide habitat vital to the maintenance of many marine invertebrate and vertebrate populations. Seventeen extant glass sponge reefs have been discovered since 1989 in the deep waters off the coast of British Columbia, Canada, the only place in the world they are known to exist. To prevent loss of these unique and critical habitats, Marine Protected Areas (MPA) are being designed around some, but not all, glass sponge reefs. However, although knowledge of modes of reproduction and connectivity between reefs would assist in the design of these areas, little is known due to depth-related difficulties with sampling and field studies, previously unknown reproductive season, and scarcity of larvae. We addressed this paucity of knowledge by studying population genetics and larval development of *Aphrocallistes vastus*, the primary reef-builder in the Strait of Georgia. Adult tissue samples bearing both gametes and embryos were collected in mid-December, indicating a December start to the reproductive season. Subsequent sampling suggests multiple pulses of reproductive activity. Population genetics provides information on dispersal distance, population structure, and genetic variability. Due to a high level of duplication in the genome and associated difficulties with utilizing microsatellite markers, a double digest Restriction Associated DNA (ddRAD) sequencing approach on the Ion Torrent Personal Genome Machine has been used to discover Single Nucleotide Polymorphisms (SNPs) de novo. An average of 1387 SNPs were discovered across 10 individuals. A selection of these were used as markers in 265 individuals sampled in and around the reefs through Genotyping by Sequencing (GBS). Specifically, SNPs were compared from individuals sampled within patches of a single reef, between patches of a single reef, between reefs, and between reef-forming and solitary individuals. Results from this work will allow for predictive models of genetic diversity in other glass sponge species, and will also provide valuable insight into the management requirements in the MPAs currently under discussion.

**Positive effects of canopy forming kelp on sponge assemblages through habitat modification**

César A Cárdenas, Simon K. Davy, James J. Bell  
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Competition, cooperation and facilitation play important roles in structuring benthic communities and the outcome of such interactions can have important implications for biodiversity patterns and ecosystem functioning. Sponges and

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algae are both common in shallow rocky shore environments and there has been considerable debate regarding the importance of sponge-algae relationships in determining sponge diversity and abundance patterns. While the majority of previous research has focused on negative interactions between these two benthic groups, there is also the potential for some sponge species to benefit from the presence of macroalgae through facilitation as a result of habitat modification. Here we aimed to examine positive interactions between sponges and algae, and the critical role that the canopy-forming kelp *Ecklonia radiata* plays in facilitating a suitable habitat for sponge assemblages. Experimental removals of *E. radiata* were conducted at two sites on rocky walls in New Zealand and monitored for 1.5 years. Relative to abundance at the beginning of the experiment, the abundance of some sponge species following canopy removal was up to 15 times lower 4-5 months weeks post removal. Canopy removal led to a community dominated by turfing algae, which corresponded with a decrease in sponge abundance and species richness. However, multivariate regressions showed that changes in ambient conditions (particularly increased irradiance) as a result of canopy removal, rather than an increased abundance of turfing algae, is likely to account for the changes in sponge assemblages. Our results highlight the facilitative role provided by *Ecklonia* canopy to sponge assemblages and how loss of canopy-forming species might have secondary effects on sponge assemblages, which could affect the flow of energy and the overall biodiversity occurring in these habitats. Our results also highlight the likely role that light, rather than direct algal competition has in explaining spatial variation for many sponge species in shallow water ecosystems.

### **Sponge biodiversity and ecology of the Van Diemen Rise, northern Australia**

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University of Waikato, Environmental Research Institute*

Australia is increasingly recognised as a global hotspot for sponge biodiversity, but there is a large knowledge gap about sponge communities in northern Australia. Such information is particularly important to manage and monitor the Commonwealth Marine Reserves network finalised by the Australian Government in November 2012. This study aims to quantify sponge biodiversity of the Van Diemen Rise and eastern Joseph Bonaparte Gulf in northern Australia and to examine spatial and environmental patterns associated with differences in community structure of sponges. Two collaborative surveys were undertaken in 2009 and 2010 as part of the Australian Government's Offshore Energy Security Initiative. Sponges were collected with a benthic sled from 65 sites across a range of geomorphic features (bank, terrace, ridge, plain, valley) and environmental variables (depth, distance offshore, substrate hardness, slope).

Approximately 700 sponge specimens were collected and assigned to 283 species, representing three classes, 53 families and 117 genera. Results showed that sponges were positively and significantly related to other taxa in respect to richness and biomass, thus highlighting their important role in habitat provision. Distance offshore and geomorphic features affected community structure, species richness and biomass. In general, sponge diversity was highest further offshore and on raised geomorphic features. Sponge assemblages collected from the same bank were more similar than those collected from different banks. There were no strong relationships between sponges and other environmental factors. The current study will help facilitate integrated marine management by providing a baseline species inventory, supporting the listing of carbonate banks of the Van Diemen Rise as a key ecological feature, and highlighting the importance of sponges as habitat providers and potential biological surrogates for monitoring activities.

**Use of underwater videography and still imagery for sponge biodiversity habitat mapping and modelling**

Andrew Carroll, Maggie Tran, Rachel Przeslawski  
*Geoscience Australia*

Understanding the distribution and abundance of sponges and their associated benthic habitats is of paramount importance for the management and monitoring of the marine estate, especially marine reserves, due largely to the role of many sponges as habitat providers for other biota. Benthic sleds or trawls can collect specimens for taxonomic and genetic research, but these sampling methods can be too qualitative for many ecological analyses and too destructive for monitoring purposes. Underwater videography and still imagery have been used by Geoscience Australia (GA) and the NERP Marine Biodiversity Hub to extract data related to sponge biodiversity patterns. Here we present three case studies to showcase how underwater imagery has been used in relation to sponges. In the new Oceanic Shoals Commonwealth Marine Reserve, sponge morphologies were characterised from still images to identify areas in which biodiversity may be high due to habitat-forming taxa. In a different study from the same region, abundance of a target sponge (*Cinachyrella* sp.) was quantified from video to investigate relationships between biology and sediment characteristics. Around Lord Howe Island, benthic habitats are being analysed to the CATAMI (Collaborative and Automative Tools for Analysis of Marine Imagery and video) national standard of classification using both video and still images. This research demonstrates the importance of integrating biological and physical data to provide unique and meaningful maps of predicted distributions and habitat suitability for key ecological benthic habitats. As one of the major groups of habitat-forming fauna in many regions, sponges are an ideal biological parameter to be considered in broadscale habitat mapping and classification.

**Lithistid demosponges of the Macaronesian Islands and Northeast Atlantic Seamounts: Diversity, distribution and phylogenetic relationships**

Francisca Carvalho, Shirley Pomponi, Paco Cárdenas, James Harris, Joana R Xavier

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Lithistid' Demospongiae sensu Pisera & Lévi, 2002 is a much understudied polyphyletic group of sponges usually found at bathyal or bathyal-like environments, such as caves, in tropical and warm temperate regions.

In this study we investigated the diversity, distribution and phylogenetic relationships of the lithistid sponge fauna of the northeast Atlantic with a focus on the Macaronesian Islands (Azores, Madeira, Selvagens and Canaries) and neighbouring seamounts (of the Great Meteor group).

Specimens were collected in the course of several research campaigns conducted by the Azores University (DB and DOP) in the Azores EEZ, by the MNHN of Paris in the seamounts southern of the Azores (the Seamount-2 campaign) and by the Harbor Branch Oceanographic Institute in Madeira, Selvagens and Canary islands.

A total of 180 specimens were analyzed and assigned to 11 species representing five families and nine genera. Eight species were recorded for the first time on the seamounts southern of the Azores, of which seven are shared with this archipelago. Three species constitute new records for Madeira island (*Neophrissospongia nolitangere*, *Discodermia verrucosa* and *Racodiscula clava*), two for Selvagens (*N. nolitangere* and *Macandrewia azorica*), and two for the Canaries, of which one is a new species for science of the genus *Isabella*. Of the 14 species found to occur in the Macaronesian islands, only three viz. *N. nolitangere*, *Leiodermatium pfeifferae* and *R. clava* are shared among all archipelagos, the remainder being restricted to one or two island groups. Similarly, only three species seem to be shared between the Mediterranean Sea and these Atlantic archipelagos: *Neoschrammeniella bowerbankii* (Madeira); *Siphonidium ramosum* (Azores) and *L. lynceus* (Azores and Canaries).

Phylogenetic reconstruction, by means of Bayesian analyses, of the mtDNA COI gene has confirmed the astrophorid affinity of the studied species (families Corallistidae, Macandrewiidae and Theonellidae). Species and genera were always retrieved as well-supported monophyletic clades. The family Corallistidae is monophyletic (posterior probability of 0.97) and a sister-taxa relationship was found between the genera *Neoschrammeniella/Neophrissospongia* and *Herengeria/Isabella*.



Ongoing efforts aimed at increasing the taxonomic and geographical coverage of this study, and including additional genetic markers, will allow us to further clarify the phylogenetic relationships and contribute to a new classification of lithistid sponges.

**Reproductive cycle and gametogenesis in the coral excavating sponge  
*Cliona delitrix***

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The excavating sponge *Cliona delitrix* is one of the most abundant and coral destructive species on Caribbean reefs. However, basic aspects of its reproductive biology and the effect of environmental conditions on its propagation are unknown. A two year study was performed on a *C. delitrix* population located in a shallow reef in Florida (USA) to describe the reproductive cycle. Tissue from tagged and randomly collected sponge individuals was sampled periodically and light microscopy used to identify, count, and measure reproductive products. TEM was also used to describe the cytology and ultrastructure of gametogenesis. The relationship between reproductive activity and local sea water temperature was examined by recording temperature values using Hobo loggers at 20m depth. *C. delitrix* is oviparous and gonochoric, except for few individuals that were simultaneous hermaphrodites. Spermatogenesis started by transdifferentiation of choanocyte chambers, whereas oogenesis appeared to start from single archaeocytes. Granulose cells may act as nurse cells that contribute to the growth and maturation of both of male and female gametes. These cells were phagocytosed by oocytes of all sizes and also occurred mixed within spermatogenic cells since initial stages of development. In addition, in late oogenesis, granulose cells formed an envelope around each mature oocyte. Oogenesis was slow, with oocytes of different cohorts co-occurring in the females year round. Spermatogenesis was faster, starting in May, when water temperature reached approximately 25°C, and lasting until October or December, depending on the year. Unlike in other oviparous sponges, the reproductive period of *C. delitrix* does not culminate with a single pulse of gamete release per year. Rather it appeared to be at least six successive spawning events. This extended spawning decreases the risk of massive offspring mortality in the case of local adverse events, and it is thought to increase the chances of successful colonization over different reef habitats. The spatial-temporal scale in which coral mortality events and *C. delitrix* reproduction and larvae attachment occur play an important role in the settlement and recruitment success of this excavating sponge.

**A modified Molybdosilica method for rapid biogenic silica content determination in keratose sponges**

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The keratose sponges (Order: Dictyoceratida) engulf a certain amount of foreign particles to strengthen sponge internal structures. Not only non-biogenic silica (n-BSi) particles, these sponges also use biogenic silica (BSi) particles such as spicules for skeleton construction. Traditionally, BSi content in sponges is obtained by measuring skeleton weight after soft tissues removed. Alternative approach is to dissolve spicules by HF or NH<sub>4</sub>F, then calculate the difference before and after treated-samples. However, in keratose sponges, such quantification may result in an overestimation of BSi by additional n-BSi particles. In the present study, a molybdosilicate method, commonly used in the quantification of diatom frustule and other BSi in sediment cores, was applied to the quantification of BSi content in sponges containing not only BSi but also n-BSi particles. This method involves the digestion of BSi by alkaline solutions and the measurements of the molybdate-silica reactive yellow by spectrophotometer. With 8.5-9 hrs digestion in 2-M KOH solutions, little dissolution on n-BSi particles has been found. The BSi contents were also measured in keratose sponge, *Spongia* sp. and the *Xestospongia* sp. which contained native skeletons. The results indicated molybdosilicate method is applicable for BSi determination in keratose sponges that containing both BSi and n-BSi particles. With this method, the knowledge on sponges with foreign spicules can be further strengthened.

**Genetic marker assessment for demosponges**

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One of the primary challenges facing the sponge community, and more broadly benthic ecologists, is the further development of databases of genetic marker data that allow for reliable taxonomic inference at the species level and above. The US-based Porifera Tree of Life project (PorToL), bolstered by numerous international collaborators, has derived several different molecular markers from a large number of sponge specimens, including many newly represented type species. These markers include mitochondrial COI, COII, ATP6, and nuclear ALG11, 18S and 28S and each has different characteristics of interest. Given the primers we have used, some have been much more likely to result in contaminants, e.g., COI and regions of 28S. Some have proven to be comparatively easier to derive, including 18S, COII and larger regions of 28S. All of these markers can help refine taxonomic and phylogenetic affinities of different

species, but the easy to obtain 18S gene appears to be particularly useful for clarifying the higher taxonomic affinities of species. However, as expected, 18S evolves too slowly to resolve the identities of closely related species. COII shows the most promise, in terms of efficiency of derivation and rate of evolution, for being a reliable genetic barcode for sponges. We present the results of experiments employing an IonTorrent Personal Genome Machine to simultaneously sequence multiple genetic markers from a set of samples.

**Challenging the specificity of the marine sponge microbiome - a biogeographical approach**

Rodrigo Costa, Cristiane C.P. Hardoim, Ana I.S. Esteves, María A. Lago-Lestón, Cymon J. Cox, Joana R. Xavier  
*Algarve University/ University of the Azores*

Because of their conspicuous diversity and potential use as sources of novel bioactive compounds, marine sponge bacterial communities have been the focus of increasing research interest. Disentangling the specificity and spatial-temporal stability of these assemblages bears implications to our understanding of the distribution of genetic and metabolic resources in marine ecosystems. Here, we determine the diversity, composition and micro- and macro-scale distribution of bacteria in the phylogenetically close marine sponges *Ircinia variabilis* and *Sarcotragus spinosulus* (Demospongiae, Irciniidae). Sampling took place at three sites in the North Atlantic: the Algarve coast (continental Portugal) and the oceanic islands Madeira and São Miguel (Azores archipelago). Cytochrome oxidase gene phylogeny showed high genotypic conservation within *I. variabilis* and *S. spinosulus* specimens from different sites. 454 pyrosequencing of 16S rRNA genes revealed that sponge bacterial communities sharply differed from those of sediments and seawater. More than 20 bacterial phyla were detected in c. 300.000 high-quality sponge-derived bacterial sequences. Usually, 80 to 100 ?species level? phylotypes belonging to ? 8 bacterial phyla were detected per sponge specimen. The examined species shared about 47% of all detected symbionts within the same habitat, whereas each sponge host maintained a considerable pool of common bacteria across habitats. For instance, *I. variabilis* specimens from all sites had 18% of all their symbionts in common. This proportion rose to 46% when only specimens from the oceanic islands were taken into account. The core microbiome of *I. variabilis* and *S. spinosulus* consisted of diverse, uncultured symbionts belonging to the phyla Acidobacteria, Actinobacteria, Chloroflexi, Poribacteria and Proteobacteria. FISH-CLSM demonstrated that prevailing symbionts were intrinsically associated with sponge cells and occurred at high densities in close proximity. Reduced diversity and selection of rare, generalist members of the sponge microbiome were observed for hundreds of bacteria cultured from the samples. However, this approach enabled the first-time detection of polyketide synthase genes in *Aquimarina* spp. (Bacteroidetes) and uncovered 10 putative novel bacterial species. Our findings

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suggest intricate and complementary roles of host genotype and environment in shaping the marine sponge microbiome, highlighting great biotechnological interest for Irciniidae sponges and their diverse bacterial symbionts.

### **Evaluation of differential protein expression in *Haliclona aquarius* and sponge-associated microorganisms under cadmium stress**

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A comparative proteomic approach was used to assess differentially expressed proteins in marine sponges after 36 h of exposure to cadmium (Cd). After separation performed by 2-D polyacrylamide gel electrophoresis, 46 protein spots indicated differential expression, and 17 of these proteins were identified by electrospray ionization quadrupole time-of-flight mass spectrometry. From the proteins identified, 76 % were attributed to sponge-associated microorganisms (fungi and bacteria), and 24 % were attributed to *Haliclona aquarius*. Some of the proteins that were identified may be related to cell proliferation and differentiation or processes of oxidative stress repair and energy procurement. An integrated evaluation based on spot expression levels and the postulated functions of these proteins allowed a more accurate evaluation of the stress caused to the sponge holobiont system by cadmium exposure. This study could provide new insights into the use of a proteomic approach in the marine sponge to assess the effects of Cd pollution in a marine environment.

### **Re-interpretations of the evolution of fibre skeletons within *Verongida***

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Matterson

*Museo Marino/HBOI/ University of Alabama at Birmingham/ NMNH, Smithsonian  
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As part of various expeditions of the Porifera Tree of Life project (PorToL) we encountered a dozen apparently "skeleton-less" specimens, which had patterns of coloration and oxidation typical of taxa of the order *Verongida* (*Demospongiae*). These taxa were divided in two groups. The first group inhabited open and cryptic habitats of shallow (15-20 m) Caribbean reefs at Bocas del Toro Archipelago, Panama. The second group inhabited sciophilous habitats on shallow reefs (0.5-20 m deep) of Moorea, French Polynesia. We combined external morphology, histological observations, and sequence data

from nuclear 18S rRNA and mitochondrial COI genes to determine their identities and phylogenetic relationships. 18S analyses revealed that none of the species studied belonged to the genus *Hexadella* (lanthellidae, Verongida), the only fibreless genus of the Order Verongida as currently recognized. The samples from Panama belong to the family lanthellidae, and are closely related to the Pacific genera *lanthella* and *Anomoianthella*, both with well-developed fiber reticulations. The samples from Moorea, included three distinct taxa, all diplodal, and with high affinities to a clade containing *Pseudoceratina*, *Verongula*, and *Aiolochoira*. The evolutionary and ecological meaning of having or lacking a fiber skeleton within Verongida is challenged under the evidence of the existence of fibreless genera, within three distinct verongid clades: a non-*Hexadella* lanthellidae clade, a distinct *Pseudoceratinidae* clade, and a distinct branch within the genus *Pseudoceratina* clade. The encounter of a fibreless *Pseudoceratina* species embedded within dead coral skeletal structures suggests that the possession of a spongin-chitin fiber reticulation is a plastic trait, that might be lost or acquired under certain environmental conditions. These results raise new questions about the ecological and evolutionary significance of the development of a fibre skeleton within verongids, and of sponges' adaptability to the environment. The variability on the fibre skeleton within well supported phylogenetic clades (i.e. clade containing *Pseudoceratina* and *Verongula* taxa) suggests the lack of phylogenetic signal both for the presence of a fibre skeleton and for traditionally morphological traits within the Verongida. Moving forward with the systematics of the order should benefit from the observation that most genera are fairly well supported within our analyses. However, the morphology and production of fibre skeleton appears to be too plastic and variable to delineate suprageneric taxa.

**Is Habitat Heterogeneity an Effective Surrogate for Temperate Rocky Reef Biodiversity?: Implications for Marine Protected Areas**

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Marine Protected Areas (MPAs) are seen as an effective means to manage and conserve marine resources, but their placement is often based on ad hoc decisions. We sought to determine the potential of remotely sensed habitat complexity as a surrogate for rocky reef sessile invertebrate assemblages (as well as fishes). We focused on a network of MPAs at Batemans Marine Park, southeastern Australia. We used high-resolution bathymetric side-scan sonar imagery to quantify features of mid-depth (20-40m) rocky reef. We explicitly examined the relationship between measures of habitat complexity and sessile invertebrate diversity and abundance. We selected Generalized Additive Mixed Models (GAMMs) to explore these relationships, to account for the spatial autocorrelation present in our data. Sponges dominated reefs and habitat complexity was a powerful predictor of their diversity and abundance; explaining

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more than 70% of the variation we observed. By far the most important predictor was vertical relief within a 75 metre radii seascape scale; it was positively associated with sessile invertebrate abundance and diversity up to a threshold of 12 metres in vertical relief. In contrast, bathymetric variables were poor predictors of fish richness and abundance. Overall, we contend that habitat complexity could be a useful and cost-effective surrogate to indirectly determine areas of conservation value for certain temperate rocky-reef assemblages. This information is valuable for future MPA development and design.

### **A sponge-driven analogue to the microbial loop supports energy and nutrient retention on coral reefs.**

Jasper de Goeij, Dick van Oevelen, Mark Vermeij, Ton de Goeij, Ronald Osinga, Wim Admiraal

*University of Amsterdam - IBED Aquatic Ecology and Ecotoxicology/ Royal NIOZ Yerseke/ CARMABI Foundation/ Maastricht University - Department of Pathology/ Wageningen University and Research Centre - Aquaculture and Fisheries*

Since Darwin's early observations, scientists have debated how coral reefs, one of the most productive and biologically diverse ecosystems in the world, acquire and maintain energy and nutrients in the marine equivalent of a desert. The largest pool of organic matter present on reefs is dissolved organic matter (DOM) from primary production, but this source of energy and nutrients is traditionally considered to be available only to prokaryotes. The transfer of DOM to higher trophic levels by free-living bacteria, a phenomenon defined as the microbial loop, is largely insufficient to explain the DOM retention on reefs. Here we show an additional pathway for DOM recycling driven by sponges, which was tested in aquarium incubation experiments and verified in a field study using  $^{13}\text{C}$  and  $^{15}\text{N}$  as food web tracer in reef cavities. To prove the existence of aforementioned energetic feedback loop on coral reefs, we sought to test three predictions of this hypothesis: (1) that sponges are capable of DOM uptake; (2) that sponges convert DOM into detritus (POM); (3) that sponge-derived detritus is taken up by detritivores and transferred to higher trophic levels. Sponges assimilate and convert DOM into rapidly proliferating cells that are subsequently shed as detritus taken up by benthic animals. This 'sponge loop' thus funnels energy and nutrients into particulate fractions, preventing the expected dispersion and loss of DOM from the reef, and facilitates secondary production. However, we suspect that the efficient shunt of nutrients by sponges may also catalyse reef degradation during eutrophication. Sponge DOM turnover is therefore likely to play a pivotal role in the cycling of energy and nutrients on coral reefs and in other oligotrophic ecosystems where sponges are abundant.

## **Sponge distribution patterns across the Indonesia archipelago**

Nicole de Voogd  
*Naturalis Biodiversity Center*

The coral reefs of Indonesia are among the most diverse in the world. No other area contributes as much to overall marine diversity, which should make it a primary target for research, protection and management. Despite this realization we still know very little about how marine diversity is spatially distributed especially among lesser-studied invertebrate taxa such as sponges. The published literature on sponge taxonomy of Indonesia remains incomplete, although more than 850 species are thought to be present. In addition, sponge faunas from different geographic regions are difficult to compare when different authors describe these.

Here I present a study on sponge distributional pattern from various regions, across the Indonesian archipelago. This study is a result of several surveys done in the past 10 years and yielded a collection of approximately 2000 specimens belonging to 400-500 species.

This study revealed that in addition to regional endemics, only few species are common, but are known having a wide Indo-Pacific tropical distribution and very broad ecological amplitude, from very pristine coral reefs to very disturbed habitats. Besides, at a regional level, sponge assemblages are spatially heterogeneous and this heterogeneity is strongly related to deterministic (environmental) processes and habitat heterogeneity. These results highlight the need to assess and protect marine areas in biodiversity hotspots such as Indonesia.

## **The *Amphimedon queenslandica* genome and the pursuit to understand why sponges aren't like other animals.**

Bernie Degnan  
*University of Queensland*

The sequencing of the first poriferan genome revealed some surprising truths about the ancestor from which evolved all contemporary animals. Specifically, comparison of the genome of *Amphimedon queenslandica*, a haplosclerid demosponge, with the genomes of eumetazoans (vertebrates, insects, cnidarians and their allies) has identified deeply conserved genomic features that have been maintained over some 700-800 million years of independent evolution. Presumably, these underpin the indispensable regulatory and structural systems necessary for animal multicellularity. In the first part of my talk I will discuss genomic features that unite sponges with the rest of the animal kingdom and show that many of these features arose along the metazoan stem before the divergence of sponge and eumetazoan lineages. These constitute the 'zootypic' genomic ground plan and are likely to be responsible for a range of canonical

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metazoan-specific traits, including cell cycle control and growth, development, somatic- and germ-cell specification, cell adhesion, innate immunity and allorecognition.

From this deeply conserved 'zootypic' genomic ground plan arose an array of distinct animal body plans, varying in complexity and size. At this time we have little understanding of how genomic differences between phyla relate to body plan differences. Arguably, comparing the sponge genome to representative eumetazoan genomes may be the best first step in identifying the genomic features underlying phyletic body plans. In the second part of my talk, I will describe our recent efforts to identify protein coding and non-coding sequences in the *A. queenslandica* genome that associate with the evolution of particular cell types or developmental processes, many of which are absent in sponges but present in eumetazoans. While identifying coding sequences that can be attributed to a phenotypic trait is relatively straight forward, understanding the regulatory networks that control the expression of these genes remains difficult. This information is largely housed in the non-coding part of genome, the so-called 'genomic dark matter'. To begin to address this issue we are using transcriptomics as a proxy for gene regulation. Developmental and morphological differences between animals can be revealed by comparative analysis of transcriptomes. For example, *A. queenslandica* possesses a near-complete set of genes to specify and build nerve cells, yet lack this cell type. This sponge does not co-regulate neuronal genes to the same extent as neural eumetazoans, suggesting that the evolution of a regulatory circuitry that underlies the co-expression of neuronal proteins was critical for the formation of this cell type. This comparative approach along with other analyses of non-coding sequences in the *A. queenslandica* genome will be discussed.

### **Pattern and process in a threatened seagrass community: habitat use by sponges in *Posidonia australis***

Marie-Claire Demers  
*University of Wollongong*

The sessile epifaunal invertebrate community associated with seagrass meadows have been poorly documented despite the alarming rate of global seagrass decline. This sessile epifaunal invertebrate community likely comprises undescribed species possibly endemic to the seagrass being investigated, *Posidonia australis*. Considering that this threatened seagrass species has endangered populations in NSW, it is crucial to uncover its sessile epifaunal invertebrate assemblage which might have a similar risk of extinction. This is the first study to expand the baseline knowledge of the spatial distribution patterns of sponges in seagrass meadows and to determine the biotic and abiotic factors driving this distribution. The first component of my research was to design and trial a method of sampling sessile epifaunal invertebrates in seagrass



ecosystems based on limited scientific background information. Then, the abundance, volume, diversity and choice of substrata of sponges were sampled within P. australis meadows of Jervis Bay, NSW. Approximately 20 sponge species were found and catalogued. The assemblage was dominated by 5 species in terms of their volume. Sponges were ?patchily? distributed and their spatial distribution was dependent on multiple factors such as seagrass health, predation and the availability of substrata. Uncovering the spatial patterns of sessile epifaunal invertebrate distribution will help conserve undescribed species potentially endemic to P. australis, as well as to understand the processes determining their distribution.

### **Low Genetic Structure of lithistid sponge species between deep sea mounts off New Caledonia**

Merrick Ekins, Dirk Erpenbeck, Kathryn Hall, Monika Bryce, John Hooper  
*Queensland Museum/ Ludwig-Maximilians-University/ Western Australian Museum/ Griffith University*

Three species of lithistid sponges, *Neoaulaxinia zingiberadix*, *Isabella mirabilis* and *Neoschrammeniella fulvodesmus* from deep sea mounts of New Caledonia were tested for the relative contribution of sexual and asexual reproductive strategies to their populations. These rare and presumably ancient sponges have a restricted distribution to seamounts in the south western Pacific. These deep sea mounts represent geographically separated islands. These sponges due to their slow growing nature, with low sexual dispersal opportunities via larvae, are expected to have sexual reproduction restricted to near neighbours. To resolve this question of sexual and asexual contributions and to assess the population structure, these sponges were tested using ITS and CO1 sequencing.

### **Sponge Barcoding v.2 - much more than just barcodes**

Dirk Erpenbeck, Kathryn Hall, Merrick Ekins, Temi Varghese, John Hooper, Gert Wörheide  
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DNA taxonomic approaches and DNA barcoding are important tools for unambiguous identification of species and subsequent assessment of biodiversity, identification of cryptic speciation, understanding of biogeography, and numerous other applications in the recent years. The Sponge Barcoding Project ([www.spongebarcoding.org](http://www.spongebarcoding.org)) aims to facilitate unambiguous species identification for sponges using standard barcoding and other molecular markers. More than five years after its foundation, the Sponge Barcoding Project continues

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to establish a reference backbone database, particularly based on samples of the Queensland Museum (Brisbane, Australia), but also many other sources, including newly described species.

Scientific interest in sponges keeps increasing, making sponges excellent subjects for DNA barcoding approaches due to their difficult taxonomy and high morphological plasticity in combination with their ecological importance as filter feeders in almost all aquatic habitats, their evolutionary pivotal position as arguably the earliest-branching metazoan phylum, and their importance for biotechnological, biochemical and pharmaceutical purposes.

Data originating from the Sponge Barcoding Project are successfully applied to a wide range of evolutionary research: 1) Identification of sponge colonization patterns in freshwater habitats; 2) Detection of endemism among marine and freshwater taxa; 3) Phylogenies of pivotal demosponge taxa; 4) Reconstruction of skeleton evolution; 5) Identification of sponge chemotypes; 6) Unravelling cryptic shallow and deep-sea species. We demonstrate in several cases to what extent sponge DNA barcoding provides a powerful tool not only for taxon discrimination but also for multiple other applications in evolutionary research, biochemistry and biotechnology.

We will also present the new user interface for the Sponge Barcoding Database with important new and updated features, tools and links, making molecular sponge identification much more accessible to the non-taxonomist.

### **Evolution and function of eukaryotic-like proteins in the bacterial sponge symbionts**

Torsten Thomas, [Ana Esteves](#), Mary Nguyen, David Reynolds, Michael Lin, Lu Fan  
*University of New South Wales*

The basic nutritional requirements of sponges are in part satisfied by the phagocytosis of bacterial food particles from the surrounding water. How bacterial symbionts, which are permanently associated with the sponge, survive in the presence of phagocytic cells is largely unknown. Here we present the discovery that (meta-) genomes of bacterial sponge symbionts encode for proteins with apparent eukaryotic origin. These so-called eukaryotic-like proteins (ELPs) are more abundant in sponge symbionts than in free-living bacteria and other symbiotic bacteria. Phylogenetic analysis reveals that bacterial ELPs from sponge bacteria have orthologs in the genome of the sponge *Amphimedon queenslandica*, and they appear to form distinct evolutionary clades. Many ELPs found in sponge symbionts contain protein motifs, such as ankyrin-repeats, that are known to mediate protein-protein interaction. This led us to investigate their molecular function. Through recombinant approaches, we show that these ELPs when expressed in *Escherichia coli* can modulate phagocytosis of amoebal cells and lead to accumulation of bacteria in the phagosome or prevent uptake by the

amoebal cells. Our results show that ARP from sponge symbionts can function to interfere with phagocytosis and we postulate that this might be one mechanism of how symbionts can escape digestion by the sponge host.

**Phylogeny drives large scale patterns in Australian marine bioactivity -  
biodiscovery is not a lottery, and Porifera is the winner!**

Libby Evans-Illidge, Murray Logan, Jane Fromont, Christopher Battershill,  
Carsten Wolff, Lyndon Llewellyn  
*Australian Institute of Marine Science/ WA Museum/ University of Waikato/  
National University of Ireland*

Biogeographic, taxonomic and phylogenetic patterns in cytotoxic, anti-infective, and CNS-protective bioactivity were retrospectively assessed using a Bayesian modelling approach with a dataset of 18 000 bioactivity measurements spanning 13 marine phyla and over 1200 sites around Australia's ocean territory. Phylum classification was the major predictor of bioactivity, with Sponges being the most bioactive phylum across all three bioassay types and most Australian bioregions. To further explore the concept of phylum assignment explaining the potential for secondary metabolism and consequent bioactivity, this study assigned each animal phylum into one of four main phylogenetic lineages according to a modern phylogenetic synthesis based largely on molecular data. The deuterostomes and the ancestral early-metazoans (Porifera) and early eumetazoans (Cnidaria) typically had a higher percentage of bioactive samples, compared to non-deuterostome samples, in most bioregions and bioassay categories examined, and finer resolution of taxonomic assignment of samples to genus did not provide any further resolution to bioactivity patterns. We propose a hypothesis that high level phylogeny, and therefore the availability of metabolic pathways and capacity, is a major determinant of bioactivity, while habitat diversity and ecological opportunity are possible secondary drivers in the activation of this metabolic machinery and bioactive secondary metabolism.

**Characterisation of vertically inherited symbiotic proteobacteria in the  
tropical coral reef demosponge *Amphimedon queenslandica***

Rebecca Fieth, Kathryn Green, Sandie Degnan  
*University of Queensland*

Marine sponges provide excellent systems for the elucidating the ways in which animal hosts utilise and interact with their bacterial symbionts. Here we show that the tropical demosponge *Amphimedon queenslandica* is a low microbial abundance species in which the adult microbiome is dominated by just a few proteobacteria taxa distinguishable by both 16S sequence and morphology. Using transmission and scanning electron microscopy, we have tracked three

predominant bacterial morphotypes throughout the sponge life cycle, from early embryogenesis through to planktonic larval release and metamorphosis into the juvenile body plan. This visual determination of vertical inheritance of the primary symbionts is consistent with results from 16S amplicon sequencing through the sponge life cycle, and together these results indicate that the most numerically dominant symbiont is a novel gamma-proteobacterium with an unusual cross-sectional star-shaped morphology. Maternal trophocytes appear to deliver both yolk and intact bacteria through the follicle of very early embryos that are housed collectively in brood chambers. In blastula stage embryos, we observe apparent phagocytosis of these same bacterial morphotypes, which continues at a low level through the remainder of development, suggesting a possible nutritional role. The numerical dominance of the star-shaped morphotype is disrupted around the time the planktonic larva settles onto the substrate and metamorphoses into the benthic form, but is recovered once the new juvenile body plan becomes established. In adults, the dominant symbiont is found along with the less abundant morphotypes irregularly dispersed throughout the host, particularly – but not exclusively – in association with connective tissue of the mesohyl. To investigate the various roles of host versus symbiont in regulating this bacterial community composition that fluctuates through the host life cycle, we are interrogating both sponge host and bacterial symbiont genomes for bacterial and inter-kingdom signalling systems.

**Cruciform cells of calcarean sponge-larvae: a gene expression study in *Sycon ciliatum*.**

Sofia Fortunato, Sven Leininger, Corina Guder, Marcin Adamski, Raymond Bannister, Maja Adamska  
*Sars International Centre for Marine Molecular Biology/ Institute of Marine Research*

Sponges possess a diverse array of larval types. The amphiblastulae of Calcarean sponges are composed of macromeres, micromeres, and four cells called “cruciform cells” or “cellules en croix” of enigmatic function. These four cells become apparent during the pre-inversion stage of embryogenesis, and are located among micromeres on the equator of the embryo, giving it tetra-radial symmetry. As early as the 1940’s it was suggested that the cruciform cells might be photoreceptive, however, no evidence to support this notion has been published.

In this study, we investigated the development and fate of cruciform cells in *Sycon ciliatum* using molecular methods. We first followed the fate of cruciform cells after metamorphosis to determine whether apoptosis is involved in their degeneration. Our result indicates that cruciform cells degenerate after settlement of the larva.

Secondly, we searched the *Sycon ciliatum* genome for genes that are involved in photoreception (opsin and cryptochrome) and sensory organ development and neurogenesis (*pax*, *six*, *eya*, *elav*, *musashi*, *soxB*, *atonal*, ANTP-homeobox) in other animals. In addition, we have identified a number of other genes expressed during cross cells development while studying various developmental processes in *Sycon*. Opsin genes could not be found in *Sycon*, as previously demonstrated for the demosponge *Amphimedon queenslandica*. Intriguingly, so is cryptochrome, which has been implicated in photoreception of *Amphimedon* larvae, and which is expressed in its sensory pigment ring. In situ analysis of gene expression demonstrated that *soxB*, *elav*, *atonal*, *musashi* and several NK genes, as well as multiple components of the Wnt pathway are expressed during formation of the cruciform cells and that *nanos* appears to be the earliest marker of their specification. On the other hand, *pax*, *six* and *eya* are not expressed in the cruciform cells. While the function of these enigmatic cells remains unclear, our results indicate that many sensory and neuronal marker genes known from other metazoa are involved in their specification. In future work, we will investigate of photosensitivity of *Sycon* larvae by recording their behavior under different light conditions to confirm whether the larvae are phototactic.

### **Sponges in a dust cloud: dredging-related pressures in NW Australia**

Jane Fromont, Christine Schönberg, Oliver Gomez, Peter Speare, Evy Büttner,  
Flora Siebler  
*Western Australian Museum/ Australian Institute of Marine Science/Stuttgart  
University*

Coastal Western Australia (WA) has unique and dense communities of diverse filter feeders that are comparatively understudied and are not found anywhere else in the world. They provide a range of bioservices, including benthic-pelagic coupling, nutrient cycling, purification of vast water masses, 3-dimensional habitats, and support for a multitude of other organisms, including commercial species. These communities are often dominated by sponges, a group that in WA contains many endemic or undescribed species that are not located in marine protected areas. Extensive proposed dredging projects pose an environmental risk to local filter feeder communities through turbidity and light attenuation, impediments to filtering activity and smothering by sedimentation. The present project aims to investigate the nature and thresholds of such risks on filter feeders. Field surveys using underwater video and SCUBA at an industry dredging project near Onslow NW Australia will characterise these benthic communities, describing densities and diversities of filter feeders before and after exposure to dredging pressures generated by excavations for an 18 km shipping channel. These data will allow insights into which growth forms, common taxa and feeding strategies will be most affected, thus informing, but also in part validating, simultaneous aquarium experiments. Response values will include impacts of shading (effects on symbionts) and monitoring of tissue:skeleton

ratios. Through fieldwork in addition to laboratory experiments we will provide critical insights into local filter feeder communities and their responses to dredging pressures. Identifying relative impacts of different types of stress in combination with thresholds and guidelines will assist stakeholders and managers alike in reducing risks and consequences from dredging operations.

**Sponge community gradients in submerged caves of the Eastern Mediterranean**

Vasilis Gerovasileiou, Eleni Voultsiadou  
*Aristotle University of Thessaloniki*

Marine caves are geomorphologically complex ecosystems characterized by strong environmental gradients resulting in zonation of sessile communities and biological impoverishment towards the inner dark sectors. Sponges dominate in cave habitats, which have been characterized as reservoirs of poriferan biodiversity in the Mediterranean Sea. In the present work spatial variability of sponge communities in two submerged caves was surveyed in a region poorly explored for its cavernicolous biodiversity, i.e. the Aegean Sea (Eastern Mediterranean). Sampling was carried out with SCUBA diving through classical (qualitative samples and scraped quadrats) and non-destructive (photo-quadrats) methods, at different sites (walls and ceilings) along the exterior-interior axis of the two caves. In total, 270 sponge specimens were examined and 126 photo-quadrats were analyzed for sponge species richness, coverage, morphological diversity, and associated macrofauna.

The cavernicolous sponge fauna consisted of 76 species, with almost half of them found in both caves. Poriferan percent coverage, species richness, and diversity increased from the exterior environment to the middle zone of the cave walls and decreased thereafter. Lower values were recorded for the corresponding sites of ceilings, with a decreasing trend from the exterior to the middle zones and then an increase towards the dark interior. Morphological diversity was generally higher in the outer environment and anterior parts of the caves with middle and inner zones dominated by encrusting and cushion shaped forms. Although the abundance of sponge associated fauna decreased towards the dark cave interior, sponge specimens functioned as habitat engineers supporting richer populations of motile invertebrates in comparison to the surrounding bare rock.

The surveyed caves can be ranked among the richest of the Mediterranean Sea, concerning their poriferan diversity. The observed spatial variability in the studied sponge community parameters, among and within cave sectors with different topographic and physico-chemical features, is indicative of a complex ecosystem with great heterogeneity, individuality and conservation value.

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**Insights into the genetic connectivity of the marine sponge *Stylissa carteri* in the Saudi Arabian Red Sea**

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Despite the ubiquity of sponges and the ecological function they play in reef ecosystems, little is known about population-level connectivity in these organisms, limiting our understanding of sponge population dynamics. Information about larval dispersal in sponges is extremely rare. Despite the scarcity of data, the prevalent hypothesis is that dispersal in sponges occurs over short distance ranges. However, this has rarely been tested. To date, microsatellite markers have only been developed for a few sponge species and no sponge population genetics studies using microsatellites have been conducted in the Red Sea. Here we present the development and use of ten novel polymorphic microsatellite markers to characterize the genetic connectivity of populations of a common reef sponge, *Stylissa carteri*, on Saudi Arabian coral reefs. By looking at genetic relatedness among individuals within reefs (<1km) and genetic patterns among reefs (>200km) and along an inshore-offshore gradient, we explore the genetic structure of this species along the Saudi Arabian coast. Finally we examine the role of life history traits such as clonally and larval dispersal potential as factors explaining the observed patterns of genetic structure.

**A new genus and four new species of Aplysinidae sponges (Porifera, Demospongiae) from the Eastern Tropical Pacific**

Patricia Gómez, Carlos A. Sánchez, Barbara González-Acosta, Claudia J. Hernández-Guerrero, Zvi Hoffman  
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A new genus and four new species of Aplysinidae are described from the Gulf of California, México. *Retidendron* gen. nov. stands out by its peculiar anastomosed skeleton in one or two planes from which a sequence of dendritic fibers arises primarily from the deep choanosome. In addition to the common traits characteristic of the Aplysinidae, the new genus has pithed and stratified-bark body fibers with no foreign detritus, that change in color at death or by exposure to air. Three new species from *Retidendron* are here presented: *R. dominator*, *R. rex* and *R. horridus*. The first features a single thick walled hollow tube or a cluster of them; the second has a massive body with short irregular off-lined

tubes at its contour; while the latter, is a four tubule-shaped sponge, where each tubule is supported by a peduncle, it has a bristled surface, featuring strongly stratified fibers. Careful out-group revision of species belonging to the genus *Aplysina*, supports a rearrangement of two of them, *Aplysina revillagigedi* from the Revillagigedo Archipelago, and *Aplysina azteca* from the Mexican Pacific, within *Retidendron* since both feature its peculiar skeletal structure. Moreover, from this review, a new *Aplysina* species was recognized: *A. airapii* which shares the same habitat of the new genus populations, while featuring a massive body with tubules on top. Descriptions of both, the new genus and the new species, are strongly supported by their morphological and ecological traits, while their hierarchical arrangement within *Aplysinidae* is supported from scrutiny of molecular data (CO1 mtDNA and ITS nrDNA).

**Genomic and transcriptomic profiling of the aggregation factor genes of the demosponge *Amphimedon queenslandica***

Laura Grice, Marie Gauthier, Selene Fernandez-Valverde, Bernard Degnan  
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Self-nonsel self recognition phenomena in sponges have drawn considerable interest for over a century, since Wilson's seminal experiments demonstrated the capacity for species-specific sponge cell reaggregation in 1907. The molecules proposed to mediate individual-level self-nonsel self recognition processes in sponges, the aggregation factors (AFs), have been the focus of extensive biochemical characterisation since they were first isolated in the 1960s. Like other molecules regulating allogeneic self-nonsel self recognition processes, the AFs are predicted to possess an underlying level of polymorphism to allow individual-specific discrimination to occur. Until now, however, relatively few details have been available regarding the genetic nature of the AFs. The sequencing of the genome of the demosponge *Amphimedon queenslandica* has allowed us to characterise the AF genes from this species. The *A. queenslandica* genome contains an 80 kb AF locus encoding multiple AF genes.

Although these genes encode a common set of protein domains, specifically Calx-beta, two types of Von Willebrand and a sponge-specific Head domain, the sequences of these domains share a low level of similarity, both between and within genes; this is in contrast to other Calx-beta-containing genes in *A. queenslandica*. Despite the high level of sequence variation within and between Calx-beta domains, the intron-exon architecture of the AFs appears to be remarkably constrained, with a significant over-representation of phase 1 introns in all genes, and the conformation of most protein domain types to a consistent modular architecture of exonic repeats. Transcriptional profiling of *A. queenslandica* across development, and in response to alloimmune challenge, suggests that individual AF genes are transcribed as, or processed into, several



shorter transcripts, which may be differentially deployed in a context- or individual-specific manner.

**Seeing the invisible: comprehensive fingerprint of the drug-like natural product metabolome**

Tanja Grkovic, Ronald J Quinn  
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We have previously reported a strategy for the generation of lead-like enhanced (LLE) extracts and fractions with a protocol that allowed the retention of lead- and drug-like constituents by selecting favourable physicochemical properties such as  $\log P < 5$ . As this requires no knowledge of structure, it achieves an ability to examine the complete diversity of the natural product metabolome. Herein, we report on the use of the LLE fraction library protocol to create proton nuclear magnetic resonance-based metabolic fingerprint of the LLE fractions and introduce structural information early in the natural product isolation workflow. This approach is illustrated using a sponge *Iotrochota* sp., where several interesting metabolites were identified on small-scale and the subsequent large-scale extraction work yielded new natural products iotrochotadines A –D, and a novel compound iotrochotazine A.

**Determining the effect of *Cliona delitrix* removal on stony coral growth offshore southeast Florida**

Ariel Halperin, Andia Chaves-Fonnegra, David Gilliam  
*Nova Southeastern University*

Excavating sponges are strong spatial competitors on coral reefs; able to attach to, excavate, and kill live coral tissue. The ecological interaction between excavating sponges and stony corals was initially considered as epizooism or infestation, but recent studies demonstrate that these organisms are in asymmetric competition where the sponges tend to dominate and overgrow entire coral colonies. Despite the known negative effects of excavating sponges on stony corals very few studies have experimentally tested the competitive nature of this interaction. In this study we determine if removal of the excavating sponge *Cliona delitrix* has an effect on the growth of the stony coral *Montastrea cavernosa*. A total of 36 *M. cavernosa* colonies currently colonized by small *C. delitrix* sponges (5 - 10 cm in diameter) were examined. Sponges were removed using a hammer and chisel from 24 of the affected coral heads, and 12 corals were left alone as controls. After sponge removal, the resultant cavities in the coral skeletons were filled to prevent future colonization by other bioeroders. Cement was used as fill material on 12 of the coral colonies, and the remaining 12 cavities were filled with epoxy. Standardized photos of each coral head were

taken after sponge removal, after the fill material had been applied, and at 6 and 12 month intervals after sponge removal. The photos were then analyzed using NCRI CPCe V3.6© software to calculate the initial area of the fill material after sponge removal, and 6 and 12 months periods post-removal. Preliminary results show coral tissue overgrowth of both fill materials after removal of the sponge. However, overgrowth occurred more frequently on colonies filled with epoxy. This study will help to determine the direct effect of the bioeroding sponge *C. delitrix* on the growth and future survival of stony corals. Additionally, we evaluate this methodology as a novel coral restoration technique that may be applied, if successful, to preserve corals currently colonized by *C. delitrix*.

**Fluid dynamics and flow in leucon-type sponge canal systems: Insights from the freshwater sponge *Spongilla lacustris***

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As sessile filter feeding animals sponges (Porifera) rely on highly efficient fluid transport systems to keep energy expenditure low. As a consequence of the high complexity of the canal system network in leucon-type aquiferous systems only limited data on flow velocities and transport rates are available to date. Obtaining unaltered experimental measurements from internal parts of the canal system is almost impossible. Therefore data for e.g. choanocytes or canal segments of different hierarchical levels are mainly based on theoretical assumptions. This is mainly due to the limited availability of morphometric and quantitative data on canal system architecture.

In our study we used the fresh water sponge *Spongilla lacustris* which as a juvenile is an ideal model system to study flow in sponge. Due to its transparency flow velocities can be determined experimentally inside canals near the surface and at the osculum. The limited architectural complexity and small body size enables the extraction of 3D canal system models from single specimens based on SR- $\mu$ CT data. Flow through the canal system was studied using a finite element modeling approach. In order to calibrate our model and verify the results of our modeling approach we conducted flow velocity measurements by particle tracking velocimetry on 10 – 14 d old hatched specimens. In order to measure flow in canal segments of the incurrent and excurrent canal system we used sandwich cultures of *S. lacustris*.

Observed flow velocities in canal segments of different hierarchical orders within the excurrent canal system were lower in our simulations than predicted based on calculations from a unidirectional strictly hierarchical model of flow for

sponges. Flow velocities ranged from ~ 0.02 mm/s to ~ 0.1 mm/s over the entire range of canal segment sizes. Only within the osculum the flow accelerates to a maximum outflow velocity between 0.7 mm/s to 1.1 mm/s. The slow increase in flow velocity within the excurrent canal system differ from previous observations in other sponges, e.g. *Haliclona* species which reported much higher and faster increasing flow velocities towards the osculum. In case of juvenile *S. lacustris* this is a consequence of the aquiferous system architecture which displays a compensating increase in available canal volume.

### **Excurrent jets and dissolved oxygen plumes from a tropical bacteriosponge**

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*Duke University/ University of North Carolina at Chapel Hill/ University of Hawaii*

On many tropical reefs, sponges play an important role in benthic-pelagic coupling and can modify the structure of physical and chemical bottom boundary layers. Water drawn into sponges is taken from a relatively broad area, processed by the sponge, and expelled in strong excurrent jet(s). Sponge pumping activity can increase mixing and exchange of momentum and dissolved constituents between the benthos and the overlying water column. To better understand these processes we made in situ velocity and dissolved oxygen (DO) measurements over *Xestospongia muta* (Giant Barrel Sponge) individuals on Conch Reef, Florida. Acoustic Doppler velocimeters (ADVs) and DO sensors were systematically moved in a two-dimensional grid pattern to construct spatial maps of water velocity and DO. Each excurrent forms both a momentum jet (with velocities >10 cm/s) and a scalar plume (with DO deficits >10%), with physical and chemical properties distinct from ambient water. Both the velocity jet and the DO plume interact with the ambient cross-flow. In a second set of measurements, the relationship between sponge pumping rates and ambient environmental conditions was investigated using multi-day time-series of water velocity, temperature, salinity, DO, PAR, and turbidity. For nearly all the records, pumping rates were largely insensitive to the measured environmental conditions, including the speed of the ambient currents. Our measurements and theoretical predictions do not support the commonly stated hypothesis that Bernoulli suction from ambient currents enhances sponge excurrents. The long-term records include periods of sharply reduced pumping and decreased excurrent DO occurring synchronously between individuals, and a day-night cycle in excurrent DO likely associated with the sponges? autotrophic symbionts.

### **What drives sponge symbioses?**

Ute Hentschel-Humeida  
*University of Wuerzburg*

Sponges (phylum Porifera) are the most ancient still living metazoan phylum with a fossil record dating back more than 600 million years in time. Many sponges are known to contain large amounts of phylogenetically diverse microorganisms that are located extracellularly in the mesohyl matrix. This presentation will seek to address the question what function/s the microbial symbionts might have in the symbiosis context and which factors might be responsible for the microbe-host interaction. Recent results obtained by -omics approaches in the author's laboratory and presented in the literature will be reviewed towards this goal. Specific emphasis will be placed on aspects of nitrogen and carbon metabolism, as well as on vitamin biosynthesis. Furthermore, candidate genes representing putative symbiosis factors will be presented.

### **Signaling in bacterial symbionts of sponges**

Russell Hill, Jindong Zan, Clay Fuqua  
*University of Maryland Center for Environmental Science/ Indiana University*

The diversity of bacteria associated with many species of sponges has now been well established but little is known about the processes whereby particular bacterial species initiate and maintain symbiotic relationships with sponges. In the dense bacterial communities present in many sponges, bacterial signaling may be important. One major group commonly found associated with sponges is the Proteobacteria. Quorum sensing (QS) is a cell density-dependent process that bacteria employ to coordinate and regulate gene expressions. Bacteria isolated from sponges are able to produce acyl-homoserine lactones (AHLs), an important class of QS molecules found mainly in Proteobacteria. We isolated 420 bacterial strains from the Florida Keys sponges *Mycale laxissima* and *Ircinia strobilina* and screened them for production of signaling compounds. Overall, 44% of these isolates are able to produce AHLs compared with only 5% from the surrounding water and 77% of the positive isolates belonged to the *Silicibacter-Ruegeria* (SR) subgroup of the *Roseobacter* clade. Genetic and chemical analysis of QS in the SR-type representative KLH11 revealed two discrete QS systems, designated SsaRI and SsbRI. The *ssal* and *ssaR* null mutants are both nonmotile, and do not synthesize detectable flagellin. Mutants of *ssal* and *ssaR* showed increased biofilm formation and this is not due solely to the loss of motility. Importantly, the *ssal* gene is expressed in sponge tissue, detected by RT-PCR and AHLs were also detectable within sponge tissue. We have established *Ruegeria* sp. KLH11 as a model system to study the complex

symbiotic relationships between sponges and microbes and propose a model of how signaling affects colonization of sponges by SR  $\alpha$ -proteobacteria.

### **SpongeMaps: an online collaborative tool for taxonomy**

Kathryn Hall, Merrick Ekins, Miranda Vidgen, Mary Kay Harper, Chris Ireland, Anthony Carroll, Leesa Habener, Dirk Erpenbeck, Gert Wörheide, John Hooper  
*Queensland Museum/ The University of Queensland/ University of Utah / Griffith University / Ludwig-Maxmillians University / Queensland Museum*

SpongeMaps is a web-based tool which allows researchers to collaborate in real-time on the taxonomy of sponges. With password-controlled access levels, SpongeMaps is able to display peer-reviewed information and open-access data publicly, while protecting unpublished and working data in member only areas. SpongeMaps hosts and displays data from individual sponge specimens, mandatorily stored and accessible in a public collection, in the following formats:

- precise and current taxonomic data, with hyperlinks, based on the authoritative resources of the World Porifera Database and the Australian Faunal Directory;
- morphological observations of specimens (and species) as MSEXcel (or CSV) documents;
- photographs, including underwater (live), deck, SEM and micrographs as low resolution jpegs for web-display (with the capacity to store high resolution images in an access-restricted area);
- precise collection data for specimens (including depth, longitude and latitude);
- DNA sequence data, directly deposited into SpongeMaps as FASTA files, or harvested from the Sponge Barcoding Database ([www.spongebarcoding.org](http://www.spongebarcoding.org)) and/or GenBank;
- chemical datasets, including specific information about chemical compounds isolated from individual sponges, such as molecular formulae (stored as SMILES codes)
- bibliographic records, including PDFs of unrestricted documents.

Centralising the storage of all these data sources for individual specimens, allows researchers to access information from a one-stop portal. Additional sources of data, such as DNA sequence alignment files or phylogenetic character matrices, may be added in future releases of SpongeMaps, depending on demand. Primarily, however, SpongeMaps enables researchers to collaborate on any specimen of sponge, comparing to other species and specimens elsewhere, in real time. Using SpongeMaps, anyone can obtain an integrated insight into known species of sponges (currently confined to the Indo-West Pacific fauna), through the public portal. Through the member-only area, sponge researchers can work together with confidence in the security of their data to collaborate on understanding the biodiversity of sponges in the Indo-West Pacific region, moving taxa through the pipeline from specimens, through OTU concepts, to defined species.

## **Ninth World Sponge Conference 2013**

*Abstracts for Oral, Speed Talk and Poster Presentations*

### **Barnacles and sponges –overview of diversity and upcoming research.**

Andrew Hosie, Jane Fromont, Kylie Munyard, Mark Castalanelli, Diana Jones  
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Barnacles are well known for their tendency to be nuisance foulers, but very little is known of their relationships with other organisms. Yet arguably, there are more species of symbiotic barnacles than there are free-living. Sponges are host to a significant portion of symbiotic species, with >100 known to be associated with sponges, either as epibionts or embedded in the sponge tissues. The majority of this diversity (~80 species) is found within the barnacle family Archaeobalanidae, which contains both epibiotic and free living species. Members of the Demospongiae are the typical hosts, with only two species known to inhabit the Calcarea and Hexactinellida. In Western Australia at least 24 species of sponge inhabiting barnacles are present, and are found across eight orders, 21 families and 45 species of sponges. From this dataset we can see that host specificity is variable among barnacles, with five species found in more than one family and order of sponge. Members of the Petrosiidae are the most common hosts with six species being host to seven species of barnacle. Most sponge species are host to only one species of barnacle. Future work will utilise molecular techniques to test host sponge diversity patterns and sponge barnacle phylogenetics across Australia and adjacent areas.

### **Biodiversity of Subtidal Sponges (Porifera: Demospongiae) in the Penghu Archipelagos (Pescadores), Taiwan**

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*National Penghu University of Science and Technology/ National Sun Yet-Sen University*

Marine sponges (Porifera), as the oldest and most primitive metazoan phylum, have attracted considerably attention from many different scientific aspects. Their functional roles in the benthic communities have been revealed in the past decades. Sponges can play both substrate builder and destroyer in the coral reef system. Many interactions between sponges and other organisms are mediated through a range of chemical compounds produced by sponges. Understanding of ecological functions of sponges and their symbiotic organisms also provide potential applications to the fields of natural product exploration and biotechnology. The sponge-related researches in Taiwan have been focused on natural product exploration; however, we realized the fundamental work on sponge taxonomy and ecology is urgently required. Subtidal sponges were photo recorded in situ and collected by SCUBA at the depth range 2 ? 20 m from 2009 to 2012 in 16 different sites near Penghu Islands, Taiwan. The sponge samples were identified into genus or species based on their morphology of skeleton and

spicules. A total of 53 species belonging to 22 families were found in this study. Number of sponge species and biodiversity index (H?) was the highest in ChingWan and SugGang (24; 3.2), the lowest in HuJing Bunker North (1; 0). The most wide-distributed sponge species is *Callyspongia* (*Euplacella*) cf. *communis* (Carter, 1881) followed by *Haliclona* (*Gellius*) *cymaeformis* (Esper, 1794), and *Aaptos suberitoides* (Brøndsted, 1934). No sponge was observed in ChiPeiYu and the reasons remain unveiled. Non-metric multidimensional scaling (MDS) analysis demonstrated that similarities of sponge assemblages collected from islands and locations apart from each other were low. Our results illustrated the accumulative number of sponge species increased as the sampling sites increased. The trend line of biodiversity of sponge assemblage in Penghu Islands is likely increasing, but far from the maximum.

**A checklist of shallow and mesophotic sponges, Agulhas bank South Africa: An ecologically important hotspot for poriferan Fauna**

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The Agulhas Continental Shelf is a broad extension of the South African coastal plain with a roughly triangular shape and a mean depth of just over 100 m. Approximately 60% of the shelf consists of hard substratum, and low/high-profile reefs exist in a number of places, both close inshore and extending offshore south of Cape Agulhas (20°E). As part of a Benthic Ocean Exploration Project, three expeditions to the shallow and mesophotic reefs on the Agulhas continental shelf were carried out during 2008, 2009 and 2010. Inshore and offshore reefs were sampled using ROV, Scuba and a dredge and were found to contain a high diversity of marine sponges, representing a highly unique fauna in the Agulhas biogeographic province. 330 samples were collected, representing 93 species of marine sponges belonging to the classes Demospongia (more than 98%) and Calcarea. This is the first comprehensive survey of marine Porifera from the Agulhas continental shelf. Half of the species inhabiting the mesophotic depths on Alphards, 45 and 75-mile banks were restricted to those depths. The diversity encountered was represented by approximately 39 genera, from 29 families, and 12 orders of Demospongia, and included 1 genus from 1 family and 1 order of Calcarea. From a perspective of conservation planning, a single reef structure on the Agulhas continental shelf is unlikely to accurately represent the regional faunal diversity, considering the high beta diversity and low biotic similarities of communities between the reefs and the limited distributional ranges of the component species. This large concentration of sponge biodiversity associated with the mesophotic and shallow reefs supports the status of Agulhas bank as an ecological hotspot, with important implication for conservation planning in this

region. Having said this, these surveys added hugely to our understanding of mesophotic reef sponges and yielded 22 new species, showing the importance of the Agulhas continental shelf as a source of taxonomic novelties.

**Demosponges in disguise: Formation of new syncytial tissue in glass sponges**

Amanda Kahn, Sally Leys  
*University of Alberta*

Within the Phylum Porifera, filter feeding is expensive—30% of energy is estimated to be used to pump water through the aquiferous system of the glass sponge *Aphrocallistes vastus*—however *A. vastus* can also take advantage of ambient currents to generate current-induced flow, thereby saving energy. Another major energetic cost for sponges is rapid turnover of cells, especially choanocytes. It has been suggested choanocytes turn over as fast as every 5 hours, equivalent to rates of turnover of gut epithelia in other animals. We investigated the variability of choanocyte turnover in sponges from different habitats and taxonomic groups. We collected the demosponges *Spongilla lacustris* (freshwater) and *Haliclona mollis* (marine) and *Sycon coactum*, a calcareous sponge, from Barkley Sound, BC. Additionally, we collected the glass sponge *Aphrocallistes vastus* at 130 foot depths in Saanich Inlet, BC. We exposed portions of the growing edges and body tissues to EdU, a thymidine analog, for periods up to 6 days. EdU was incorporated into new cells of demosponges and the calcareous sponge within 6 hours and turnover of whole chambers occurred from 24 hours onward, up to 3 days. In contrast, very few new nuclei labeled in the main body of *A. vastus*, a syncytial sponge whose flagellated chambers are unusual in being populated by anucleate collar bodies rather than cells. However, growing tissues at the lip of *A. vastus* revealed a surprising finding: EdU-labeled ‘cells’ formed a band in the lip of the osculum, where new skeletal growth was also observed. Moreover, the labeled cells are much more like demosponge choanocytes than the chambers of the adult glass sponge. These findings prompt a completely new view of glass sponge syncytia: that chambers are first formed with similar structure to that of cellular sponges, but then grow in size, recessing the nuclei below collar bodies as they form part of the main body of the sponge. The implication is that choanocytes, with nuclei, are expensive, and that the glass sponge syncytial tissue may have evolved strictly as a cost-saving mechanism for a food-poor, deep-sea environment.



**Shallow-depth sequencing of the genome of several sponges using  
advanced multiplexing**

Ehsan Kayal, Niamh Redmond, Allen Collins  
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We are witnessing a flood of genomic and metagenomic sequencing projects as a direct result of a broader accessibility to genome-based data generation methods, commonly referred to as “next-generation sequencing”. Such outburst of massive sequence data promises a revolution in our understanding of biological systems at many levels, from cellular and developmental biology to systematics and phylogeny to ecology and community biology. However, one obvious impediment to progress in achieving this goal is the lack of fully developed bioinformatics tools to handle, not to mention interpret, the large amount of sequence data generated. In addition, despite the relative decrease of the per sample cost, any comparative genomic project involving many samples requires a significant starting budget. Fortunately, for systematic and phylogenetic purposes partially sequenced genomes can already provide significant amount of phylogenetically informative molecular characters. In fact, some studies suggest that the phylogenetic information of sequence data plateaus way before the complete genome is covered. With these facts in mind, we developed a low-cost multiplexing sequencing protocol applicable to both PCR amplicons and total genomes. We applied our protocol to several sponge species where we sequenced either from the total genome or PCR-amplified mitochondrial genome, all in a single Illumina lane. We also examined the impact of whole genome amplification approach in this context. We present our preliminary data on the genomic coverage and discuss the relevance of such method for systematics and phylogenomics studies.

**Sponges as important sources of nitrate on an oligotrophic continental  
shelf**

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*Commonwealth Scientific and Industrial Research Organisation/ Western  
Australia Museum*

In situ field measurements of dissolved inorganic nitrogen (DIN) release by sponges in south-western Australia revealed nitrate release rates of 0.022 to 0.743  $\mu\text{mol g dry weight}^{-1}$  (dry wt)  $\text{h}^{-1}$  and ammonium release rates of between 0.002 and 1.366  $\mu\text{mol g dry wt}^{-1}$   $\text{h}^{-1}$ . The highest and most consistent rates of nitrate release were among the Thorectidae (0.324 to 0.725  $\mu\text{mol g dry wt}^{-1}$   $\text{h}^{-1}$ ) while mycalid and verongid sponges were highly variable (0.024 to 0.743  $\mu\text{mol g dry wt}^{-1}$   $\text{h}^{-1}$ ). The ratio of nitrate to ammonium in released nitrogen ranged from 0.1 to 197.0 indicating a wide range of nitrogen release modes by sponges from predominantly ammonium, to very efficient nitrate producers. The study more

than doubles the number of temperate sponge species recorded to release nitrate. Nutrient concentrations near the seabed sponge assemblages were higher at low wind speed (a proxy for turbulent mixing). These observations and our measurements of nitrogen release rates from sponges are consistent with the hypothesis that primary production in the region depends on wave induced mixing at the seabed for resupply of remineralized nitrogen to a nutrient impoverished water column and that sponges make an important contribution to these fluxes. Based on known biomass of sponges in south-western Australia we calculate that sponges may contribute DIN of 1.8 to 3.2 g N m<sup>2</sup> yr<sup>-1</sup>. Taking into account the distribution of sponge habitat across the continental shelf to 100 m depth, this constitutes a contribution of 10% to 18% of the total recycled nitrogen flux required from the benthos to balance a regional nitrogen budget.

### **Arsenic ecology in the Red Sea sponge *Theonella swinhoei***

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Marine environment contains low amounts of arsenic (~5ppb), mostly as arsenate. Marine organisms contain higher arsenic concentrations, mainly as arsenobetaine or methylated arsenic. We show that the coral reef sponge *Theonella swinhoei*, a common Indo-Pacific Ocean (including the Gulf of Aqaba) sponge, contains high amounts of arsenic and barium with a bioaccumulation factor of over 106. It contains high microbial abundance, with bacteria occupying up to 40% of its body volume. Sponge fractionation to bacterial and sponge-enriched fractions and their analysis by ICP-AES showed that 60-80% of arsenic and barium were found in the bacterial fraction.

To study the pathway leading to such high accumulation, three strategies were pursued; 1) Chemical analysis of water-soluble arsenic species. 2) Culturing of arsenic-resistant sponge-associated bacteria. 3) Elemental analysis of whole sponge and individual bacteria. Arsenic speciation by ICP-HPLC-MS examined sponges fractionated into three cell groups: Sponge cells; *Entotheonella* sp. and cyanobacteria cells; unicellular bacterial cells. The dominant form of arsenic throughout the fractions was arsenate. The arsenic was located almost exclusively in the *Entotheonella* sp. and cyanobacteria fraction, most of it not soluble.

Culture media were designed to select for a variety of arsenic-modifying bacteria according to literature. Representatives of Actinobacteria, Alphaproteobacteria and Gammaproteobacteria were isolated and identified by their 16SrRNA gene. 47 isolates grew in the presence of arsenate while only three were isolated in presence of arsenite. Arsenic resistance assays on arsenate-grown bacteria revealed 13 isolates that tolerated concentration up to 100mM. This is 20 folds higher than original culturing conditions.

SEM-EDX analysis revealed the bacterial symbiont in which most arsenic and barium were located. The elements were seen as solid granules. X-ray diffraction of these granules showed that both elements were mineralized and their structures were elucidated.

To conclude, this research shows the localization of arsenic and its forms in *T. swinhoei* holobiont. Arsenic-tolerant sponge-associated bacteria were isolated and identified. Further work into the specific pathway arsenic follows from the environment to form granules inside the bacterial symbiont, and the modifications that occur on the way, are necessary to uncover this fascinating interaction.

### **Biogeography of calcinean sponges (Calcarea)**

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Molecular and morphological analyses have recently shown a strong morphological signal for Calcinea, which allowed the proposal of a new classification, with new diagnoses and new genera. Included in this proposal is the monophyletic genus *Borojevia* gen. nov., and the revised concepts for monophyletic *Ascandra*, and *Clathrina*. On the basis of molecular phylogenetic reconstructions of these three genera using sequences of the internal transcribed spacer (ITS), we performed a primary BPA cladistic analysis to assess the historical relationships of the areas in which these lineages are currently distributed. The BPA datamatrix was analysed with the softwares Nona and Winclada. Provinces adopted are those in the MEOW system (Marine Ecoregions of the World). The phylogeny of *Ascandra* included six species from five provinces. For *Borojevia* gen. nov., four species from three provinces were analysed. *Clathrina* is a speciose and widespread genus, which permitted inclusion of 19 species from 12 provinces in our analysis. Our results for *Ascandra* revealed a more derived Amphi-Atlantic track, following a basal Transisthmian track (one area cladogram: nine steps, CI=100, RI=100). For *Borojevia* gen. nov. only an Amphi-Atlantic track was recognized (one area cladogram: seven steps, CI=100, RI=100). *Clathrina* exhibits a more complex scenario, including two main clades. One of these clades comprises an Anti-Tropical track, while the other includes three tracks, a basalmost Trans-Pacific track, followed by a Tethyan track, and an Amphi-Atlantic track in the most derived position (one area cladogram: 54 steps, CI=0.68, RI=0.73). Amphi-Atlantic tracks emerge as a recurrent evolutionary phenomenon in the biogeography of Calcinea.

**Investigations on abundance and activity of microbial sponge symbionts using quantitative real-time PCR**

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Marine sponges are hosts to dense and diverse microbial consortia that are likely to play a key role in the metabolic processes of the host sponge due to their enormous abundance. Common symbioses between nitrogen transforming microorganisms and sponges indicate complex nitrogen cycling within the host. Of particular interest is determining the community structure and function of microbial symbionts in order to gain deeper insight into host-symbiont interactions. We investigated the abundance and activity of microbial symbionts in two Mediterranean sponge species using quantitative real-time PCR. An absolute quantification of functional genes and transcripts in archaeal and bacterial symbionts was conducted to determine their involvement in nitrification and denitrification, comparing the low microbial abundance (LMA) sponge *Dysidea avara* with the high microbial abundance (HMA) representative *Aplysina aerophoba*. Absolute quantification of archaeal *amoA*-genes provided evidence of highly abundant ammonia-oxidizing archaea (AOA) in both HMA and LMA sponge species. Interestingly, detected *amoA*-transcripts indicated the activity of AOA only in the HMA representative. The variable abundance of AOA in chimneys within the *A. aerophoba* colony suggested an irregular distribution of symbionts. Moreover, denitrifying bacteria could be identified in *A. aerophoba* by using the functional gene marker, *nirK*. A high abundance of nitrite-reducing bacteria were detected based on cell numbers, and transcript counts revealed the bacterial activity. An experimental inhibition of pumping activity of *A. aerophoba* led to decreased activities of aerobic archaeal nitrifiers, whereas anaerobic denitrifying bacteria showed enhanced activity during non-pumping periods. This study revealed strong effects of pumping activity on nitrogen cycling in sponges, emphasizing the role of interactions between sponges and their symbionts.

**Origin and early evolution of myosin heavy chains and the neuromuscular system**

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From jellyfish to humans, neurons interact with muscles and sensory organs, enabling animals to react to their surroundings in a fast and complex manner. Sponges however lack both neurons and muscles but possess aneural photo-

and chemosensory structures in their larva and contractile cells in their adult. Using transcriptome sequencing, we identified conserved genes in the demosponge *Tethya wilhema* that are involved in the development and function of neurons and muscles in non-poriferan metazoans. We aim to gain insight into the origin of the neuromuscular system, which was central to the evolutionary success of animals, by investigating the possible roles of these genes in *T. wilhema*. In particular, we developed in situ hybridisation methodology in this sponge and determined that the two myosin heavy chain (myhc) genes - non-muscle myhc and striated-muscle myhc - are differentially expressed. While bilaterian non-muscle myhc genes function during basic cellular processes of non-muscle cells (e.g. cell division, shape change and migration) and during vertebrate smooth muscle contraction, bilaterian striated-muscle myhc are specifically expressed in vertebrate striated, and in both smooth and striated muscles of protostomes. Determining the origin of “muscle” and “non-muscle” myhc genes could thus provide insights into the origin of muscle cells. In *T. wilhema*, we found that non-muscle myhc has a broader expression pattern than striated-muscle myhc and is particularly expressed in cells displaying prominent slow shape changes, such as multipolar archeocytes, sclerocytes, and pinacocytes. In contrast, the striated-muscle myhc gene seems to be exclusively expressed in a specific multi-perforated cell type at the apopyle of the choanocyte chambers. While this cell type is not involved in sponge body contraction, it seems to change shape in order to regulate the diameter of the apopyle and thus the water flow through the choanocyte chamber. In this context, changes in cell shape may be needed which are faster than those occurring during whole body contraction. Hence, the striated-muscle myhc gene may have evolved an early role in fast cell shape changes and later been adopted by the typical muscle machinery during the evolution of muscle cells. More generally, our results suggest sponge whole-body contractions and muscle contractions of other metazoans are unlikely to be synapomorphic.

### **Mitochondrial genomics and phylogenomics of sponges**

Dennis Lavrov  
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Because of their ancient origin and early diversification, sponges can be expected to harbor large molecular and genomic diversity, most of which remains explored. As a part of the Porifera Tree of Life (PorToL) project, our lab has determined mitochondrial genome sequences from ~100 species of sponges representing the four major groups in the phylum (Calcarea, Hexactinellida, Demospongiae, and Homoscleromorpha). We found that sponge mitochondrial genomes are not only different from those of other animals but also highly variable within the phylum. In particular, mitochondrial genomes differ in size, genome organization, genetic code, gene content, presence/absence of introns, tRNA structures and editing, rates of nucleotide substitutions, and proliferation of

repetitive elements among the major groups of sponges. Within these groups the tempo and mode of mitochondrial evolution appear to be more uniform, resulting in strong phylogenetic signal in mitochondrial dataset. Here I describe the overall organization of mitochondrial genomes in major lineages of sponges and show how both mitochondrial genomic features (e.g., gene content and gene arrangement) and mitochondrial sequence data can be informative for understanding phylogenetic relationships and evolution of sponges.

**Synonymization of *Crambe* and *Monanchora*, with a taxonomic revision of the *Crambe arbuscula* complex, and description of three new species from the Tropical Western Atlantic**

Eduardo Leal Esteves, Thiago Silva de Paula, Gisele Lôbo-Hajdu, Eduardo Hajdu

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*Monanchora* Carter, 1883 was defined by the presence of two categories of subtylostyles in combination with anchorate isochelae and other crambeid microscleres, with the exception of asterose desmas whose presence defined *Crambe* Vosmaer, 1880. Both genera were long suspected to be synonyms for the lack of positive morphologic traits to clearly diagnose the former genus. *Crambe arbuscula* (Duchassaing & Michelotti, 1864) (new combination) was the only Crambeidae species in the Tropical Western Atlantic for decades (as *M. arbuscula*). This is one of the most variable demosponges and may be identical in spicule complement and in the chemistry of Crambescidin alkaloids to *C. crambe* (Schmidt, 1862), type species of *Crambe*, from the Mediterranean/eastern Atlantic. Our preliminary molecular results, based on Maximum Likelihood analysis of 16 S rRNA of selected Caribbean/South American and Mediterranean *Crambe*/*Monanchora* spp., revealed the polyphyly of the latter genera and monophyletic species within “*C. arbuscula* complex”: *C. arbuscula* (Florida to São Paulo, SE Brazil), *C. sp.nov.1* (SE Brazil) and *Microcionina incertae sedis* (Espírito Santo to Santa Catarina, S Brazil). Therefore, we formally propose to abandon the use of *Monanchora*, pending a comprehensive phylogenetic review of all *Crambe* s.l., wherefrom a new (sub)generic scheme may emerge. *Crambe arbuscula* was redescribed after examination of 130 specimens from throughout its distribution and two other new species are described from Bahamas (*C. sp.nov.2*) and NE Brazil (*C. sp.nov.3*). *Crambe arbuscula* can be differentiated from all other *Crambe* spp. without microscleres by the presence of subtylostyles up to 300 by 9 µm in average. Alternatively, it can be recognized by the presence of sigmoid chelae with laterally extended fimbriae, or anchorate isochelae with claws covering up to 29% of shaft length, always in combination with the latter microscleres when present. *Crambe sp.nov.1* has anchorate isochelae with claws covering 43% of

shaft length, sigmoid chelae are absent. *Crambe* sp.nov. 2 has strongyles in two categories as the only spicules and unique Crambescidin alkaloids. *Crambe* sp.nov. 3 has sigmas larger than 20  $\mu\text{m}$  and a second category of choanosomal subtylostyles. *Micronionina incertae sedis* has subtylostyles in a plumose to isodictyal arrangement and bipocillae as microscleres.

### **Taxonomy and distribution of some sponges of Cebu, Philippines**

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Twenty-nine species of sponges from Cebu were evaluated for their biomedical potential based on published literature. Fourteen species were cited as containing a variety of new and known bioactive compounds. These belong to 14 genera, 11 families and 6 orders. An assessment of the fourteen sponge species based on the number of compounds present, range of their bioactivity and their presence in the different stations around Cebu revealed that *Hyrtios erectus* (Keller, 1889) has the most number of compounds with bioactivity ranging from anti-microbial and anti-fungal to receptor-interaction-based to cytotoxic. This is followed by *Biemna fortis*, *Stylissa massa* and *Cribrochalina olemda* having at least two categories of the bioactive compounds. On the sponges? distribution in Cebu, *Hyrtios erectus* is widely distributed, present in four of the six stations. This is followed by *Plakortis lita*, present in three station and *Biemna fortis*, *Stylissa massa*, *Callyspongia* (*Cladochalina*) *aerizusa* and *Luffariella* cf. *variabilis* present in two stations. Of the six stations, the one in San Francisco in Camotes Islands has nine of the fourteen sponge species with bioactive properties.

### **Synergistic Effects of Crude Oil and Corexit Dispersant on a Sponge Holobiont System**

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Rebecca Vega Thurber  
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Following the worst oil spill in US history, "Macondo" well crude oil from the BP Deepwater Horizon spill and Corexit 9500 dispersant were applied in experimental dosing of the common reef sponge, *Cinachyrella alloclada*, found in both the Gulf of Mexico and many Caribbean reefs. Physiological monitoring of this potentially new model sponge species included baseline descriptions of a) tissue ultrastructure by scanning and transmission electron microscopy, b) profiling the sponge "microbiome" and c) high throughput RNA-sequencing of the

*C. alloclada* transcriptome. SEM revealed novel, possible reproductive structures. Under closed aquaculture conditions, *C. alloclada* individuals ( $n > 75$ ) were dosed with sublethal amounts of oil or 10:1 oil/Corexit mixtures for 1, 24 and 48 hours. Unexpectedly, microbial communities of the same sponge host diverge into two distinct 16S rRNA clades after PCA analysis. Illumina RNAseq of dosed and control samples generated over 50 million reads and 30,000 aligned contigs after de novo assembly. Additionally, over 8000 transcriptome sequences matched previously identified sponge sequences, and 483 of these appear to be differentially expressed. The latter were analyzed by KEGG pathways that highlight the induction of specific gene responses, specifically increased expression of protein transport and breakdown, cytochrome P450, and DNA repair responses, RAC1 and SOS, to oil and dispersant for transcripts associated with Ras-regulated signaling proteins. Many of these genes appear responsible for cell proliferation, morphology, and transport, and oncogenic roles in cancer. Predicted metabolite turnover analysis demonstrated differential metabolism of sulfur-containing and phenolic compounds.

### **Sponge sensory systems: Do sponges have primary cilia?**

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Sponges respond to stimuli with coordinated behaviours - indicating their ability to sense their environment. Yet, a sensory system in sponges has not yet been described, including what cells are used to collect and transduce sensory information from their environment. Primary cilia are non-motile cilia found on almost every cell in vertebrates and on most invertebrate cells, and perform a range of sensory functions. Here we show that sponges have pairs of short, non-motile cilia lining their oscula, which are oriented perpendicular to the water flow and appear to have the attributes of primary, or sensory, cilia. In the freshwater sponges *Ephydatia muelleri* and *Spongilla lacustris* we demonstrate that these cilia function as mechanosensory organelles to coordinate behaviour. Three cationic channel blockers that inhibit the sensory function of primary cilia in other organisms either reduced or inhibited the stereotypical 'sneeze' response of freshwater sponges. This sneeze behaviour results in the inflation and contraction of the canal system in response to chemical or mechanical stimuli, and is thought to flush out waste and debris from the canal system. The three cationic channel blockers also caused lengthening of the cilia in the osculum, but not the flagella of the choanocyte chambers. Chloral hydrate - thought to remove cilia from cells - also abolished the sneeze response, and after five days of washout from the drug the behavior returned. That sponge primary cilia and cilia in vertebrates have a similar pharmacology suggests either an ancient origin for mechano-sensory cilia or the convergence of sensory function based on a similar



molecular toolbox. This is the first demonstration of a sensory system in adult sponges that functions without the use of conventional nerves.

**Are there “keratose” demosponges in the Phanerozoic fossil record?**

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Recent phylogenetic research revived the classical taxonomic unit “Keratosa” into two major groups, the Verongimorpha (Verongida, Halisarcida, Chondrosida) and Keratosa (Dictyoceratida, Dendroceratida), which form the sister group of other demosponges (e.g. Erpenbeck et al. 2012). However, comparing with the unequivocal appearance of siliceous sponges at the beginning of Cambrian (e.g. Steiner et al. 1993; Yang et al. 2005), the earliest reliable fossil of a keratose taxon, Vauxiidae Walcott, 1920 was only documented in the Middle Cambrian Burgess Shale. In the following periods of Phanerozoic, fossils of “keratose” sponges are rarely reported except from the Middle Devonian microbialites from Boulonnais, Northern France (Reitner et al. 2001). The main problem is the fossilizing mode of the organic skeleton-fibers of the “keratose” demosponges. It has been discovered by recent investigations (Ehrlich et al. 2010) that some Verongida sponges possess organic skeleton-fibers made of chitin. Chitin is a very stable polysaccharide and exhibits a strong resistance against microbial degradation. In this investigation, we checked the fossil record of possible Keratosa in the Tommotian archaeocyatid reefs from the Lena River (Siberia), Middle Devonian microbialites from northern France, Lowermost Triassic microbialites of the Tethyan realm and microbial-bivalve reefs from European Triassic Muschelkalk. Within these occurrences we have found suspected keratose sponge structures which are preserved as irregular automicrite patches, exhibiting calcite cemented networks of fibers which resemble to dendro- or dictyoceratid sponge skeletons. This preservation is comparable with the other reef-dwelling siliceous sponges which also appear in situ in the Devonian and Triassic samples. The skeletal fibers are distinguishable from bioturbation, fenestral fabrics and compacted peloidal structures by fiber diameter, regularity and network pattern. This implies a previously ignored potential of aspicular sponges to be recorded as body fossils in certain condition. Further study on the taphonomy and paleobiology of these fossils are critical for our understanding on sponge evolution.

**Disease and environmental stress in a coral reef sponge**

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Approximately two thirds of *Ianthella basta* populations from the Great Barrier Reef and Torres Strait show visible evidence of a disease like syndrome. Symptoms of the syndrome include discolored, necrotic spots, tissue degradation, exposure of skeletal fibers and disintegration of feeding cells. To ascertain the role of microbes in the disease process, profiles of bacteria, viruses, fungi and other eukaryotes were compared between healthy and diseased sponges. A very low diversity of microbes was evident with both healthy and diseased sponge communities dominated by three strains: an Alphaproteobacteria, a Gammaproteobacteria and a Thaumarchaea. Bacterial cultivation, community analysis by DGGE (bacteria and eukarya), sequencing of 16S rRNA clone libraries (bacteria and archaea) and direct visual assessment by electron microscopy failed to reveal any putative pathogens. In addition, infection assays could not establish the disease in healthy sponges even after direct physical contact with affected tissue. These results provide evidence that microbes are not responsible for the formation of brown spot lesions and necrosis in *I. basta*. Further manipulative experiments were undertaken to determine the role of environmental stressors (sediment and temperature) in the formation of brown spot lesions. Although sponge health deteriorated under thermal stress, neither elevated temperature nor sedimentation induced brown spot lesions. Overall, the microbial community of *I. basta* remained stable under varying stressors, with the same three symbionts always observed. Future work will examine auto-immune dysfunction and senescence in the sponge host by identifying genes that are differentially expressed in sponges displaying disease-like symptoms.

**The contribution of siliceous sponges to the marine silicon cycle.**

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The buffer capability of the ocean to palliate increasing levels of atmospheric CO<sub>2</sub> largely resides in diatoms using much dissolved CO<sub>2</sub> through photosynthesis to support cell growth, concomitantly with much silicon (Si) to build the shell protecting the cell. As diatoms account for about 40% of ocean primary production and recycle the anthropogenic CO<sub>2</sub> excess, the coupling between the carbon (C) and Si marine cycles is receiving notable attention. Unfortunately, the understanding of how Si is cycling in the ocean has grown in

association with the idea that diatoms are the only relevant biological players, being other Si users, such as sponges, radiolarians and choanoflagellates, unimportant. By investigating Si budgets associated with siliceous sponges, we have discovered this group to have a substantial, overlooked impact on the Si cycle. On continental shelves and slopes, Si standing stocks per bottom area in sponge communities may equal and even surpass those in the diatom communities of the overlying water column. Sponge uptake systems consistently saturate at concentrations that are orders of magnitude higher than those of diatoms. Although this feature makes shallow-water sponges to be limited by DSi availability, deep-sea populations are able to impact notably regional Si budgets. Additionally, sponge longevity and resistance of sponge spicules to dissolution in seawater cause sponges to lock DSi into biogenic silica (BSi) for periods that are orders of magnitude longer than those estimated for diatoms. Consequently, sponge populations are net sinks, favoring massive export of Si to the geological cycle. More importantly, the fact that most sponge DSi demands, BSi standing stocks, and Si exports are concentrated on continental margins has important implications for modeling local and regional budgets. Therefore, our work uncovers a relevant, sponge-driven Si sub-cycle that is strongly decoupled from the C cycle, shows extremely slow turnover rates, and challenges the traditional view of a marine Si cycle in perfect equilibrium. By systematically neglecting Si fluxes through sponges, the widely accepted Si cycle model has led to a biased oversimplification of real cycling processes in the ocean and it is overestimating the levels of connection between Si and C cycles.

**Should we be managing for sponges, too? Emerging opportunities for sponge research to contribute to the management of coral reefs under a changing climate**

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Coral reef management is going through significant adaptation, if not transformation. All around the world coral reefs are in decline, despite often decades of dedicated management effort and investment. Much of this decline is driven by forces beyond the direct influence of reef managers - not least among them climate change - forcing a reconsideration of the objectives and expectations of reef management. Resilience has emerged as a key concept for this recalibration, promoting a shift in focus to the dynamic nature of coral reef systems, the importance of protecting processes and the sustainability of ecosystem services. Despite these important adaptations, coral reef management still has an overt and often exclusive focus on corals. Yet, in many places (notably the Caribbean) sponges can be prominent components of the benthic community and important providers of habitat. This is likely to be

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increasingly true for other reef areas as the climate changes. Understanding the role of sponges in the coral reefs of the future, and appreciating the role of sponges in the coral reefs of today, is important to effective management of coral reef ecosystems. The emerging use of resilience frameworks, and increasing focus on ecosystem services and the resilience of reef-dependent communities, creates opportunities for sponge research to contribute more substantively to coral reef management. Increasing application of integrated system modeling to the design of management strategies provides mechanisms for the importance of sponges to be tested and incorporated into management considerations.

### **Biodiversity associated with marine sponges using next generation sequencing and traditional approaches.**

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Marine sponges are an important element of marine coastal ecosystems and in some ways they may be regarded as ecosystems themselves. They provide habitats for some organisms but are hostile to others. Next generation sequencing methodologies have recently been employed to document the bacteria associated with sponges. Here we extend this approach and present information on both the prokaryotic and eukaryotic fauna associated with *Haliclona indistincta* and *Dysidea fragilis* from the same location in Galway Bay, west coast of Ireland. By producing 16S and 18S ribosomal RNA gene sequence data, using the 454-sequencing platform, from the surrounding seawater and from separated cell fractions from each sponge we are beginning to tease out the diversity associated with these sponges and the operational taxonomic units that are unique to each sponge species. Metagenomic data will be presented along with results from traditional taxonomy of visible fauna and from transmission electron microscopy.

### **Significant shifts in sponge-associated bacterial community and metabolic profiles of sponge *Aplysilla rosea* challenged by *Vibrio natriegens***

Mohammad Mehbub, Chris Franco, Wei Zhang  
*Flinders University*

As the single best producers of marine natural products, it is hypothesized that many of these secondary metabolites from sponges are produced by sponge-associated bacteria, or as a result of the interactions of sponges with their symbiotic bacteria and foreign bacteria. The exact interactions between sponge and bacteria, and how these interactions impact on the biosynthesis of bioactive metabolites, however, remain unresolved questions. The aim of this study is to

simulate such interactions by challenging a marine sponge *Aplysilla rosea* with a non-pathogenic foreign marine bacterium *Vibrio natriegens* in controlled aquarium setting for an understanding of potential impact on sponge-associated bacteria community and its metabolic profiles. Microbial communities associated with sponges were analysed by pyro-sequencing and TRFLP, and the sponge metabolic profiles were analysed by GC-MS and LC-MS for both apolar and polar metabolites, respectively. When *A. rosea* explants were challenged by *V. natriegens* at  $5 \times 10^6$  cells/ml in a closed aquarium system for 48 hours, significant shifts in its associated microbial community and metabolic profiles were found within 24 hours. In the controlled condition, *A. rosea* contains a diverse bacterial classes including Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria, Flavobacteria, Actinobacteria, Epsilonproteobacteria, Betaproteobacteria, Plactomycetacia, Lentisphaerae, Deltaproteobacteria, Clostridia, Bacilli, Gemmatimonadetes, Acidobacteria and Bacteroidia. After 24 hours challenge, the control group was dominated with Cyanobacteria whereas treated group was dominated with Gammaproteobacteria (e.g. *Cowellia* sp. and *Vibrio* sp.) with a significant bacterial diversity shift. After 48 hours treatment, the control group was dominated with Alphaproteobacteria whereas treated group was dominated with both Alphaproteobacteria and Gammaproteobacteria. The changes in sponge metabolic profiles correlated with the shift in sponge-associated bacteria community, indicating that some of sponge-derived metabolites are produced as a result of the sponge-bacteria interactions. The results support the notion that sponge-bacteria interactions play important role in producing sponge-derived metabolites.

**The boring sponges of Abrolhos Bank, Brazil, with a comparison between the community structure of the inner and outer reef arcs.**

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Boring sponges are among the major bioeroders in reef ecosystems and deserve growing attention worldwide due to their potential use as biomonitor of reef health, specially under the ongoing global seawater acidification, water quality degradation and overfishing. The Abrolhos Bank is the largest reef complex in the South Atlantic, comprising a mosaic of benthic megahabitats that includes soft bottoms, rhodolith beds, coral reefs and volcanic islands. Although this reef system has been regularly monitored in the last two decades and despite considerable qualitative sampling in the 1990's, its sponge fauna remains poorly known. We examined ~500 sponge samples from Abrolhos deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro, specifically searching for boring sponges. On March 2013 we conducted the first effort to quantify boring sponges in the area by sampling a non-protected area at the inner reef arc

(Pedra de Leste) and a protected area at the outer reef arc (Parcel dos Abrolhos). The number of specimens of each species was recorded in 10 (10x1 m) randomly distributed transects on the reef tops (2–8 m depth), totaling 100 m<sup>2</sup> at each site. Seven boring species were recorded: *Cervicornia cuspidifera*, *Spherospongia vesparium*, *Siphonodictyon* sp., *Siphonodictyon coralliphagum*, *Cliona carteri*, *C. delitrix* and *C. varians*, with the latter four being recorded in transects. A significant difference in boring sponges abundance was recorded between inner and outer arc reefs, with a total of 0.3 vs. 1.2 specimens.m<sup>-2</sup>, respectively. The abundance of *Cliona carteri* and *C. delitrix* was significantly lower at the inner arc than at the outer arc (0.19 vs. 0.75 specimens.m<sup>-2</sup> and 0.09 vs. 0.44 specimens.m<sup>-2</sup>, respectively). *Cliona carteri* dominated at both sites, followed by *C. delitrix* and *S. coralliphagum* (0.02 and 0.01 specimens.m<sup>-2</sup>). Although *C. carteri* was the most abundant species, it perforates mostly non-coral substrates (e.g. crustose calcareous algae), while *C. delitrix* was the major borer of corals.

Drivers of such evident cross-shelf differences in the community structure of boring sponges still remain unclear and will be investigated in future studies.

**Stelligeridae revisited: an investigation into Stelligeridae Lendenfeld, 1898 (Porifera: Demospongiae) using molecular and morphological characters.**

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The classification of Demospongiae is undergoing significant reorganization and refinement in light of numerous phylogenetic studies. Traditionally sponge classification has been based primarily on spicule morphology and skeletal architecture, yet both of these character sets can be subject to secondary loss, parallel evolution and convergence. The difficulty in differentiating whether skeletal characters indicate a common evolutionary origin (homologous) or whether they are a consequence of convergence or parallel evolution (homoplasious) has resulted in skeletal characters being interpreted differently among sponge taxonomists. In recent years a growing number of molecular studies have challenged the traditional, morphology-based classification. Previous molecular studies indicated a close relationship between some taxa assigned to Heteroxyidae Dendy, 1905 (*Halicnemia* Bowerbank, 1864; *Higginsia* Higgins, 1877) and some Hemiasterellidae Lendenfeld, 1889 (*Paratimea* Hallmann, 1917 and *Stelligera* Gray, 1867). In the current study we further investigated this relationship using molecular and morphological characters. Our results support the resurrection of Stelligeridae sensu Morrow et al., 2012 for the clade containing *Halicnemia*, *Higginsia*, *Paratimea* and *Stelligera*. Heteroxyidae

and Hemiasterellidae are polyphyletic, as previously suggested by molecular studies. We demonstrate that many of the taxa assigned to Heteroxyidae on the basis of the possession of smooth or acanthose microxea are actually more closely related to other heteroscleromorph groups and propose several changes to the classification of Heteroscleromorpha.

**Screening Apolar extracts from South Australian marine sponges for Cytotoxicity properties against three human breast cancer cell lines**

Shuang Peng, Wei Zhang, Barbara Sanderson  
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Marine sponges [Porifera] are the single best novel marine natural products, account for 30% of all marine natural products discovered by far. The oceans in South Australia have largely untapped resource of marine sponge with an estimated number over 800 species, which accounts for approximately over 58% species in Australia.

The aim of this study is to screen these untapped marine sponges in South Australian water for their anti-breast cancer activity. Thirty-five marine sponge species were collected and subjected to crude apolar extracts. These extracts were tested against three breast cancer cell lines (T47D, MCF7 and MDA-MB-231) using Crystal Violet assay. 60% of crude apolar extracts showed cytotoxicity activity to these three breast cancer cell lines. Crude apolar extracts from five sponges (Tedania sp, Chondropsis cf. arenifera, Chondropsis sp1, and Chondropsis sp2) have significant ( $p < 0.05$ ) Cytotoxicity result compared with untreated cells. The IC50 values range between 12.43 to 25.89 $\mu\text{g/ml}$  (24h treatment), 2.03 to 12.92 $\mu\text{g/ml}$  (48h treatment) and 1.02 to 3.55 $\mu\text{g/ml}$  (72h treatment). These apolar extracts showed significant ( $p < 0.05$ ) difference on cancer cell lines compared to a normal breast cell lines 184B5 (IC50: 12.11 to 23.36 $\mu\text{g/ml}$ , 48h treatment). Flow cytometry was used to assess the induction of apoptosis and potential cell cycle arrest. Apolar extracts induced apoptosis at 10 $\mu\text{g/ml}$  in MCF7 and MDA-MB-231 cell lines, and arrest cell cycle in G1 and S phase compare with untreated cells.

**The stress of reproduction: gene expression along the life cycle of the Mediterranean sponge *Crella elegans* (Demospongiae, Poecilosclerida)**

Alicia. R Pérez-Porro, María J. Uriz, Gonzalo Giribet  
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Next generation sequencing (NGS) has become a powerful tool to generate valuable genomic and transcriptomic data in non-model animals in recent years. The new cost-effective ways to obtain genomic and transcriptomic libraries have been added to the development of new bioinformatic tools for de novo assembly

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and analysis in absence of a reference genome. Given this a Pandora's box has been opened: we can now ask new questions about the biology of non-model organisms. Classical ecological studies about sponge reproduction have been key to comprehend the biology of this taxon. With the addition of gene expression studies based on NGS data we can now contribute new genetic information to the understanding of the whole process. We obtained samples of the Mediterranean encrusting sponge *Crella elegans* at six different stages along its annual reproductive cycle: 1) non-reproductive tissue before the reproduction season, 2) tissue with spermatid cysts, 3) tissue with embryos, 4) tissue with larvae, 5) post-reproduction degraded tissue after larval release, and 6) free swimming larvae. For each sample/stage we built cDNA libraries that were sequenced using the Illumina® platform. The differential gene expression of a set of genes related with stress (e.g., heat shock proteins) and reproduction (e.g., protein tyrosine kinases) was calculated in order to obtain a pattern of the expression fluctuation of those genes along the reproductive cycle under natural circumstances. Such a level of understanding of the differential gene expression could become a standard tool in future studies to assess thermal stress and its possible effects in the biology of sponges.

### **You've got a friend in me: High stability of *Ircinia*-associated bacterial communities over time and space**

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Sponge-associated bacterial communities are complex and little is known about the abiotic and biotic factors that determine their structure. To assess the relative importance of host species, environmental conditions and geographic distance in shaping symbiotic assemblages, we targeted 3 sympatric Mediterranean sponges (*Ircinia fasciculata*, *I. variabilis* and *I. oros*) that each harbor a bacterial community composed of a specific mix of generalist symbionts. We sampled sponge populations for each species over temporal (3-month intervals for 1.5 years) and spatial (6 locations, 100 to 800 km apart) scales and characterized their bacterial communities using replicated clone library and terminal restriction fragment length polymorphism (T-RFLP) analyses of 16S rRNA gene sequences. Multivariate statistical analyses and nMDS plots confirmed the host specific structure of *Ircinia*-associated bacterial communities and revealed their maintenance over time (despite annual temperature fluctuations of 12°C and seasonality in irradiance) and space (despite geographic distances of over 800 km). Some variability within each host species was observed, though restricted to changes in rare symbiont taxa during warmer months and spatial differentiation among a few populations of *I. variabilis*. The overall stability of host-symbiont



interactions across large temporal and spatial scales further supports the hypothesis that symbiotic communities are primarily structured by factors specific to each host species, notably, the evolutionary history of each host species, which favor the persistence of stable symbiont communities despite local variability in the external environment. Exploratory studies over spatio-temporal scales are critical to understand the underlying processes that drive sponge-bacteria interactions, to diagnose their resilience to global change, and to predict the ecological implications of holobiont disruption.

**Habitat-based assessment of mesophotic sponge gardens of the Murion Islands, Northwest Australia using towed video and AUV optical imagery**

Ben Radford, Jamie Colquhoun, Oscar Pizarro, Stefan Williams  
*Australian Institute of Marine Science/ Colquhoun/ Australian Centre for Field Robotics*

We present a collaborative benthic habitat survey of northern Ningaloo Marine Park and Muiron Islands management area, northwest Australia. Seabed surveys were conducted by the autonomous underwater vehicle (AUV) 'Sirius' captured high resolution optical imagery and associated physical datasets in different types of terrain and conditions. Spatially registered imagery was analysed via human observer informed automated classification methods. These data combined with multibeam bathymetry was used to develop spatially explicit habitat models detailing the fine scale distribution of sponges and filter feeders. It was also used to investigate the correlation between sponge communities and depth, substrate and light.

The versatility of AUV technology for marine benthic surveys is highlighted by the ability to acquire targeted, repeatable high resolution optical images over a large spatial scale. Current challenges lie in developing more efficient ways for data analysis, storage, visualization and processing, to provide timely and cost-effective outputs relevant to conservation managers and policy makers. While currently outside Ningaloo Marine park boundary, this survey details significant biodiversity associated with the sponge dominated, abundant filter feeding communities in waters off the Murion Islands.

**Sudden sponge occurrences in the aftermath of severe extinction events in the Phanerozoic**

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The Phanerozoic earth history is characterized by critical intervals like five severe extinction events. They were based on severe interruptions of ecologically established environments. Discussed are various reasons, however, long lasting

volcanic eruptions (Large Igneous Provinces) may have played the most important role. These eruptions often caused high CO<sub>2</sub>, CH<sub>4</sub> and SO<sub>2</sub>/H<sub>2</sub>S concentrations in the atmo- and hydrosphere resulting in, e.g. oceanic acidification, fast changes of global temperature and low oxygen/anaerobic levels in the oceanic water column. Ocean water chemistry and nutrient balance were heavily disturbed and changed. It is conspicuous that in the early phases of ecological recovery of shallow to mid-shelf environments sponge communities often associated with microbial build ups played a significant role. These communities are often dominated by e. g. lyssacinoid hexactinellids, mainly tetractinellid and ?keratose? demosponges. These communities resemble deep water sponges which have moved from a deep water setting into a shallower water environment. Shortly after the extinction events these ecological niches were not yet reoccupied by shallow water organisms. Reasons probably were that nutrients like water chemistry and illumination of the shallow sea were almost comparable with deep water conditions. This pattern is well documented in a presumable deep water sponge community of the Hirnantian extinction (End Ordovician glaciation) in a mid-shelf environment of southern China. The most critical extinction, the Permian-Triassic (P/T-) boundary, was likely caused by the Siberian Trap flood basalts. The early Triassic recovery was characterized by small microbial build-ups often associated with ?keratose? demosponges and lyssacinoid hexactinellids. The End Triassic extinction event was likely related with the Central Atlantic Magmatic Province (CAMP). In the lowermost Jurassic (Hettangian) of the Tethys realm a significant increase of sponge diversity is seen. Remarkable are deep water hexactinellid- and demosponge communities which settled on top and margins of drowned dead Triassic reefs and shallow pelagic shelf areas. In all observed cases deep water sponges in shallow water niches disappeared after 2 million years at the latest and were replaced by typical shallow organisms.

### **Bacterial profiling of closely related shallow to deep-sea *Hexadella* (Porifera, Verongida) species by Illumina sequencing**

Julie Reveillaud, Loïs Maignien, A. Murat Eren, Amy Apprill, Mitchell L. Sogin, Ann Vanreusel

*Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole/ Woods Hole Oceanographic Institution/ Marine Biology Section, Ghent University, Belgium*

Microbial communities play a key role in the physiology of the sponge host, and it is therefore essential to understand the stability and specificity of sponge-symbiont associations. In particular, the relative influence of the environment and the sponge host on the composition of the symbiont community remains unclear. Here, we exploited the high sequencing depth capacity offered by the Illumina technology, together with a well-resolved host phylogeny, to attempt an

exhaustive characterization of host-specificity patterns in sponge microbiota, including members of the rare biosphere, in the phylum Porifera.

We analyzed 82,560,660 high-quality V6 region tags of the bacterial 16S rRNA gene to profile the microbiome of seven closely related shallow to deep-sea water *Hexadella* sponge species, and of the distantly related sponge *Mycale*. In addition, we examined the bacterial community of the deep-sea background seawater where both *Mycale* and the widespread deep-sea taxa *H. cf. dedritifera* were sampled. While our data did not reveal any co-speciation patterns between the *Hexadella* species and their associated microbial communities, we confirmed sponge specific and sponge-species specific bacterial communities. In addition, we found a striking diversity of microbes associated with the sponges, spanning almost 50 bacterial phyla. We then explored diversity within dominant and functionally important members of the sponge microbiome. Overall, our results show that sponge-bacteria are really diverse and highly specific communities controlled by both host and environmental-related factors.

### **Molecular machinery of germ line, sex determination, and vitellogenesis of sponges**

Ana Riesgo, Susanna López-Legentil, Sally P. Leys, Gonzalo Giribet  
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The recent publication of two sponge genomes revealed a remarkable molecular complexity that was unexpected when considering the apparently simple sponge body plan. Regarding the reproductive biology of sponges, which can either be oviparous/viviparous and hermaphroditic/gonochoristic, usually the aquiferous system plays an essential role in the production and transfer of gametes, yet carnivorous sponges reproduce without such a system. Whether this diversity is accompanied by a different molecular machinery cannot be inferred by simply comparing the genomes of two species. Regarding germ line specification, only eight genes have so far been described in sponges although approximately twenty were found in other metazoans. Very few is known about the rest of genes involved in the production and nourishing of gametes. Using NGS, we sequenced eight sponge species belonging to the four extant classes (Hexactinellida: *Aphrocallistes vastus*, Calcarea: *Sycon coactum*, Demospongiae: *Chondrilla nucula*, *Ircinia fasciculata*, *Petrosia ficiformis*, *Spongilla lacustris*, and Pseudospongosorites *suberitoides*, and Homoscleromorpha: *Corticium candelabrum*), and focused on the identification of the germ line, sex determination, and vitellogenic genes. Out of the 20 genes described to participate in the determination of the germ line, we found eleven genes in sponges. The genes *vasa*, *nanos*, *maelstrom*, *PL10*, *mago nashi*, *piwi*, *tsunagi*, and *smaug* were present in all species, while the genes *germ cell-less* and *pumilio* were absent in two species, and *boule* was not detected in one species. Paralogy was detected in *piwi*, *boule*, *PL10*, *nanos*, and *mago nashi*. The sex determination gene *DMRT1* was found exclusively in *Corticium*

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candelabrum, while FEM-1 appeared in all datasets, sometimes showing 2 paralog sequences. All species datasets, with one exception, contained the precursor of the pheromone attractin and also, vitellogenin was found in all species but one, even though the sequences diverged greatly among species. All sponges examined possessed a highly complex molecular machinery for germ line determination and gamete production, both being also very similar among species and highly conserved when compared with other metazoans. Interestingly, the only representative with an eumetazoan sex determinant (DMRT1) is the homosclerophorid species *C. candelabrum*, which possesses spermatogenic cysts containing all stages of spermatogenesis, similar to other eumetazoans. The specific function of all these genes is not fully understood, but the acquisition of the germ line, sex determination, and vitellogenic molecular machinery is of undeniable importance for understanding the evolution of reproduction in sponges.

### **Integrative transcriptome analysis of two Red Sea sponges, *Stylissa carteri* and *Xestospongia testudinaria***

Taewoo Ryu, Ute Hentschel, Timothy Ravasi  
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Two Red Sea sponges, *Stylissa Carteri* and *Xestospongia testudinaria*, are attractive species due to their properties as primitive multicellular organisms, great difference in abundance of residing microbial communities, and close interaction with surrounding reef species. However, their gene repertoire, the key to understand their characteristics, is still unknown. In this study, we analyzed transcriptome of these sponges using massive high-throughput sequencing techniques. We also compared our data with transcriptome of other published sponges from different geographical locations. We identified many conserved gene toolkits and protein domains that are essential components of metazoans. We also identified many lineage-specific genes that are not characterized so far. Gene repertoire between two sponges shows remarkable difference that reflects the different characteristics of them. Our analysis results provide valuable resources for deeper understanding of evolution of basal multicellular organisms and interaction between host and residing microbial organisms.

**Temporal dynamics and tissue specificity of sponge-associated bacteria in  
*Aplysina aerophoba***

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A diverse microbiota has been found inhabiting marine sponges with implications in secondary metabolite biosynthesis and other aspects of host metabolism. The stability of the sponge holobiont has been tested under different environmental conditions; however, natural dynamics and intraspecies variability over time are largely unknown. Here, we investigated temporal variation in bacterial symbionts in two tissue layers (i.e., ectosome and choanosome) of *Aplysina aerophoba* over a two-year period in the northwestern Mediterranean Sea. We sampled five different specimens every two months to assess seasonal bacterial patterns using terminal restriction fragment length polymorphism (T-RFLP). A total of 258 unique microbial symbiont terminal restriction fragments (T-RFs) were identified from *A. aerophoba* (126 using the restriction enzyme *HaeIII*, and 132 with *MspI*). Sponge-associated bacteria exhibited remarkably tissue specificity; about 50% of T-RFs were specific of each tissue layer (49.2% for *HaeIII* and 48.5% for *MspI*). T-RFs were similarly distributed between the inner and the outer sponge layer for *HaeIII* (26.2% and 24.6%, respectively), while the ectosome were enriched in T-RFs in contrast to the choanosome for *MspI* (31.8% and 19.7%, respectively). Bacterial profiles from the external region seemed to be affected by a large temporal scale (i.e., warm water vs. cold water seasons). However, symbiont communities from the inner part varied in a smaller scale (i.e., months), variability restricted to months of warmer seasons, coincident with elevated thermal regimes. Despite certain temporal shifts in sponge microbiota, symbiont consortia showed considerable stability most of the year with no interannual changes. This study contributes to the assessment of temporal dynamics of sponge-microbe associations over multiple years and improves our understanding of the organization and functioning of these complex host-symbiont interactions.

**Impact of global change on sponge bioerosion: from experimental  
evidence to monitoring guidelines**

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Reef corals are seriously suffering from human and environmental impacts. However, to date we have no evidence that the bioeroders in reef ecosystems experience negative effects in the same way, whereas new experimental data for bioeroding sponges rather suggest the opposite. In an experimental setup at Orpheus Island (central Great Barrier Reef), the zooxanthellate bioeroding

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sponge *Cliona orientalis*, one of the most abundant and detrimental bioeroders at Australia's Great Barrier Reef, was exposed to different combinations of lowered as well as elevated levels of carbon dioxide partial pressure and temperature. Our results prove a significant enforcement of the sponges' bioerosion capacity with increasing pCO<sub>2</sub> (decreasing pH) inherent to ocean acidification, while temperature had comparatively little effect. This implies that in a high-CO<sub>2</sub> world, tropical reef ecosystems are facing the combined effect of weakened coral calcification and accelerated bioerosion, resulting in critical pressure on the balance between biogenic carbonate build-up and degradation. Despite the importance of the issue and an urgent demand for information for reef authorities and modellers of reef health, we lack data and particularly time series supporting impact scenarios over larger scales. Monitoring data are needed, and we recommend targeting only the most dominant species per area, which usually belong to the *Cliona viridis* species complex. These mostly occur in encrusting form and are easily spotted and quantified. Line intercept transects should be used, being the most simple, cheap, accurate and reliable of three tested methods. International monitoring programs in the league of Reef Check are highly encouraged to include this approach of recording bioeroding sponges in their existing protocols.

### **Bio-silica from sponges: Towards new applications in nano-biotechnology and biomedicine**

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Bio-silica, a self-adapting and -repairing material that forms the inorganic skeleton of the siliceous sponges (*Demospongiae* and *Hexactinellida*) is characterized by exceptional mechanical and optical properties (1). A breakthrough in understanding sponge spicule formation came with the demonstration that the axial filament protein silicatein acts as an enzyme which catalyzes bio-silica synthesis at low precursor concentrations (2). Bio-silica has a hierarchical architecture – a result of its mechanism of formation: Self-cleavage of the immature pro-silicatein into a pro-peptide and the mature silicatein triggers this molecule not only to become enzymatically active but also to acquire structure-guiding and structure-forming properties (3). The initially formed soft bio-silica undergoes an aging/hardening process (syneresis), mediated by aquaporin channels, and a process of “precision bio-silica molding” (1). The bio-silica nano-spheres fuse together by “bio-sintering”. The spicules can transmit light, both in vitro and in vivo (2) like technical optical fibers. In the demosponge *Suberites domuncula* both a light generation (luciferase) and a light perception system (cryptochrome) have been identified. Recently we found that this sponge which can flash in a rhythmic manner contains nocturnin, a light/dark-controlled

gene that controls the half-life of mRNA (4). The enzymatically formed bio-silica, along with the enzymes/proteins involved, allows novel applications in nano-biotechnology, nano-optics and nano-medicine (5). Recent developments in the biotechnological/biomedical applications of silicatein and its bio-silica product, including its application in bone repair and 3D printing (“rapid prototyping”), will be presented. (Supported by EU FP7 grants “SPECIAL” no. 266033 and Marie Curie Training Network “BIOMINTEC” no. 215507; WEGM: ERC Advanced Grant no. 268476).

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### **Anti-predator and anti-microbial defences in tropical Pacific sponges**

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Sponges, like any other sessile benthic organisms, have to deal with several ecological threats like predation, fouling, or competition. To overcome these threats, they have evolved a wide array of defensive mechanisms, such as morphological or chemical defenses. The aim of this study was to determine how common sponges from Guam and surrounding Micronesian islands employed secondary metabolites as defense against predators and microbial pathogens. To test for antipredatory defenses choice feeding assays were conducted in the field with omnivorous fishes and in the laboratory with the pufferfish *Canthigaster solandri*. Therefore, sponge crude extracts were incorporated at natural concentrations into an artificial diet and offered simultaneously with food containing solvent only to potential fish predators.

In addition, the same sponge extracts were tested at natural concentrations for possible antimicrobial activity in disc diffusion assays against ecologically relevant bacterial isolates. Bacteria had previously been isolated from biofilms of reef rock and crustose coralline algae. Disc diffusion assays were used to evaluate the antibacterial properties of the different sponge extracts. Crude extracts of both *Xestospongia carbonaria* and *Stylissa massa* revealed very pronounced antimicrobial defense against all bacteria classes.

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A subset of the investigated sponges was further analyzed with regard to dynamic defensive strategies, such as activated or induced defenses. Each of these represents a trade-off between costs, availability, risks and efficacy of defense. Eight different sponge species were artificially grazed in four different ways to yield activated, non-activated, induced and non-induced extracts, which were subsequently tested for antimicrobial properties against bacteria and antifeeding activities against the pufferfish *C. solandri* and the urchin *Diadema* sp. Three of the eight sponges revealed induced or activated defenses against fish feeding, while most artificially grazed sponges induced higher anti-microbial defenses in most tested bacteria. Furthermore, we investigated whether allocation of defense metabolites followed strategies such as the Optimal Defense Theory.

### **Molecular palaeobiology of ‘Lithistid’ demosponges: A new classification**

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‘Lithistida’, a polyphyletic group of recent and fossil sponges, provide the richest fossil record among demosponges and produce bioactive compounds useful for pharmaceutical research. ‘Lithistid’ sponges possess a firm to rock-hard skeleton of irregularly articulated siliceous spicules (desmas) as their defining morphological feature, but although previously all assigned to its own order, they have been shown to be not monophyletic. The most recent morphology-based classification of extant ‘lithistid’ sponges comprises 13 families and 41 genera.

By integrating morphological and molecular data, we can benefit from the well-documented ‘lithistid’ fossil record to elucidate the evolutionary history of demosponges. However, molecular studies on ‘lithistid’ sponges are rare or suffer from poor taxon sampling. Therefore, the phylogenetic relationships of this polyphyletic group are to a large extent unresolved and morphology-based hypotheses remain untested. This study therefore aims to investigate the molecular phylogeny of ‘lithistid’ sponges in more detail, reconstruct the evolution of their spicule morphologies and test morphological-based systematic hypotheses.

With a sampling of more than 300 specimens, covering all known families and 25 genera, this work presents the to date largest systematic study of ‘lithistid’ sponges, using independent mitochondrial protein-coding and nuclear ribosomal markers in addition to a newly constructed morphological character data matrix.

Our resulting phylogenies suggest that I) at least eight out of 13 families belong to the order Astrophorida; II) monotypic family Vetulinidae is the sister group to Spongillina; III) family Desmanthidae is polyphyletic and shows affinities to



halichondrid taxa (Dictyonella and Topsentia); IV) family Scleritodermidae is recovered as a separate clade to Astrophorida and Spirophorida.

Based on our molecular and morphological results, we recommend a re-classification of most lithistid sponge taxa to their closest relatives within the Demospongiae. Incongruences between our molecular trees and previous morphological hypotheses can be explained by convergent evolution and secondary spicule loss during evolution, which consequently has occurred multiple times in both mega- and microscleres in 'lithistid' and astrophorid sponges.

Our molecular phylogeny of 'lithistid' sponges, together with their rich fossil record, can be used to provide unambiguous calibration points for relaxed molecular clock estimates to reconstruct timing and radiation patterns within demosponges.

**Genetic diversity of the Indo-Pacific barrel sponge *Xestospongia testudinaria* (Haplosclerida: Petrosiidae)**

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*Xestospongia testudinaria* (Haplosclerida: Petrosiidae) is a large and widespread barrel sponge species in the Indo-Pacific. Although several studies have been conducted on its ecological role and bioactive compounds, less attention has been given to the genetic diversity of this iconic species. Molecular phylogenetics has repeatedly proven useful for unravelling genetic diversity, cryptic speciation, and phylogeography in sponges. Consequently, we applied nuclear and mitochondrial molecular markers to assess the genetic structure of *Xestospongia testudinaria* in the Indo-Pacific. At the same time, we aimed at testing to what extent this species' genetic structure correlates with its geographic distribution patterns. Likewise, we test whether the genetic structure observed has been affected by the paleohistory of the Indo-Pacific, which has been shown to shape the genetic structure of other marine organisms in this region.

We find no apparent correlation between geography and genetic variation in the *Xestospongia testudinaria* specimens investigated from twelve localities in the Indo-Pacific (Tanzania, Saudi Arabia, Taiwan, Thailand, West Java, Central java, East Java, Bali, North Sulawesi, Southeast Sulawesi, Great Barrier Reef Australia, and Solomon Islands) even after applying a faster-evolving mitochondrial marker ATP6 (Adenosine triphosphate synthase F0 subunit 6) or a nuclear marker, an intron of ATPS-? (Adenosine triphosphate synthase ? subunit). Furthermore, *Xestospongia testudinaria* shares several haplotypes with

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Xestospongia bergquistia and Xestospongia muta (dominating barrel sponge species in the Caribbean Sea). We discuss the implications of our findings for the taxonomic status of barrel sponges in the Indo-Pacific and the Caribbean Sea.

### **Ontogeny of choanocyte chambers during metamorphosis in Amphimedon queenslandica**

Shunsuke Sogabe, Nagayasu Nakanishi, Bernard Degnan  
*University of Queensland*

The choanocyte chamber is an instrumental component of the poriferan bauplan. It is comprised of choanocytes, a cell type that is structurally very similar to choanoflagellates, the sister group to the Metazoa. This has led to the proposition that these cells are homologous and that the first metazoan cell type was the choanocyte. The existence of extant colony-forming choanoflagellates (e.g. *Salpingoeca rosetta*) with proliferative properties similar to sponge choanocyte chambers (where *S. rosetta* colony formation and growth occurs via multiple mitotic events, consistent with previous observational studies undertaken on sponge choanocyte chamber formation) lends further support for this idea. Studying the formation of choanocyte chambers during the metamorphosis of the haplosclerid demosponge *Amphimedon queenslandica*, we find that choanocyte chamber ontogeny and growth to be more complex than often appreciated. During normal metamorphosis at 25°C, choanocyte chambers begin forming around 48 hours after settlement and initiation of metamorphosis. By 72 hours, the aquiferous system is functional and the average choanocyte chamber has increased significantly in size and cell number. Using cell tracker CM-Dil to follow cell lineages during metamorphosis, we demonstrate that the larval ciliated epithelial cells undergo a set of serial transdifferentiation events to ultimately become choanocytes in early postlarval chambers. These chambers display various Dil labeling patterns, where a single chamber can be completely labeled, unlabeled, or partially labeled, consistent with choanocyte chambers being comprised of cells from multiple larval cell origins and lineages, and not solely the product of proliferation of a single progenitor cell. Using anti-phosphorylated-histone-H3 antibody as a marker of mitotic nuclei, we find that *A. queenslandica* choanocyte chambers also grow by the division of choanocytes within the chamber. There is also evidence of extensive fusion of choanocyte chambers in 2 to 3 day postlarvae. Finally, there is evidence for the recruitment of external choanocytes into mature chambers. Together these results demonstrate multiple cell lineages and mechanisms contributing to the ontogeny and growth of choanocyte chambers in *A. queenslandica*, suggesting that this process may not be directly comparable to colony growth in choanoflagellates.

**'Putative effects of prawn trawling on sponge diversity and distribution in a temperate Australian gulf**

Shirley Sorokin, David Currie  
*SARDI Aquatic Sciences/ GHD*

The distribution and diversity of sponges in Spencer Gulf was evaluated by analysing the bycatch from commercial prawn trawlers. Quantitative samples of bycatch were collected from 120 stations over three nights in a whole-of-gulf survey. A total of 395 taxa including fish, benthic invertebrates, seagrasses and algae were collected. Sponges were represented by 105 taxa. A cumulative species model suggests that these sponge taxa represent a small fraction of the total sponge fauna in the gulf. Sponge biomasses were found to be highest in the north and south of the gulf, and were significantly lower in the central section of the gulf, where commercial prawn trawling has been concentrated over the last 20 years. This pattern was similar for both abundance and species richness data. Spatial patterns of biomass, abundance and richness were inversely correlated with historical prawn trawling effort. These findings collectively suggest that the cumulative effects of prawn trawling in Spencer Gulf have had a negative impact on the composition and diversity of the gulf's sponge fauna.

**Sponge chemical mediation: tracing the signals in the water column**

Eva Ternon, Olivier Thomas  
*University of Nice-Sophia Antipolis*

Because marine sponges are sessile filter feeders, they have selected all along their evolutionary history original metabolic pathways directed towards the production of secondary metabolites exhibiting an ecological role in the ecosystems. Most of these compounds have been identified as defensive allomones against non symbiotic bacteria or macro-organisms competitors. Whether these compounds are kept within the organism or released into the water column is still a matter of debate and a better assessment of the sponge secondary metabolites that are dissolved or in the particulate matter is urgently required in order to propose preliminary answers to these questions. Assessing the concentrations of non volatile organic derivatives in seawater clearly needs analytical development and we screen several processes already described for this purpose. We consequently developed a highly efficient column filtration that enabled us to quantify toxins produced and released by the Mediterranean marine sponge *Crambe crambe*. Furthermore, this process allowed us to trace the impact of different biotic and abiotic stressors controlling the production and the release of secondary metabolites in the water column.

**Assembling the Poriferan Tree of Life: Integrative Taxonomy and Systematics Confirm New Hypotheses of Sponge Evolution**

Bob Thacker, April Hill, Malcolm Hill, Niamh Redmond, Christine Morrow, Cristina Diaz

*University of Alabama at Birmingham/ University of Richmond/ Smithsonian Institution/ Queen's University/ Harbor Branch Oceanographic Institute/*

The highly collaborative research sponsored by the NSF-funded Assembling the Porifera Tree of Life (PorToL) project has considerably changed our understanding of phylogenetic relationships within the Phylum Porifera with increased taxon sampling and data from additional molecular markers. PorToL researchers have falsified earlier phylogenetic hypotheses, discovered novel phylogenetic alliances, found phylogenetic homes for enigmatic taxa, and provided a more precise understanding of the evolution of skeletal features, secondary metabolites, body organization, and symbioses. Our analyses of over 300 nearly complete 28S ribosomal subunit gene sequences, as well as over 500 partial 28S sequences, provide specific case studies that illustrate how our dataset confirms new hypotheses of sponge evolution. We recovered monophyletic clades for all four classes of sponges, as well as the four major clades of Demospongiae (Keratosa, Myxospongiae, Haploscleromorpha, and Heteroscleromorpha), but our phylogeny differs in several aspects from traditional classifications. In most major clades of sponges, families within orders appear to be paraphyletic. While additional gene and taxon sampling are needed to establish whether this pattern results from a lack of phylogenetic resolution or from a paraphyletic classification system, many of our results are congruent with those obtained from 18S ribosomal subunit gene sequences and complete mitochondrial genomes. These data provide further support for a revision of the traditional classification of sponges.

**Assessing the metabolic pathways of sponge alkaloids through feeding experiments**

Olivier Thomas, Marie-Aude Tribalat  
*University of Nice Sophia Antipolis*

The main economic value of marine sponges is undoubtedly associated to the chemical diversity of their secondary metabolites. Indeed, the original architectures of the small compounds produced by these sessile filter feeders may find applications in several industries and mostly in the pharmaceutical sector. In consequence, sponges are producers of unique high value added biomolecules and deserve attention for this purpose. However, our knowledge on the metabolic pathways leading to these often complex natural products is still very limited. Because molecular biology and biochemistry have today failed to

respond these key issues mainly because of the complex interactions within these old organisms, we decided to revisit the old fashioned feeding experiments with radiolabelled starter units to identify the precursors of well known sponge alkaloids like oroidin, crambescidins and saraines. Our results will help in the understanding of these original metabolic pathways and they will also give some clues towards an increased production of these original compounds.

**Chemoautotrophic fixation, translocation and turnover of inorganic carbon in the cold water coral encrusting sponge *Hymedesmia coriacea* (Tisler Reef, NE Skagerrak)**

Fleur C van Duyl, Richard Doggen, Sabine K. Lengger, Tomas Lundälv, Christina Müller, Stefan Schouten

*Royal Netherlands Institute for Sea Research/ Sven Lovén Centre for Marine Sciences*

Many sponges harbor a rich community of microorganisms of which the benefits for the sponge host are often poorly understood. We studied the fixation of dissolved inorganic carbon (DIC) by sponge associated chemoautotrophic microorganisms, the translocation of this carbon to the sponge *Hymedesmia coriacea* and the total turnover of chemoautotrophically fixed carbon in the sponge holobiont. *H. coriacea* grows encrusting over branches of the stony coral *Lophelia pertusa* at 90-115 m depth on Tisler reef, NE Skagerrak (Norwegian Shelf). Live sponge material was collected by ROV and kept in running seawater aquaria at the in situ temperature of 8°C in a climate room. Lipid and fatty acid analysis revealed that *H. coriacea* lives associated with bacteria and archaea. We incubated live sponges with <sup>13</sup>C-labelled DIC and assessed the incorporation and fate of labeled carbon in the sponge. The  $\delta^{13}\text{C}$  of the sponge bulk material increased by 3‰ in 1.5-2.5 days in experiments with a single sponge piece per incubation chamber after addition of 600-900  $\mu\text{M}$  <sup>13</sup>C-DIC and by 8‰ in the an aquarium with multiple sponges after addition of 2353  $\mu\text{M}$  <sup>13</sup>C-DIC. Average DIC fixation rates ranged from 0.5-0.7  $\mu\text{g C.g dry weight}^{-1}\text{sponge.h}^{-1}$ . Addition of ammonia did not stimulate or inhibit the DIC fixation rates. The increase of  $\delta^{13}\text{C}$  in sponge bulk organic matter coincided with evident <sup>13</sup>C enrichment of the polar lipid fatty acids C16:1 $\omega$ 7c (20‰) and C18:1 $\omega$ 7c (8‰) suggesting that chemoautotrophic nitrifying bacteria were involved. The tetraether lipids from sponge-associated Thaumarchaeota showed ambiguous results with less than 15‰ enrichment. Striking increases in labeling of up to 41‰ in long chain (>C23) fatty acids, which can be considered as biomarkers for sponges, indicate that sponge cells were also labeled within 1.5-2.5 days. After labeling, limited loss of <sup>13</sup>C from the sponge holobiont was recorded in a through-flow aquarium during 22 days, suggesting a slow turnover (>60 days) of sponge compounds (0.3-1.5%.d<sup>-1</sup>). We conclude that the sponge host rapidly uses compounds derived from its associated microbes and modifies them into constitutive compounds with a long turnover time.

**A blueprint strategy for improving the classification of the Porifera**

Rob Van Soest

*Naturalis Biodiversity Center*

. The past decade has seen the publication of many molecular studies testing the comprehensive morphological classification presented in the *Systema Porifera*. With numerically small and homogeneous groups there are encouraging improvements of the classification based on integrative studies, but with the larger groups - despite all the admirable efforts – frequent mismatches between the *Systema* classification and molecular results have become apparent, leading to a widely advocated pessimistic view about the employability of morphological characters. I do not share this pessimism about the lack of good morphological markers. Indeed, certain types of generalized characters which previously were considered synapomorphies may need to be rejected as probable plesiomorphies, but a majority of morphological markers shows intricate derived states in recognizable transformation series, especially when SEM and other ultrastructural research tools are employed. Regrettably, truly integrative studies are still few and far between and are under-represented among the many molecular studies. New or revised taxa have been erected on molecular markers in which the definitions were merely an enumeration of all morphological characters found in their members. Such definitions are not operational in a classification. For me as an editor of the WPD, the current situation is problematic because recent studies do contain serious indications that parts of the Porifera classification need to be changed, but due to the often incomplete or uncertain results these changes cannot (yet) be implemented. Simultaneously, we see an intensified exploration using SCUBA and ROVs of poorly investigated habitats and regions yielding a multitude of newly discovered taxa which must be assigned to well-established recognizable taxa in a stable classification. Morpho-molecular studies are urgently in need of a coordinated and cooperative effort to stepwise improve the classification without causing instabilities. I present recommendations for a concerted and rigorous approach in five areas of research activities: (1) objectivity by concentrating on types, (2) comprehensiveness, (3) quality control by expert taxonomists, (4) uniformity of methodology, (5) integration of morphological markers. I suggest a Do's and Don't's list for researchers in this field.

**Sponge symbioses in the shadows: Specialized co-existence between  
Plakortis and Xestospongia in cryptic habitats of the Caribbean**

Jan Vicente, Sven Zea, Russell Hill

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Cryptic habitats such as caves and crevices can harbor highly diverse and densely packed sponge communities, resulting in beneficial epizoic sponge associations as a strategy to deal with competition for the limited space. Alternatively, in this crowded environment sponges may establish highly specialized and intimate associations with other sponge species. We report two new species-specific symbiotic relationships involving species of *Plakortis* and *Xestospongia* from cryptic habitats of the Caribbean. Sponge specimens were identified with the use of molecular markers [cytochrome oxidase sub-unit 1 (COI), 28S rRNA and 18S rRNA gene fragments], spicule analysis and histological sections with SEM. The molecular phylogeny of the sponges involved in the association suggests that *Plakortis* sp. 1 and 2 are likely new species. We found *Plakortis* sp. 1 always living as a thin veneer of tissue over *Xestospongia deweerdtiae* (Lehnert & Van Soest, 1999) which was originally described living freely on the deep fore reef and reef caves of Jamaica. Interestingly, *X. deweerdtiae* tissue grew within the mesohyl and part of the surface of *Plakortis* sp. 1, unlike the basibiont-epibiont association previously documented for sponge pairs. Spicule measurements conducted on associated vs. free-living *X. deweerdtiae* revealed a significantly smaller spicule size for associated individuals implying a cost in terms of silicon availability, or a benefit in terms of a lower investment in skeleton for support and defense. Assuming there is need for larger spicules for either body support or defense, these results suggest that the association could be beneficial for *X. deweerdtiae*, allowing it to invest less energy in spicule synthesis. Possible ecological advantages for *Plakortis* sp. 1 are still unclear. Intriguingly, in Puerto Rico, *Plakortis* sp. 2 is associated not only with *X. deweerdtiae*, but has been found in association with another Petrosiid sponge *Xestospongia* sp. 3. This study reveals a new specialized symbiotic association between two distantly related sponges that is likely an alternate strategy of adaptation for life in reef caves.

**Genetic key components of spicule formation in calcareous sponges**

Oliver Voigt, Marcin Adamska, Kasia Sluzek, Gert Wörheide, Maja Adamska  
*LMU Munich, Department of Earth and Environment Sciences, Palaeontology &  
Geobiology/ Sars International Centre for Marine Molecular Biology*

Calcareous sponges are the only sponges which form calcite spicules. The spicules comprise a substantial proportion of the biomass of these sponges, and

by supporting the tissue enables them to form larger bodies. Furthermore the spicules and their arrangement are the main characters used in the taxonomy of the group. Without doubt, the ability to produce calcite spicules can be considered a key innovation predating the radiation of extant Calcarea.

We identified some essential genetic components involved in the process of calcification in the emerging model system *Sycon ciliatum* and verified our findings by additional data from *Leucosolenia complicata*. For this, we identified candidate genes from available genome and transcriptome data, and verified their involvement in biomineralization by combining calcein staining methods with RNA in situ hybridization. Our results are discussed in the context of the evolution of biomineralization in sponges and other metazoans.

**A Bacterial Symbiont of Japanese Marine Sponge *Discodermia calyx*,  
Produces Biologically Active Metabolites**

Toshiyuki Wakimoto, Yoko Egami, Yu Nakashima, Ikuro Abe  
*Graduate School of Pharmaceutical Sciences, The University of Tokyo*

Marine sponges have provided many secondary metabolites exhibiting complex structures and specific biological activities. In particular, the members of the family Theonellidae are exceptionally rich sources of structurally unique polyketides and peptides. For instance, the *Discodermia* and *Theonella* genera contain diverse biologically active metabolites, including calyculins and discodermins from the *Discodermia* genus and onnamides and polytheonamides from the *Theonella* genus. Interestingly, certain classes of these metabolites were structurally similar to the metabolites of terrestrial bacteria, which have suggested the bacterial symbiont as the real producer of these sponge metabolites. Indeed, marine sponges has been reported to be the complex consortium composed of symbiotic microorganisms, which can contribute up to 40% of the sponge biomass. However, the uncultivability of most symbionts has hampered the understanding of the producer organism, despite its potential to produce bioactive molecules.

In this study, we first tried to obtain the biosynthetic gene cluster of two cytotoxic compounds, calyculins and calyxamides isolated from the Japanese sponge *Discodermia calyx* by a metagenome mining approach. With the desired PKS-NRPS hybrid gene clusters in hand, further attempts to identify the symbiotic bacteria having these two gene clusters were conducted by fluorescence in situ hybridization and laser microdissection for PCR analysis. As a result, both two gene clusters were encoded by same filamentous bacterium, which was identified as *Entotheonella* sp. based on the 16S rRNA sequence. An abundance of filamentous bacteria in *Theonella swinhoei* had been already observed and identified to be the candidatus *Entotheonella palauensis*, as the theopalauamide-containing bacteria.



Moreover, kasumigamide, a linear peptide originally isolated from a fresh water cyanobacterium, was also identified from *D. calyx*. The single cell analyses of complex microbial assemblage revealed that kasumigamide is also biosynthesized by same *Entotheonella* sp.. This is the solid proof of the long-suspected question regarding who the real producer of unique sponge metabolites is, demonstrating that there are indeed the talented but uncultured bacteria commonly residing in at least two genera, *Discodermia* and *Theonella*. In this presentation, the biosynthetic pathway and origin of all three metabolites derived from the Japanese marine sponge *D. calyx* will be discussed.

### **Can microbial symbionts help sponges soak up the pressures of climate change?**

Nicole Webster, Fan Lu, Craig Humphrey, Sven Uthicke, Torsten Thomas  
*Australian Institute of Marine Science/ Advanced Wastewater Management  
Centre, University of Queensland/University of New South Wales*

Environmental conditions which disturb the distribution, abundance or function of sponge microbes will have significant effects on host fitness and survival. Climate change scenarios predict increases in sea surface temperatures and decreases in oceanic pH during the coming century. A combination of experimental research and data from sponges collected at natural CO<sub>2</sub> seeps is used to explore the impacts of elevated SST and OA on sponge microbial symbiosis. From experimental analysis, metagenomic sequencing revealed an abundance of mobile genetic elements, restriction-modification systems, CRISPRs, eukaryotic-like proteins, membrane transport systems and substrate utilisation in control sponges (28°C) but these functions were largely absent in heat stressed samples (32°C). In contrast, thermally stressed sponges were rich in proteins with EAL domains and proteins associated with flagellar biosynthesis. To test whether sponges in the early stages of heat stress had substantial changes in expression we also undertook a metaproteomic approach. Specific COG functional groups like amino acid transport and metabolism were relatively overrepresented in the control sponges, post-translational modification was overrepresented in the early stages of heat stress and translation / transcription were overrepresented in the advanced stages of heat stress. The functional changes observed in the early stages of heat stress suggest that whilst symbionts persist in the host during this time, they no longer carry out normal symbiotic functions. This breakdown of symbiotic function impacts upon the sponge holobiont resulting in a necrotic phenotype. Analysis of symbiont communities from 2 sponge species at the CO<sub>2</sub> seep site revealed highly conserved bacterial populations within each species but clearly distinct symbiont communities along the pH gradient. It is likely that these specialised microbial populations contribute to the sponge's ability to survive these different pCO<sub>2</sub> environments. Sponges at CO<sub>2</sub> seeps have had a lifetime of exposure to high CO<sub>2</sub> therefore these sites are "natural laboratories" for OA research and allow

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us to answer questions of long-term acclimatization. The application of genomic, transcriptomic and proteomic approaches to model marine symbioses allows us to explore the functional implications of environmental stress for sponges and thereby better predict how they will acclimate and adapt to a changing climate.

### **The young and the restless: sponge larval settlement behaviours**

Steve Whalan, Muhammad Azmi Abdul Wahab, Dianne Tapiolas, Cherie Motti  
*Marine Ecology Research Centre, Southern Cross University/ AIMS@JCU(2),  
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Institute of Marine Science(4)*

The motile larval phase is one of the more intriguing processes contributing to patterns of population maintenance, species distributions, and more broadly biogeography. While post-settlement processes play a role in shaping sponge populations, the larval phase underpins both temporal and spatial patterns of these populations. Of key importance are processes that influence larval behaviours at settlement; more specifically, do habitat and con-specific related cues facilitate larval settlement? Information on whether sponge larvae actively choose sites to settle is limited, but examples of marine invertebrate larvae settling in response to specific cues, suggests larval settlement is far from a random process. Indeed, some marine invertebrate larvae are unable to settle unless the right habitat (cue) is encountered; this behaviour invoking creative titles such as the “death before dishonour hypothesis”. Conversely, other marine invertebrate larvae are content to settle without apparent settlement cues. Intermediate strategies are also observed with some larvae delaying settlement until optimal habitats are encountered, but retaining the ability to settle in sub-optimal habitats if they become desperate (desperate larva hypothesis). What do sponge larvae do? The dearth of information for sponge larval settlement is a conspicuous gap hindering our understanding of sponge population ecology. This talk will present data on sponge larval behaviour for *Luffariella variabilis*, which shows evidence of settling in response to conspecific cues. Through a series of controlled lab based experiments, testing responses to isolated compounds from conspecific adult and larval *L. variabilis*, this talk will outline the capacity of larvae to optimise their choice of settlement to conspecifics. In doing so, this talk will begin to piece together the premise that some species can actively choose settlement sites, clearly showing that larval settlement for some sponges is far from random, and providing perceived advantages for individual fitness and more broadly population persistence.

## **The Past, Present and Future of Sponge Science**

Clive Wilkinson

*Reef and Rainforest Research Centre in Australia*

This talk will look back at sponge research from when I started in 1970 and then go back further in evolution of sponges and how they are perceived now. That early research built on decades of fundamental research; I was fortunate to meet some of those people. Throughout this research history, I have been asking: where do sponges fit in the 'inverted cones' or 'evolutionary trees' of life? And what roles did their microbial symbionts play in that evolution? Many of the great names in sponge research have assisted me and have remained as friends; that illustrates that the sponge community has many great people who have the capacity for great companionship.

My research spanned many scales from large scale benthic ecology at ocean basin scales to the ultra-microscopy of microbial symbioses. However these all encompass the general concept of the 'sponge community'. Like many organisms, including humans, the whole animal is a complex combination of the parts; in the case of sponges, these parts include sponge cells plus eubacteria, cyanobacteria, zooxanthellae, dinoflagellates, worms, crustaceans etc. etc. etc. Our early research was limited by the 'tools' we had at our disposal; those limits eventually forced me to abandon research on microbial symbionts and move to ecology. However, as we will see in this conference, sponge researchers now have a wide range of biochemical tools especially in DNA analyses to answer some of the questions that we could not attempt just 20 years ago.

### **Tethya wilhelma: an emerging marine model for early-branching metazoans**

Gert Wörheide, Dirk Erpenbeck, Gaurav Shimpi, Oliver Voigt, Sergio Vargas,  
Jörg U. Hammel, Michael Nickel

*Ludwig-Maximilians-Universität München, Dept. of Earth and Environmental  
Sciences/ Universität Jena*

Model organisms – organisms that are easy to culture, can experimentally be manipulated under laboratory conditions, and whose genome sequences are readily accessible – have revolutionized our understanding of many processes that drive evolution. An in vivo marine model system for arguably the most ancestral animal lineage, the sponges, has yet to be established but is thoroughly needed to address questions about the origin and early evolution of all animals. Our laboratories succeeded to cultivate *Tethya wilhelma*, one of the few marine sponge species that can easily be maintained permanently under laboratory conditions. *T. wilhelma* has already proven its high scientific value and fulfills important criteria as an in vivo model: it has a small body size, easily reproduces (mainly asexually by budding) throughout the year, and a wealth of

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experience and background data exists on its physiology, development and evolution, such as contraction, reproduction, movement and skeletal dynamics. Importantly, it has been shown that it is possible to knock-down genes by RNAi in *T. wilhelma*. Here we report on our ongoing efforts to analyze its genome and metagenome, and to establish this species as a marine early-animal model organism, amenable for experimental manipulation.

### **Good Neighbors: Sponges collaborate with adjacent heterospecific sponges to keep the neighborhood safe for all**

Janie Wulff  
*Florida State University*

Communities dominated by sessile invertebrates often appear to be structured primarily by competition between neighbors for the limiting resource of hard substratum space. On Caribbean mangrove roots, competition influences a typical successional sequence of early colonizers on bare roots that are eventually out-competed by late colonizing effective spatial competitors. Apparent corroboration of the primary importance of competitive interactions in structuring these communities is provided by results of experiments in which individuals of coral reef sponge species that were transplanted onto mangrove roots were soon overgrown by the more rapidly expanding mangrove sponges. However, a comparison between communities developed on artificial mangrove roots through colonization by larvae vs. colonized by transplanted pieces of adult sponges reveals that sponge species that can be characterized generally as effective competitors actually collaborate with each other in preventing colonization by more dangerous enemies.

### **Predicting suitable habitat for the bird's nest sponge *Pheronema carpenleri* (Hexactinellida, Pheronematidae) in the Northeast Atlantic**

Joana R Xavier, Jorge Marco, Javier Diaz-Castillo  
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Hexactinellids or glass sponges constitute a predominantly deep-sea sponge group typically occurring at bathyal and abyssal depths. Some species form dense populations, sponge grounds and reefs that are classified as Vulnerable Marine Ecosystems (European Union Council Regulation no. 734/2008) and as threatened species and habitats (OSPAR agreement 2008-6) of utmost conservation importance.

Aggregations of the birds' nest sponge *Pheronema carpeniteri* (Thomson, 1869) have been reported for several areas of the Northeast Atlantic such as: on the Porcupine Seabight, West coast of Portugal, in the Ibero-Moroccan Gulf, on the Azores islands, and on the continental slope off Morocco. However, the distribution and extent of these populations and the eco-geographical factors shaping its distribution are ill known.

In this study we used a maximum entropy (Maxent) approach to model and predict the habitat suitability of *P. carpeniteri* throughout the Northeast Atlantic. Model evaluation was assessed by generating 10 random sets comprising 75% training and 25% test data, of a total of 124 presence records. We found an excellent fit of the models with training and test AUC above 0.9. Depth, temperature, salinity, slope, and deep-sea current velocity are the factors contributing the most for the species distribution. By co-locating the species presence records and the environmental factors we were able to enlighten this species' niche. Most records of *P. carpeniteri* were found between the following environmental ranges: 800 and 2000m depth, 3.5–10.7° C temperature, salinities of 35-26 PSU, 0.2-4% slope and current speed of 0.5-7.3 cm/s. The areas with the highest habitat suitability index (0.95-1.00) are found along the European and West Africa margins, and on the slopes of the Macaronesian islands and seamounts where the range of the above-mentioned environmental conditions are slightly broader. Interestingly *P. carpeniteri*'s predicted distribution closely matches that of other VME indicator-species such as the cold-water corals *Lophelia pertusa* and *Madrepora oculata*.

We show that statistical modelling methods are powerful tools to enlighten ecological aspects of deep-sea species and that this approach may constitute a way to develop a system of predictive management for the identification and conservation of VMEs.

**Phylogenetic Diversity Comparison of Culturable Actinobacteria  
Associated with Marine Sponges (*Mycale* sp. and *Styliss* sp.) and Ascidians  
(*Styela plicata* and *Molgula manhattensis*) in Six Isolation Media**

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The diversity of culture-dependent actinobacteria was investigated and compared between two sponges (*Mycale* sp. and *Styliss* sp.) collected from Fujian province and two ascidians (*Styela plicata* and *Molgula manhattensis*) collected from Hainan province under the same isolation conditions. Five actinobacteria-selective isolation media and one general bacteria isolation medium were selected to isolate and culture actinobacteria from both sponges and ascidians.

The phylogenetic affiliation of the actinobacterial isolates was assessed using a combination of 16S rRNA gene amplification-restriction fragment length polymorphism (RFLP) analysis and 16S rRNA gene sequencing. A total of 198 purified actinobacteria were cultivated, including 87 from sponges and 111 from ascidians. There are 38 different RFLP patterns and the phylogenetic analysis based on the sequencing results reveal a high and distinctive diversity of actinobacteria associated with these two sponge species and two ascidian species, with six and seven actinomycetes genera, respectively. Apart from *Streptomyces* isolated from both sponge species, four other genera were found only in the *Mycale* sp. sponge compared to one species found only in the *Stylissa* sp. sponge. Similarly, five species-specific genera were isolated from ascidian *Styela plicata* but only one was isolated from ascidian *Molgula manhattensis*. The 16S rRNA sequence analysis showed that two actinobacteria isolated from sponge and eight isolates from ascidians are probably novel strains with similarity lower than 97% compared to the nearest type strain. Most actinobacterial genera from the sponges are also found in ascidians except for *Arthrobacter*. There are two ascidian-specific actinobacteria genera *Rhodococcus* and *Micromonospora*. The study indicates that each sponge or ascidian contains a large set of unique actinobacteria and shares a few common actinobacteria genera. However, there are four actinobacterial genera found in sponge *Mycale* sp. and ascidian *Styela plicata*. In particular, the diversity of ascidian-associated actinobacteria is high. Together with sponges, ascidians are promising sources of novel marine actinobacteria currently underexploited for the discovery of new bioactive marine natural products.

### **Why does the genome of the sponge, *Amphimedon queenslandica* encode highly complex and diversified innate immune receptor systems?**

Benedict Yuen, Sandie Degnan  
*University of Queensland*

The tropical demosponge *Amphimedon queenslandica* lives intertidally on coral reefs, where it faces constant environmental challenges that include tidal fluctuations, strong competition for very limited space with other benthic organisms, and the need to decode a myriad of microbial signals to discriminate symbiont from food, and friend from foe. Such challenges may be met through the evolutionarily ancient innate immune system that confers the ability on animals to discriminate self from nonself, and to respond to internal and external stressors that may impact cellular function. The fully sequenced genome of *A. queenslandica* is allowing us to address questions about the innate immune genes that may underlie the complex interactions between the sponge and its environment. Here we present our findings of an unexpectedly large and diverse repertoire of potential innate immune receptors and regulators in the *Amphimedon* genome, which includes large expansions of several innate

immune gene families. Notable among these is an expanded set of 134 Nucleotide-binding domain and Leucine-rich repeat containing genes (NLRs), a family of cytoplasmic pattern recognition receptors. Their structural similarity to NLRs in other organisms, and their extensive sequence diversity, suggest a role in immunity that could serve as a powerful toolkit at the front line of interactions between sponges and their environment. Also present is a similarly expanded array of 338 Scavenger receptor cysteine-rich genes that have broad functions in extracellular immune recognition. Large expansions such as these bear striking similarities to the elaborate immune repertoires of more morphologically complex invertebrate deuterostomes, such as sea urchins and lancelets. In light of this, we also draw upon gene expression data to suggest ways in which Amphimedon may exploit its astonishing immune receptor diversity to help it cope with the myriad of challenges faced by a marine sessile animal with a biphasic lifecycle.

### **Marine Sponge Biotechnology: An Ocean of Discoveries for New Industrial Products**

Wei Zhang, Raymond Tham

*Flinders Centre for Marine Bioproducts Development, Flinders University*

Marine sponge (Porifera) is the evolutionary oldest, first multicellular animal dated back to nearly 700 million years ago (MYA). They have been proven to be the single best source of marine natural products, contributing up to 30% of all marine natural products discovered by far. With such a high diversity of secondary metabolites, marine sponges possess unique biosynthetic pathways and a myriad of enzymes that catalyse many biological reactions to produce these diverse chemical structures of industrial significance. Australia with one of the longest coastlines in the world is one of the top sponge hotspots that remain largely unexplored for these new industrial products opportunities.

This presentation will present an overview on the ocean of discoveries for potential new industrial products from marine sponges. These products include marine drugs for anticancer, neurodegenerative diseases, antiviral and antibiotics; marine biomaterials such as biosilica; agriculture pesticides from marine sponge-associated bacteria; biosensors for environmental monitoring and assessment of global climate changes; and new enzymes for industrial biotechnology. Drawn on the past 12 years of researches in the authors' laboratory, several case studies of marine sponge biotechnology will be discussed to highlight the unprecedented opportunities for new industries.

With so many new industrial product opportunities from marine sponges, the challenges in translating the product discovery into commercialization outcomes are in many ways the lack of sustainable production methods. This presentation will discuss different production pathways and their latest development, especially the development of sponge aquaculture and in vitro sponge cell culture in bioreactor. While significant progresses have been made, the commercial biotechnology production is yet to come.

**SPEED TALK ABSTRACTS**

**Marine sponge *Hymeniacidon heliophila* as biomonitor of hydrocarbons pollution in the South Western Atlantic**

Daniela Batista Silva, Karla Telinni, Adriana Nudi, Arthur Scofield, Nubia Floriano, Angela Wagener  
*Pontifícia Universidade Católica do Rio de Janeiro*

The present study was the first to evaluate the potential of marine sponge *Hymeniacidon heliophila* as biomonitor of PAH contamination in Rio de Janeiro, Brazil. Samples (sponges and waters) were collected in August 2010 from three areas in the polluted Guanabara Bay as well as in one reference site (Itaipu). Additionally, *Perna perna* was sampled for comparison with *H. heliophila*, since the mussels are widely used to assess PAH contamination. The determination of 16 PAH (USEPA) compounds and its alkylated homologues were obtained by GCMS (? 38PAH). No correlation was found between PAH in the biota and lipid content, mussel size or sponge volume. Both species provide similar information on total PAH concentration: the highest and lowest values were observed in the most and least polluted stations, respectively. Dibenzothiophenes, phenanthrenes and its alkylated compounds were dominant PAH in *H. heliophila* and *P. perna* at almost every site. Sponge showed slight tendency to accumulate high molecular weight (heavy) PAH in relation to light PAH, while the mussel showed an opposite pattern. *Hymeniacidon heliophila* showed to be a good PAH contamination monitor in estuary waters, and may be used in wide regional surveillances since the species can be found from North Carolina (USA) to Santa Catarina (Brazil).

**Taxonomic and environmental related sponge spicule dissolution**

Marco Bertolino, Marco Bertolino, Riccardo Cattaneo-Vietti, Maurizio Pansini, Giorgio Bavestrello  
*Università degli Studi di Genova - Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV)/ Università Politecnica delle Marche - Dipartimento di Scienze della Vita e dell'Ambiente (DISVA)*

Recent studies put in evidence an unexpected role of sponges in the ocean silica cycling and availability. Sponge skeletons, made by siliceous spicules, function as an important Si sink that drastically delays Si recycling. In this study we have compared by SEM analysis the dissolution of spicules dispersed in the sediment of different geographic localities: hexactinellid spicule mats from Terra Nova Bay (Ross Sea, Antarctica), demosponge spicule mats from Argentine fjords (Mar del Plata, South Atlantic Ocean), and spicules dispersed in the coralline sand of Banka Is. (North Sulawesi, Indonesia). Spicules



coming from the tropical sediments were recorded deeply etched by deep circular marks on their surface that develop in tunnels reaching the axial canals that results enlarged. On the contrary spicules coming from Antarctic and Mar del Plata cold waters were intact, rarely showing a negligible enlargement of the axial canal. In these spicules the superficial dissolution pits were never observed. To check if the recorded differential solubility may be due to water temperature or to a qualitative difference of different kinds of spicules we have experimentally documented the dissolution of spicules coming from an Antarctic hexactinellid spicule mat and from a Mediterranean specimen of *Geodia cydonium*. *Geodia* spicules, obtained by dissolving sponge tissue in boiling nitric acid, and hexactinellid spicules, obtained by cleaning portion of the mat with hydrogen peroxide, were separately packed in a plankton net (50  $\mu$ m) and left for eight months at 15 m depth in the Ligurian Sea. After this period Antarctic spicules showed a deep reduction by dissolution (about 50% in volume) due to an impressive enlargement of the axial canal producing sometimes spicules virtually empty. On the other hand *Geodia* spicules remained completely unaltered. This bulk of evidences indicates a complex scenario where spicule dissolution is related to different parameters including water temperature and "quality" of silica composing the spicules of different sponge taxa. These data have to be considered for a correct evaluation of the sponge role in the ocean silica cycle.

### **Population structure and dispersal of the coral excavating sponge *Cliona delitrix* within the Greater Caribbean Sea**

Andia Chaves-Fonnegra, Kevin Feldheim, Jesse J. Secord, Jose Lopez  
*Nova Southeastern University/ Field Museum of Natural History. Pritzker  
Laboratory for Molecular Systematics and Evolution. The Field Museum*

Excavating sponges of the genus *Cliona* strongly compete with living corals, often killing and bioeroding entire colonies. During the past few decades the increase of excavating sponges on Caribbean reefs has been correlated with pollution and coral degradation. However, important aspects affecting their distribution, such as dispersal capability and population structure remain mostly unknown. One of the most destructive species of Caribbean reefs is *Cliona delitrix*. Thus, the aim of our study was to determine levels of genetic connectivity and dispersal of *C. delitrix* across the Greater Caribbean Sea, and within the Florida reef track. Ten species-specific microsatellite markers have been developed and are being used to genotype 442 individuals from 12 different locations. Preliminary results using six microsatellites and 222 individuals showed that there are high levels of overall genetic differentiation. Using two clustering admixture methods; with and without assigning prior geographical locations of individuals, we found four main clusters (k): 1) Belize; 2) South Caribbean (Panamá, Colombia and Curazao); 3) Bahamas and some locations from the Florida reef track; 4) Florida reef track. Possible gene flow and ecological dispersal occur between some of the populations in the Florida reef

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track, and between populations in the south Caribbean. These results support the influence of water current patterns on genetic connectivity in the Florida reef track, specifically for marine sponges and other invertebrates. We also confirmed previous allozyme studies that found the dispersal range of *Cliona delitrix* to be greater than 10-100m.

### **Community structure of archaea and bacteria in two Indonesian sponges**

Nicole de Voogd, Daniel FR Cleary, Ana RM Polónia, Newton CM Gomes  
*Naturalis Biodiversity Center/ Universidade de Aveiro*

A key goal in ecology is to understand how communities are structured. This includes understanding fundamental parameters of diversity such as richness and abundance distributions, and the variation in composition across local and large spatial scales. Of all biological communities, prokaryotes (Archaea and bacteria) are the most ecologically important, but also least understood. Prokaryotes play crucial roles in nutrient dynamics, for example, oxidising organic compounds such as nitrogen and sulphur to biologically available inorganic forms. In the present study we assess prokaryote communities in four distinct coral reef biotopes, two abiotic (sediment and seawater) and two biotic (the sponges *Stylissa massa* and *Xestospongia testudinaria*) in three coral reef systems in Indonesia, namely the Jakarta Bay Thousand Island system, the Spermonde reef system off Makassar, South Sulawesi and the Berau reef system on the northeast of Borneo. Coral reefs are diverse and highly productive marine ecosystems that provide a number of important ecological services including food provision and limiting coastal erosion. Research in coral reefs has tended to focus, however, on popular eukaryotic taxa such as corals and fishes. Importantly, recent advances in molecular techniques, such as pyrosequencing, now enable us to assess prokaryote communities at an unprecedented level of detail. Communities of archaea and bacteria were thus assessed using barcoded pyrosequencing. Initial results show pronounced variation among biotopes and within the same biotope (e.g., sponge species) from different regions.

### **Environmental impacts on sponge pumping measured using a micro-thermistor**

Alan Duckworth, Damien Jorgensen, Christine Schönberg, Nicole Webster,  
Andrew Negri  
*Australian Institute of Marine Science*

Sponges actively pump water through their aquiferous system to deliver food and oxygen and to remove waste products. Therefore pumping is essential for metabolism, but rates can be negatively influenced by environmental factors

such as temperature and sedimentation, resulting in decreased flow rates or even arrest. Some species can tolerate extended periods of non-pumping but if the environmental pressure is not reduced or removed, death may result. Sponge flow rates could therefore be a useful bioindicator to monitor stress responses. Although difficult to quantify experimentally, one method is using a micro-thermistor. A micro-thermistor uses the power dissipated in a heated experimental probe, placed in the exhalant flow, to calculate flow rates; a control probe placed away from the sponge compensates for changes in ambient water temperature. We developed a micro-thermistor with multiple experimental probes that can measure simultaneously flow rates of different individuals or from different exhalants within a single sponge. This provides quantitative measurements on the effects of environmental pressures on sponge pumping rates. Sponge flow rates will be examined during controlled experiments to assess responses of sponges to sedimentation and turbidity.

### **Sponge biomass and bioerosion rates increase under ocean warming and acidification**

James K.H. Fang, Matheus A. Mello-Athayde, Christine H.L. Schönberg, David I. Kline, Ove Hoegh-Guldberg, Sophie Dove

*The University of Queensland/ Australian Institute of Marine Science, Oceans Institute, The University of Western Australia/ Coral Reef Ecosystems Laboratory, School of Biological Sciences and Australian Research Council Centre of Excellence for Coral Reef Studies, The University of Queensland*

The combination of ocean warming and acidification as a result of increasing atmospheric CO<sub>2</sub> is considered to be a significant threat to calcifying organisms and their activities on coral reefs. How these global changes impact the important roles of decalcifying organisms (bioeroders) in the regulation of carbonate budgets, however, is less understood. To start addressing this important question, the effects of a range of past, present and future climate scenarios on the excavating sponge *Cliona orientalis* Thiele, 1900 were explored over twelve weeks in early summer on Heron Island, in the southern Great Barrier Reef. *C. orientalis* is a widely distributed bioeroder on many reefs, and hosts symbiotic dinoflagellates of the genus *Symbiodinium*. Sponge biomass production and bioerosion rates were similar under pre-industrial and present day (control) ocean temperature and acidification scenarios. Under low and high CO<sub>2</sub> emission scenarios, however, *Symbiodinium* population densities were significantly reduced with sponge replicates within the high treatment exhibiting strong bleaching. Despite these changes, biomass production rates increased and the ability of the sponge to erode coral carbonate materials increased. Our study reveals that *C. orientalis* is likely to grow faster and have higher bioerosion rates than at present, even with significant bleaching. Assuming that our findings hold for excavating sponges in general, increased sponge biomass coupled with

accelerated bioerosion may push coral reefs towards net erosion and negative carbonate budgets.

**$\gamma$ -aminobutyric acid and L-glutamic acid induce contraction on the demosponge *Chondrosia reniformis* Nardo 1847**

Dario Fassini, Maria Daniela Candia Carnevali, Francesco Bonasoro  
*Dipartimento di Bioscienze*

The mesohyl of the demosponge *Chondrosia reniformis* Nardo 1847 mainly consists of collagen, which is responsible of typical cartilaginous consistency of this sponge. It has been reported (Bonasoro et al., 2001; Wilkie et al., 2006) that following specific stimuli this species is able to change the mesohyl mechanical properties by modifying the interactions between the collagen fibrils. Indeed the sponge is able to stiffen the mesohyl following localized mechanical stimulation (Fassini et al., in preparation) and to undergo modulated creeping processes, i.e. phenomena of gradual loss of tensility which depend on the environmental conditions and generates long and slender outgrowth (Fassini et al., 2012). The present work is addressed to: 1) demonstrate the presence of expansion/contraction activity in *C. reniformis*; 2) describe by means of a biomechanical approach the effects of the exposure to  $\gamma$ -aminobutyric acid (Gaba) and L-glutamic acid (Glu) on both the expansion/contraction activity and the mesohyl mechanical properties. Our data reveal the presence of evident and complex expansion/contraction activities in *C. reniformis*. The exposure of the sponge to Gaba and Glu 1mM results in a general “contraction” of the whole sponges within few minutes. There are no differences in the contraction phenomenon between the two treatments in terms of magnitude and time of response; in contrast Glu is able to generate a higher tensile force during the “contraction phase” with respect to Gaba. Data related to changes in stiffness reveal a dose-dependent stiffening response to Glu treatment, whereas Gaba does not appear to induce any significant change in stiffness. Although these data show that both Gaba and Glu are able to induce similar “contraction phenomena”, they suggest the possible presence of different mechanisms of action still to be explored.

**Sessile fauna of a temperate, shallow water Western Australian environment.**

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*Western Australian Museum/ CSIRO Wealth from Oceans Flagship/  
Universidade Federal do Rio de Janeiro*

Temperate Western Australia (WA) has high biomass, high productivity shallow benthic ecosystems largely dominated by macroalgae, yet much of the area is bathed in the relatively low nutrient, warm, southward flowing Leeuwin current. Both rocky reefs and seagrass beds are important habitat types in these nearshore, shallow coastal waters but, until recently, few studies had quantified the diversity and abundance of macrofauna in these habitats. We examined four locations on the west coast of Australia and conducted counts of sessile and motile invertebrates along three 5 metre<sup>2</sup> transects at 32 sites, 24 of which were reef sites and eight of which were seagrass. We found a total of 178 sessile species on reefs and 40 in seagrass. In both habitats sponges dominated the sessile invertebrate fauna. At seagrass sites the sponge fauna was not dominated by any one species, although a species of *Mycale* accounted for 17% of the individuals recorded in this habitat. However, on reef sites the calcareous species, *Leucetta prolifera*, accounted for 32% of the individuals recorded. This species was first described by Carter (1878) with a WA type locality (Fremantle), and has not been reported since. Overall, we found a high proportion of calcareous sponges with 29 species recorded. This high proportion is in marked contrast to comparative quantitative surveys in tropical regions of WA where numbers of calcareous species have been low. We present the following findings: the parameters that best explain the distribution and composition of the sessile fauna at the locations studied, the dominant species in reef and seagrass habitats, and the implications that calcareous sponges are an important component of these communities in the face of ocean acidification.

**Implementing sponge physiological and genomic information to enhance the diversity of its culturable associated bacteria**

Adi Lavy, Ray Keren, Markus Haber, Inbar Schwartz, Micha Ilan  
*Tel Aviv University*

Until recently, culture of sponge-associated bacteria used conservative methods: standard culture media inoculated with environmental samples, followed several weeks of standard incubation conditions. The coral reef sponge *Theonella swinhoei* is a high microbial abundance sponge, with bacteria occupying up to 40% of its body volume. Some of these bacteria are known to be the source of natural products found in the sponge. This research aims to increase diversity of cultured bacteria from *T. swinhoei* and identify isolates producing antibacterial compounds, by using a wide variety of culturing media (some new in sponge microbiology), and different culture conditions. Culturing media, either rich or poor in nutrients, were inoculated with bacterial fraction of a sponge sample. One set of plates experienced aerobic conditions, while another was sealed in microaerophilic containers for one of three durations. DNA samples of 619 sponge-derived bacterial isolates were analyzed. These isolates belong to five Phyla, ten classes and 44 genera. The specificity of the obtained isolates to culturing condition varied considerably. Some genera were specific to a single

type of medium while others grew on several media. We found that 66% of all genera were specific to one type of culture medium and 61% of all genera were specific to one type of aerobic treatment. Antibiotics addition to source plates inhibited growth of fast growing bacteria, which allowed isolating 32% of the genera, with specificity to this culturing condition. Phylogenetic clustering was observed in all microaerophilic treatments, as well as in two out of three poor nutrient media. Clustering suggests these bacteria cannot quickly adapt to any artificial culturing condition, indicating their successful culture would require a large variety of conditions. In our experiment 18 isolates had similarity values of less than 97% to any known type strains, which suggests new species cultured, if not new genera. Preliminary results of antibacterial streak assay reveal that some of the newly isolated bacteria secrete antibacterial compounds. This study shows that different culturing media compositions, combined with aerobic treatments, should be considered when searching for novel bacteria. Such newly isolated bacteria could prove useful sources for industrial important compounds.

**Phylogenetic relationships within *Mycale* Gray, 1867 inferred from molecular data, and implications for morphological character evolution**

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*Universidade Federal do Rio de Janeiro*

Since its inception, *Mycale* Gray, 1867 has gone through numerous revisions and modifications. Currently, this taxon is one of the richest among sponge genera, including 249 species distributed in eleven subgenera. Alternative cladistic hypotheses about evolutionary relationships among these subgenera were originally proposed solely on the basis of morphological data. These pointed out that some subgenera lacked clear apomorphies, namely *M. (Aegogropila)*, *M. (Carmia)* and *M. (Mycale)*, possibly being polyphyletic. In a recently published phylogenetic hypothesis for ten species representing five subgenera, the partial sequences of 28S and 18S rRNAs used as molecular markers, proved to be exceedingly invariable. The aim here was to generate a phylogenetic hypothesis for *Mycale* subgenera using a fragment of the 16S rRNA of the mitochondrial genome, believed to be more variable than its nuclear paralogous. The evolutionary relationships among 26 species representing ten subgenera was analysed by Bayesian Inference (BI) and Maximum Likelihood (ML) methods, and ancestral character states were reconstructed for selected morphological characters. Subgenera *M. (Zygomycale)*, *M. (Arenochalina)* and *M. (Naviculina)* were recovered as monophyletic. In turn, *M. (Carmia)*, *M. (Aegogropila)* and *M. (Mycale)* confirmed their suspected polyphyly. Three well defined clades were recovered: clade A, comprising *M. (Carmia)*, *M. (Zygomycale)* and some species of *M. (Aegogropila)*; clade B, with *M. (Arenochalina)* and the remaining *M. (Aegogropila)*; and clade C, with *M. (Raphidoteca)* and two species of *M.*

(Mycale). Results are partially in accordance with recently published molecular phylogenies: 1) Mycale (Anomomycale) was recovered as an outgroup to Mycale; and 2) the controversial basal position of *M. (M.) laevis*, which, in addition to a highly divergent sequence, suggests this species does not belong in Mycale. Most morphological characters proved to be homoplastic. The majority of homologous morphological characters were those diagnostic for some subgenera: the occurrence of isochelae and naviculichelae in *M. (Zygomycale)* and *M. (Naviculina)*, and the choanosomal skeleton arrangement in *M. (Arenochalina)*. Additional morphological synapomorphies were: 1) abundant slime in clade B; and 2) two categories of subtylostyles plus markedly curved anisochelae I in clade C. Character evolution in clade A appears to comprise several losses or reversions.

### **Systematics & evolution in the marine Haplosclerida**

Grace McCormack, Kelly Stephens, Niamh Redmond  
*National University of Ireland Galway/ Smithsonian Institution*

The Haplosclerida are considered to be one of the most difficult groups of sponges from a systematics point of view having large numbers of species living in a wide number of habitats and possessing a very simple (and plastic) morphology. Molecular data has indicated that the suborders and many families, genera and even species within the monophyletic marine Haplosclerida are polyphyletic, being in serious disagreement with their classification based on morphology. Yet, despite the morphological simplicity of this group patterns of skeletal arrangement are also clear, making identification reasonably unambiguous to experienced taxonomists. How can these two approaches be reconciled? Given the levels of diversity and divergence evident in this group, should the marine Haplosclerida be considered a Subclass within the Demospongiae? I will discuss the evolutionary patterns shown by this group of marine sponges by reviewing existing molecular and morphological data for the group and by presenting new data from *Haliclona* species. Divergent evolutionary patterns evident in the mitochondrial and ribosomal genes of some marine haplosclerids will also be considered.

### **Overview of the biodiversity and distribution of the Class Homoscleromorpha in the Tropical Western Atlantic**

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de Janeiro*

In this study we revise the current knowledge on the diversity and distribution of the Class Homoscleromorpha in the Tropical Western Atlantic (TWA). Twenty

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species of *Homoscleromorpha* are currently recognized in the TWA, belonging to the genera *Plakortis* (*P. angulospiculatus*, *P. halichondrioides*, *P. insularis*, *P. microrhabdifera*, *P. simplex*, *P. zyggompha*, *P. ?bahienese?*, *P. ?petrupaulensis?*, *P. ?potiguar?*, *P. ?spinalis?*), *Plakinastrella* (*P. microspiculifera*, *P. onkodes*, *P. ?globular?*), *Plakina* (*P. elisa*, *P. jamaicense*, *P. monolopha*, *P. trilopha*, *P. tetralopha*, *P. versatilis*) and *Corticium* (*C. quadripartitum*) (quotation marks indicate undescribed species; MS in preparation).

Three of these species are cosmopolitan and probably represent complexes of cryptic species: *Plakina monolopha*, *P. trilopha* and *Plakortis simplex*. *Plakortis angulospiculatus* is the most widely distributed in the TWA, being recorded from the U.S. (Florida), Caribbean (Cayman Islands, Colombia, Cuba, Dominican Republic, Jamaica, Panama, Venezuela) and NE Brazil (from Rio Grande do Norte to Bahia State). Two species are restricted to the Brazilian coast and oceanic islands: *Plakortis microrhabdifera* occurs in Fernando de Noronha and Potiguar Basin, and *Plakinastrella microspiculifera* in São Pedro e São Paulo, Fernando de Noronha, Atol das Rocas, Potiguar Basin, Minerva Seamount and Trindade Island. Other two are restricted to the Caribbean: *Plakina jamaicense* (Jamaica, Belize) and *Plakina versatilis* (Panama, US Virgin Islands). Most species (8/19, or 42%) are known from a single area in Brazil (*Plakortis ?bahienese?*, *P. insularis*, *P. ?petrupaulensis?*, *P. ?potiguar?* and *P. ?spinalis?*) or in the Caribbean (*Plakina elisa*, *P. tetralopha*, *Corticium quadripartitum*). Undetermined species of *Oscarella* were recorded from Brazil (Atol das Rocas, Fernando de Noronha, Abrolhos, Búzios, Cabo Frio) and the Caribbean (Belize, Cayman Islands, Panama), but none of them was identified to species level so far.

We are currently describing new species of *Oscarella* and *Plakina* from Brazil and we estimate that the biodiversity of *Homoscleromorpha* in the TWA is much higher than the 19 species currently known. The reasons for the current underestimation are the low size, cryptic habits and low number of morphological characters of *Homoscleromorpha* species and the low number of taxonomists making collections directed exclusively or primarily to homosclerophorids.

### **Habitat characterization of deep-water *Aphrocallistes beatrix* reefs in Aviles Canyon (Cantabrian Sea) from underwater ROV images**

Rios Pilar, Muñoz Araceli, Sanchez Francisco, Cristobo Javier  
*Instituto Español de Oceanografía/ TRAGSA-SGP*

The study area is located on the Aviles Canyon System in the Cantabrian Sea, constituted by three main canyons, a marginal platform and a relevant rocky outcrop. The canyons are, from east to west: La Gaviara Canyon, El Corbiro Canyon and Aviles Canyon where is focused our research.

This submarine canyon crosses the entire continental slope from the rise, and incises on the continental shelf, only 12 km away from the shore line. The sharp



pathways changes indicate that the location of the canyon system is structurally-controlled. The canyon walls are affected by well-developed drainage features that form a network of gullies. The Aviles Canyon is characterized by a V-shaped bottom, and a well defined axial incision (~75 km long), with well differentiated walls. Its head is at a depth of about 128 m, and reaches the abyssal plain at 4766 m.

On this study we try to identify the reasons why this deep sea canyon system may provide suitable environmental conditions for cold water reef growth.

In order to correctly identify the different facies in the areas of interest previously identified with the acoustic remote sensing techniques, direct visual transect sampling was carried out using Liropus 2000 ROV (a SuperMohawk, manufactured by Sub-Atlantic Ltd). In addition to transects, we also made some detailed close up inspections in order to identify and obtain samples of species. Owing to the great length of transects the vessel was dynamically positioned to follow the ROV.

Glass sponges (Porifera: Hexactinellida) produce a skeleton of nearly pure glass (SiO<sub>2</sub>) that remains long after the sponge has died, forming a substrate for future generations of sponges.

The genus *Aphrocallistes* presently contains only two recognized species, *A. beatrix* and *A. vastus*. Several small *A. beatrix* reef complexes were discovered at Calafrio area in the Aviles canyon. These deep sea sponge reefs habitats occur between 814 and 1199 m. Video and still images provided information to understand the dominant species of the reef is the sponge, which considerably increase the habitat's complexity and biodiversity in relation to other facies described in the canyon.

## **Marine to Medicine**

Howard Shawcross

*Department of Commerce*

Marine biodiscovery research within Western Australia is designed to capitalise on the uniqueness and profusion of the States marine biodiversity and to assist the development of a marine component to Western Australia's emerging biotechnology industry. The growth of the Western Australian Institute of Medical Research (WAIMR), WA Marine Science Institution (WAMSI), Western Australian Chemistry Centre and an unprecedented focus on the marine estate both for conservation purposes and in response to development of the oil and gas industry, provides an unequalled opportunity for exploration of marine biodiversity in Western Australia and examination of applications across all sectors. The main hurdle limiting progress in the marine biotechnology sector in Western Australia has been a lack of a legislative framework to underpin security for biodiscovery investment. The Western Australian Government has been working towards a biodiscovery legislative framework to enhance global investment in the State's biodiscovery industry as well as facilitating legal certainty for research and

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commercialisation The legislative framework is now progressing at the highest levels within the State Government and a biodiscovery Bill is expected to be introduced into WA State Parliament in late 2013. Building on the examples of Queensland and the Northern Territory, it is anticipated that the proposed legislation will encourage true collaboration between WA's well established research, small industry and government sectors. The State has a world class research base through its 4 public universities; a cluster of Cooperative Research Centres, Centres of Excellence and Australia's most advanced Phase 1 clinical trial facility, Linear Clinical Research Limited. The State's capabilities in the areas of research into food security, grain crops, aquaculture, Alzheimer's and microalgae for biofuels, lifestyle diseases including diabetes, liver disease and cancer will be the building blocks of a fully functional biodiscovery industry. The Department of Commerce acknowledges the key role that the Western Australian Museum though its marine bioresources library has undertaken to streamline the policy and regulatory requirements for access and benefit sharing, and to re-invigorate the use of WA Marine bioresources in discovery programs.

### **Phylogenetic analysis of bacteria isolated from sponges using a diffusion-growth-chamber**

Georg Steinert, Susanna Whitfield, Mike Taylor, Peter J. Schupp  
*Carl von Ossietzky University of Oldenburg/ University of Guam Marine  
Laboratory/ University of Auckland*

Sponges are well known for their diverse and species-specific bacterial communities, with some of these bacteria producing bioactive compounds that are of pharmacological interest. However, most of these bacteria resisted cultivation due to complex biochemical and metabolic host-specific interactions that are not reproducible using common cultivation procedures. We therefore recently adopted the diffusion-growth-chamber (DGC) technique in a bid to isolate these rare, uncultured sponge-associated bacteria. Semi-permeable growth chambers containing sponge homogenate-enriched medium were placed in the reef sponge *Rhabdastrella globostellata* with the aim to culture previously uncultivated sponge-specific bacteria in situ. By utilizing the DGC method it was possible, for the first time, to cultivate in vivo in sponges and subsequently isolate known and novel bacteria in vitro. More than 270 16S rRNA gene sequences were obtained during this study. BLAST analysis of sequences against the NCBI database resulted in identification of 21 novel OTUs at 0.03 genetic distance cut-off and <97% 16S shared sequence identity with any previously cultured bacterium. These novel strains belong to four phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Alpha and Gamma), and we have assessed their possible phylogenetic positions within monophyletic sponge-specific sequence clusters are still unknown. Here, we present a comprehensive

analysis of the DGC-derived bacterial strains and, and report evidence for the sponge specificity of some strains.

**Corexit dispersant 9500 increases the impact of crude oil on coral reef sponge larvae**

Nicole Webster, Andrew Negri, Andrew Heyward, Steve Whalan  
*Australian Institute of Marine Science/ Southern Cross University*

Accidental oil spills from ships or rigs are key perceived threats to tropical biota from industry activities. Sponges are an abundant and functional component of reef and inter reef ecosystems. The motile larval phase of sponges moves throughout the water column and may be vulnerable to oil contamination. We investigated the effects of water accommodated fractions (WAF) of crude oil and the dispersant Corexit 9500 on metamorphosis, gene expression and microbial symbiosis of the larval phase of *Rhopaloeides odorabile*. Corexit 9500 facilitated the entrainment of 100-fold more hydrocarbons into the WAF but there was little difference in the entrainment of PAHs between dispersed and non-dispersed treatment. Larvae were visibly unaffected by crude oil at 100% WAF (107 µg l<sup>-1</sup> PAH) as evidenced by normal swimming behaviour, successful metamorphosis and high survivorship. In contrast, larvae exposed to oil dispersed with 10% (v/v) Corexit 9500 were visibly deformed and had significantly reduced motility, metamorphosis or survival at 25% WAF (18 µg l<sup>-1</sup> PAH). A molecular level response was detected in larvae after 2 h exposure to 50% dispersed oil whereas it took 24 h to invoke a comparable response for oil alone. The molecular stress response was primarily comprised of increased expression of heat shock proteins and oxidative stress genes. Interestingly the 1.8% WAF of Corexit 9500 alone (28 mg formulation l<sup>-1</sup>) caused 100% mortality of *R. odorabile* larvae after 24 h (the lowest % WAF of all treatments to cause toxicity). The sponge larvae tested exhibited tolerance to moderate concentrations of the crude oil, they become more vulnerable to oil and Corexit 9500 in combination and were sensitive to Corexit 9500 alone, which may have implications for oil spill responses near coral reefs.

**Location, location, location: using novel surfaces to optimise sponge larval recruitment for reef restoration and aquaculture.**

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Settlement of marine invertebrate larvae is often triggered by chemical and physical cues associated with surfaces that signal optimal habitats. Determining the chemical cues implicated in larval settlement is a significant focus of sessile

invertebrate larval settlement studies, with many larvae (including sponges) settling in response to habitat related chemical cues. There has been less effort in determining sessile larval settlement to physical cues, such as surface micro-topographies. This is surprising because research has determined the importance of surface micro-topography for biofouling marine invertebrate larvae; the theoretical framework of this work built around Attachment Point Theory. The underlying premise of Attachment Point Theory is that larval attachment is enhanced with increasing availability of attachment points (i.e. surface micro-topography) and decreased with fewer points of attachment. Matching the micro-topography profile to larval size can provide a surface that enhances settlement by reducing the potential of detachment from hydrodynamics and providing refuge from potential predation. Armed with this background, is it possible to optimise sponge larval recruitment using artificial surfaces with inbuilt micro topography? We trialled novel, custom engineered artificial surfaces with a range of surface micro-topographies to establish if sponge larval settlement could be optimised without the use of habitat related chemical cues. Our results indicate that for some species larval settlement can be optimised with this technology, and may provide a simple and effective method to facilitate large scale sponge larval recruitment for use in aquaculture (sponges as bio-materials) and reef restoration projects.

**Potential of using marine sponge *Euryspongia* sp. to investigate the impact and adaptation to elevated ocean temperature and reduced pH due to global warming and ocean acidification**

Wei Zhang, Kuo Yang, Susanne Zeile, Jingjing Wang, Shirley Sorokin, Jason Tanner

*Flinders Centre for Marine Bioproducts Development, Flinders University/ SARDI Aquatic Sciences*

Extreme changes forced by global warming, ocean acidification and pollution of the ocean may directly affect the diversity of marine organisms and indirectly affect human health and resources. Early warning systems, in the form of environmental triggers, are needed to alert environmental management of these potential changes and to prevent any major environmental disasters. With their strong filter-feeding capacity, sponges as the iconic and ubiquitous “living fossils” not only take in their food, but also uptake and accumulate dissolved or suspended pollutants that may be present in the surrounding water. In this study we investigated the short-term effects of reduced sea water pH and elevated ocean temperature due to global warming and ocean acidification on sponge primmorphs obtained from the south Australian sponge *Euryspongia* sp. We have established controlled conditions that enable this species to be kept in aquaria for several months. We also have established in vitro culture technology of sponge primmorphs from *Euryspongia* sp., and tested a variety of different

conditions in vitro that can be used to observe changes on a cellular level resulting from a decrease in pH and a rise in temperature over time. Using a range of lower pH values (pH 7.4, 7.6 and ambient), primmorphs were cultured in natural seawater for one week the effects of increased CO<sub>2</sub> and temperature were evaluated by morphological studies and survival with the aim of estimating CO<sub>2</sub> tolerance threshold on a cellular level. Preliminary results indicated that primmorphs react in a highly sensitive manner to elevated temperature and reduced pH. Sponge cells have the ability to adapt to elevated temperature with a gradual increase, when compared to a sudden increase. These results will enable us to establish advanced tools for environmental risk assessment and monitoring, as well as development of environmental adaption strategies.

**POSTER ABSTRACTS**

**Antibacterial and Cytotoxic Activities of Secondary Metabolites Produced by *Aptos suberitoides* associated bacteria**

Syamsudin Abdillah, Syarmalena, Fatmah Zuraiha

*Master of Pharmaceutical Sciences Programe Faculty of Pharmacy, Pancasila University*

A study had been conducted for antibacterial and cytotoxic activities against secondary metabolite of *Artemisia salina* resulted from the association between sponge *Aptos suberitoides* and bacteria. The experimental study was conducted by isolating sponge *Aptos suberitoides*-associated bacteria, which were then extracted using n-hexane, ethylacetate and methanol as solvents. The bacteria were isolated with pour plate method and the resulting secondary metabolites were brought under antibacterial assay for their activities against the bacteria *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* and cytotoxic activities against *A. salina*. Conclusion. All extracts showed weak antibacterial activities with inhibiting diameter ranging from  $6.32 \pm 0.27$  –  $9.95 \pm 0.09$ . An assay for *A. salina* showed that ethyl-acetate extracts were more toxic than methanolic extracts and n-hexane, with an LC50 value of 21,93 $\mu$ g/mL, 65.02  $\mu$ g/mL, and 178.22 $\mu$ g/mL, respectively.

**Bioactivity test of Secondary Metabolites associated fungi with *Aptos suberitoides* from Tanjung Pecaron Situbondo**

Syamsudin Abdillah, Syarmalina, Mieysa Intan Permatasari

*Master of Pharmaceutical Sciences Programme, Faculty of Pharmacy, Pancasila University*

Sponges, an important part of the coral reef ecosystem, have a bioactive potential that have not been widely used. Sponges compound an active substances with higher percentage than other which produced by land plants, so in this research aims to isolate the fungi that associated with *Aptos suberitoides* which generate secondary metabolit derived from *Aptos suberitoides*, using direct seed and pour plate methods. Three isolates produced by direct seed method and five isolated derived from pour plate method, the total was eight isolates. Antimicrobial activity for supernatants from shake fermentation of eight isolates using starter 10% and 50% have shown DDH ranges 6,67 mm - 9,67 mm for microbial test of bacteria and 6,88 - 13,42 mm for microbial test of fungi. While tests on BSLT, indicated by LC50 values, using starter 10% were within the range of 518,192  $\mu$ g/mL ? 913,901  $\mu$ g/mL and using starter 50% were in the range of 333,960  $\mu$ g/mL ? 787,360 $\mu$ g/mL. In sum, result of antimicrobial test from supernatants derived from shake fermentation has a higher DDH than DDH

resulted from extraction. The similar result also produced from of BSLT test that showed supernatants with shake fermentation has a higher LC50 values than after extraction.

### **Come on baby light my larva: exploring sponge larval fluorescence**

Muhammad Azmi Abdul Wahab, Tracy Ainsworth, Nicole Webster, Steve Whalan  
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Fluorescence techniques are increasingly employed in marine science to address questions pertaining to genomics, proteomics, symbiosis and ecology. Apart from being able to produce “pretty pictures”, fluorescence microscopy can shed new light onto existing biological and ecological frameworks by providing a quick and effective method of viewing specimens of interest in real-time. With constant improvements in technology and accessibility, fluorescence microscopy is increasingly utilised for both field and laboratory based research. We present applications of fluorescence used in our work on multiple species of tufted parenchymellae sponge larvae from the Great Barrier Reef (GBR). We used epi-fluorescence and confocal microscopy to explore sponge-microbe associations during embryogenesis and maternal investments to larval energetics in optimal and sub-optimal habitats. Interestingly, we also recently discovered variations in the intensity of auto-fluorescence of the larval sponge posterior pigmented ring (light receptive apparatus) between four species of co-occurring GBR sponges. If these variations correspond to larval sponge light sensitivity, we hypothesise that species depth distributions and habitat selection may, in part, be determined by innate light detection capability.

### **A morphological and molecular revision of Phyllosponginae: how similar are different foliose sponges from the Australian tropics?**

Muhammad Azmi Abdul Wahab, Jane Fromont, Nicole Webster, Steve Whalan, Nikos Andreakis  
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The delineation of valid taxonomic units is critical in inferring phylogeographic and genealogical relationships amongst species and populations, and underpins effective management and conservation of marine biodiversity. Marine sponges represent key residents of benthic ecosystems in the tropics because of their abundance, diversity and ability to influence community-level processes such as

seawater filtration and substrate consolidation. Sponge taxonomy can be challenging, with many sponge groups exhibiting extreme morphological plasticity induced by local environmental conditions. Foliose sponges of the sub-family Phyllospongiinae (Dictyoceratida, Thorectidae: Strepsichordaia, Phyllospongia and Carteriospongia) are commonly found in intertidal and subtidal habitats of the Indo-Pacific. Being aspiculated, these sponges are sometimes difficult to differentiate due to the lack of other reliable morphological characters (e.g. spicules) traditionally used for species delineation. We use molecular phylogenies inferred from the nuclear Internal Transcribed Spacer 2 region (ITS2) and morphometrics (19 characters; 52 character states) to identify evolutionarily significant units (ESUs; sensu Moritz) within foliose Phyllospongiiniids collected from seven geographic locations across tropical eastern and Western Australia. The ITS2 topology was congruent with cladistic analysis of morphological characters supporting the expected taxonomic relationships at the genus level. However, phylogenies inferred from the sole ITS2 marker revealed multiple sequence clusters corresponding to eight ESUs some of which were characterized by distinct morphological features and specific geographic distribution range. Our results are discussed across three topics in the light of 1) taxonomic incongruences within this study, 2) hidden sponge diversity and 3) vicariant events associated with the last glaciation cycles responsible for the geographical distribution patterns of the different ESUs.

**Marine sponges as biomonitors of heavy metal pollution in the South Western Atlantic**

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In this study we evaluated the potential of the marine sponges *Hymeniacion heliophila* and *Paraleucilla magna* as biomonitors of heavy metal pollution in Rio de Janeiro, SE Brazil (SW Atlantic). Heavy metal concentration in both species was compared along a decreasing gradient of pollution from the heavily polluted Guanabara Bay to the less impacted Cagarras Archipelago and Redonda Island. Seven specimens of each species were sampled in each site by SCUBA diving in December 2007 and from January to February 2008. In general, heavy metal concentration in both species decreased significantly along the pollution gradient. Out of 16 elements analyzed, 10 were more concentrated in *H. heliophila* and 11 in *P. magna* collected from heavily polluted sites in Guanabara Bay when compared to specimens from the coastal islands. Only Cd was more concentrated in specimens from the less polluted sites. *Hymeniacion heliophila* accumulates 11 elements more efficiently than *P. magna*. This difference may be related to their skeleton composition, histological organization, life cycle and symbiotic bacteria. Both species can be used as a biomonitors of metallic



pollution, but while *Hymeniacidon heliophila* was more effective in concentrating most metals, *Paraleucilla magna* seems more indicated to detect recent pollutant levels due to its shorter life cycle.

### **Fouling and quorum sensing inhibitory potential of marine sponges from Brazilian coast**

Daniela Batista Silva, Ana Carvalho, Rafaela Costa, Sergey Dobretsov, Ricardo Coutinho

*Instituto de Estudos do Mar Admirante Paulo Moreira/ Marine Science and Fisheries Department, College of Agricultural and Marine Sciences, Sultan Qaboos University*

Biofouling causes serious industrial problems, however, toxic biocides that are used to its control are not effective against some marine bacteria and diatoms. Quorum sensing (QS) is a bacterial population density dependent gene regulatory mechanism important for the regulation of their adhesion, growth, and biofilm formation. Therefore, compounds that inhibit QS provide an opportunity to control biofilms and biofouling. In this study, we screened dichloromethane (non-polar) and water: methanol (1:1) (polar) extracts from six marine sponges collected in Arraial do Carbo, RJ area for their ability to inhibit biofilm formation of *Pseudomonas aeruginosa* PA01 and QS of *Chromobacterium violaceum* CV017 in laboratory experiments. Additionally, inhibition of larval settlement of the bryozoan *Bugula neritina* was screened. Our results showed that non-polar extracts from *Arenosclera brasiliensis* and polar extracts from *Darwinella* sp. have QS inhibitory properties. No toxicity against the QS report has been observed. Only extracts from *Aplysina fulva* inhibited biofilm formation. Polar extracts from *Dysidea robusta*, *Dysidea etheria* and *A. brasiliensis* showed strong ability to prevent *B. neritina* settlement. In experiments with these extracts all larvae died after 1 hour. In conclusion, this study suggests that natural products from marine sponges have promising QS and antibacterial activities and may potentially be used in the future for antifouling applications.

### **Behavior of nudibranchs (Mollusca, Gastropoda) in relation to secondary metabolites from marine sponges**

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In Brazil, records on feeding of nudibranchs are based on descriptions made from field observations. The lack of experimental studies limits the understanding of the behavior of nudibranchs with their prey, both in relation to food preference as well as a possible chemical mediation in predator-prey interactions. The aim

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of this study was to evaluate, through laboratory experiments, participation of secondary metabolites from marine sponges in the behavior of chemical attraction of nudibranchs *Cadlina rumia* and *Tyrinna evelinae*. The collections of sponges and nudibranchs were made in Cabo Frio, Rio de Janeiro, Brazil. At the first moment, experiments were made using powder of lyophilized sponge *Dysidea etheria* added to artificial food and offered to nudibranchs *C. rumia* and *T. evelinae*. Two bioassays using the crude extract of the sponge, were conducted to evaluate the performance of secondary metabolites as chemical signaling for nudibranchs. In the first bioassay, the crude extract of the sponge was incorporated into artificial foods offered to *C. rumia*. In the second bioassay, the crude extract of the sponge were incorporated in mimics of natural dry marine sponges and offered to *T. evelinae*. In the assay with powder of lyophilized sponge, *C. rumia* showed no preference for food found in their natural habitat, or no recognition. The nudibranch *T. evelinae* showed preference for the food found in the field. In the first test with the crude extract, *C. rumia* did not recognize chemical signaling. In the second test with crude extract due to the low sample size was not possible to observe a definite answer from the behavior of *T. evelinae*. It was observed difficulty on working experimentally with nudibranchs, mainly due to the low sample size obtained. The experiments of chemical attraction showed that existing methods in the literature are not applicable to trials with nudibranchs and sponges. New approaches need to be investigated to test the hypothesis. Thus, it was not yet possible to verify if the crude extract of the sponge has a direct influence on the behavior of nudibranchs *C. rumia* and *T. evelinae*.

### **Climate Change and Tropical Sponges: The Effect of pH on Sponge Morphology and Ultrastructure.**

Holly Bennett

*Victoria University of Wellington*

At present, atmospheric carbon dioxide (CO<sub>2</sub>) levels are around 400 ppm, a level significantly higher than anything seen in the previous 650, 000 years, and rising at a rate 100 times faster than in the past. As the partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) in seawater rises due to this increasing atmospheric CO<sub>2</sub>, the Intergovernmental Panel on Climate Change (IPCC) predicts that by 2100 the pH of surface waters will have decreased 0.14–0.35 units and sea surface temperatures will have risen by 1–3 °C. It is projected that as a result of this global climate change, the reef building corals we see today will be extinct by the 21st century. Already, coral cover is declining at unprecedented rates. As coral cover declines, there is increasingly reports of shifts from coral to sponge dominance on reefs. Sponges are important structural and functional components of coral reef systems. As well as being top spatial competitors, sponges undertake suspension feeding (facilitating the flow of carbon to higher trophic levels), primary production (via

associations with photosynthetic microbes) and bioerosion and/ or the consolidation of reef framework. Despite this, and regardless of the fact they appear to be increasing on coral reefs globally, very little is known about the likely effects of climate change on sponges. Here I will present the results from the first stage of my PhD, looking at the effects of elevated pCO<sub>2</sub> on sponge skeletal material through histological analysis of specimens collected from a volcanic CO<sub>2</sub> seep in Papua New Guinea. Sponge morphologies, and therefore structure, often reflect adaptation to surrounding environmental conditions. Therefore, comparisons between spicules, spongin, mesohyl and canal systems of sponge specimens collected from high CO<sub>2</sub> and adjacent control sites are currently being made. Using standard histological procedures, the material is being studied for any degradation or adaptive effects of increased CO<sub>2</sub> to sponge structure, this will provide insight into the effects of long-term exposure to elevated pCO<sub>2</sub> on sponges.

### **Three-dimensional sponge aggregates in the Mediterranean Sea coralligenous accretions**

Marco Bertolino, Marielvira Matrone, Barbara Calcinai, Carlo Cerrano, Maurizio Pansini, Giorgio Bavestrello

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It is already known that sponges not only compete for substrate occupation but can also cooperate each other producing multi-specific assemblages, with epilithic and endolithic species. These patterns of growth may play a key role in the Mediterranean coralligenous bioconstructions. Coralligenous accretions, produced by the continuous deposition of coralline algae accumulating during hundreds to thousands of years, represent one of the most important habitats of the Mediterranean Sea. The three-dimensional complexity of these bioconstructions allows the formation of heterogeneous microhabitats that offer the opportunity to settle and thrive to a large number of benthic species. Many of them are inconspicuous (cryptic) and hard to recognise. Sponges, in particular, with more than 270 recorded species, are the most diversified animal group of the coralligenous community. The study of this taxon was performed after a gentle dissolution of the coralligenous substrate by soaking it in diluted HCl for several days. We could thus ascertain that several sponge species are strictly associated to form aggregates occupying different levels of the three-dimensional substrate.

Here we present some quantitative data on the sponge assemblages observed after dissolving 10 blocks of coralligenous substrate, each of about 1 litre volume, collected from the coralligenous rims of Portofino Promontory (Ligurian Sea) at 15 and 30 m depth. The pinacoderm of each sponge species is in contact with that of the others, without apparent development of a collagen barrier. The

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sponge species involved in the aggregates were in total 31 (10 encrusting, 16 insinuating and 5 perforating). The most frequent sponge species in the coralligenous accretions are four: three insinuating (*Triptolemma simplex* (Sarà, 1959), *Jaspis incrustans* (Topsent, 1890), *Spongosorites intricatus* (Topsent, 1892)) and one excavating (*Pione vastifica* (Hancock, 1849)). At least one of them is present in all the studied aggregates.

Our data demonstrate a positive correlation between the species diversity of a block (from 7 to 27) and the number of recorded aggregates (from 2 to 22). The average number of species present in the aggregates is 2.75. Also depth seems to affect these cryptic sponges: at the upper level (15 m) the largest number of aggregates is composed of encrusting and insinuating species while at the deeper level (30 m) the most common aggregates are formed by insinuating and boring species.

### **Sponge spicule flux in Antarctic water column studied by sediment trap**

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In Antarctic ecosystems, siliceous sponges play a pivotal role at different levels. They are one of the main components of the benthic biota showing species diversity and biomass levels comparable with those of tropical habitats. Demosponges and hexactinellids play a crucial role in the benthic-pelagic coupling absorbing large amounts of dissolved silica that is accumulated in the opaline spicules of their skeleton.

When sponges decay, the biogenic silica stored in their skeletons sunk in the sediments where, under the action of the currents, it accumulates in thick mats representing secondary biogenic substrata for benthic organisms. These free spicules show a negligible rate of dissolution representing therefore a net depletion of the dissolved silica from the system.

The fluxes of biogenic silica in Antarctic waters have been mainly studied with particular regard to diatom frustules while no quantitative data are available for sponge spicules.

In this paper we present the daily fluxes of sponge spicules in a sediment trap placed on a mooring 461 m depth on a bottom of 514 m depth in Terra Nova Bay (Ross Sea) from February 2003 to February 2005. The trend of the spicule sink, ranging from 7 to 19000 spicules m<sup>-2</sup> d<sup>-1</sup>, is strongly correlated with that of total sediments with maximum values during the austral summer and minimum during winter. Spicule fluxes are significantly different among the years: the annual maximal values are always recorded in February with values of 5000, 19000 and 8000 spicules m<sup>-2</sup> d<sup>-1</sup>, respectively for 2003, 2004 and 2005.

The spicule weight, obtained from the biovolume, indicates that these structures represents 1-2% of the total sediments. From a quantitative point of view, demosponges and hexactinellids contribute with a similar amount to the total spicule number. Also the entire spicules and the fragments are almost evenly represented in the trap, suggesting an equivalent supply from living sponges and from sediment resuspension

**Transitions from coral to sponge dominated reef states: an example from an Indo-Pacific reef**

Andrew Biggerstaff, James Bell, David Smith  
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Transitions away from coral dominated reef systems to alternate states have been widely discussed in the scientific literature, most extensively for transitions to algal dominance. However, the potential for transitions to sponge-dominated reef systems have gained much less focus. Coral reef systems in the Wakatobi Marine National Park (S.E. Sulawesi, Indonesia) represent an ideal study site for examining the potential for transitions from coral to sponge dominated systems. The Sampela reef system has seen a reduction in coral cover from over 30% to less than 10% (2003-2010) in conjunction with a 50% increase in sponge density between 2003 and 2010, whilst Hoga reef sites, situated only 1.5 km from Sampela, have a coral cover of 35% with only half the sponge density of Sampela reef sites. In addition, the Sampela reef system has a sedimentation rate four times higher than that of Hoga contradicting previous findings that sponge abundance is negatively correlated with the level of sedimentation. The sponge assemblages surrounding Hoga and Sampela are heavily dominated by a single species: *Lamellodysidea herbacea* with this species representing 42% of the overall sponge abundance, and whose abundance has increased significantly in recent years. Here I present the preliminary results of a three year study that will examine temporal and spatial variation in the abundance of *L. herbacea* at both Hoga and Sampela reef sites in relation to measures of sedimentation and turbidity, to assess the potential for this sponge to increase in abundance at other sites in the future.

**Orpheus Island Research Station**

Haley Burgess, Sue Kelly, John Chandler  
*Orpheus Island Research Station, James Cook University*

Orpheus Island Research Station is located within the sheltered water of Pioneer Bay and offers unique access to the inshore islands and reefs of the central Great Barrier Reef. Reached via a short boat ride from the mainland, with easy access to a variety of reef management zones and accommodation for 58

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persons in two separate accommodation blocks, OIRS is ideally suited to meet the needs of both education and research groups.

Station facilities include a lecture theater, dive shed and several laboratories; Filtered and unfiltered seawater is supplied to most lab areas; additionally, the wet lab is equipped with 30m of bench space, fumehood, autoclave and laminar flow; the dry lab/microscope room has 15m of bench space, -80° freezer, centrifuge and 12x microscopes including a dissecting and a binocular microscope equipped with a camera and video; the raceway area has 6 large flow through and 2 circular aquaria tanks; there are 5 temperature control rooms able to control air and humidity levels within .5% of a set point which are also supplied with additional 2 lines each able to control water temperature within 1% of their set point. Out in the field researchers have access to our 120m aquaculture line, JCU's deep sea research vessel, 35 radio receivers linked into the FAIMMS network and 10yrs worth of data from local weather stations associated with the GBROOS network.

Our new 10m catamaran has been specially designed to cater to the needs of researchers and small groups of up to 8pax visiting the outer reef areas such as Bramble Reef, as well as transporting large groups around the Palm and Hinchinbrook Island area. Additionally visitors are able to use our 5 self-drive vessels or hire our barge with or without skipper.

We would love to host your next expedition at OIRS.

### **Effects of simulated dredging and trawling on Australian sponges – a pilot study**

Evy Büttner, Flora Siebler, Jane Fromont, Muhammad Abdul Wahab, Christine Schönberg, Franz Brümmer

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Australia supports unique and dense filter feeder communities that are comparatively understudied. These communities are commonly dominated by sponges, representing important functional components of benthic habitats playing active roles in benthic-pelagic processes including nutrient cycling and purification of vast water masses. Additionally, they form 3-dimensional benthic structures providing habitats for other organisms including commercial species. As these communities are often located out of marine protected areas, trawling and coastal development pose environmental risks through physical damage, increased turbidity and shading from resuspended sediments, impeded filtering activity and smothering by sedimentation. We investigated the responses of five tropical, shallow-water sponges to simulated trawling and dredging stresses in flow-through aquaria at Orpheus Island Research Station, central Great Barrier

Reef. *Carteriospongia foliascens*, *Neopetrosia exigua*, *Cymbastela stipitata*, *Cliona orientalis* and *Paratetilla* sp. were subjected to the following treatments:

- daily covering with sand,
- daily covering with mud,
- an initial 24h application of clamps to cause squeezing,
- a single clean cut removing a significant portion of tissue,
- a single application of superficial abrasion.

After eight days, sponges were sub-sampled for chlorophyll and compositional analyses (i.e. salt, tissue, spongin, skeleton). Micro-computed tomography, a powerful tool to study various aspects of sponge biology, was used. All sponges had strong regenerative capabilities after physical damage, as demonstrated by the healing of cuts and abrasions after a few days. Squeezes caused the most damage in more highly organised sponges such as the radially built *Tetilla* sp., and in the brittle *N. exigua*, which lost the damaged tissue prior to healing. Sediment treatments caused a reduction in chlorophyll concentrations in the first four sponge species which are photosymbiotic, while the non-photosynthetic *Paratetilla* sp. exhibited an increase in carotenoids when squeezed, likely induced by a fungal infection. In summary, our results indicate that partial loss of tissue is generally repaired quickly, with squeezes often leading to tissue necrosis, and smothering by finer sediments resulting in more pigment loss than by coarser sediments. In comparison, the bioeroding *C. orientalis* appeared to be least affected, which may have implications for bioerosion levels in damaged and stressed filter feeder communities.

### **Influence of environmental variation on symbiotic microbial communities of two temperate sponges**

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Symbiotic relationships are critical to the success of many organisms. Sponges are an important component of temperate subtidal marine ecosystems, with a range of important functional roles and extensive symbiotic relationships with microorganisms. However, despite the importance of sponges, little is still known about their relationships with these symbiotic organisms, and specifically the role these symbionts play in sponge physiology, feeding and adaptation to local environmental conditions. Earlier studies on the effects of environmental factors on sponge symbionts have yielded contrasting results potentially because some sponges harbor more stable bacterial communities than others. Light is a factor that is thought to strongly influence the distribution patterns of sponges, primarily though its indirect effect on algal abundance. However, light is also likely to influence the diversity and abundance of sponge microbes, since many are phototrophic, while others may be light sensitive. Changes in light intensity may

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alter relationships between sponges and their symbionts, which may have an important role in determining the abundance and distribution patterns of some sponge species. Here we used 454 pyrosequencing of 16S rRNA genes to analyse the effect of light intensity on the bacterial communities in specimens of *Tethya bergquistae* and *Ecionemia alata* collected on the south coast of Wellington, New Zealand. We discuss the effect of light intensity on the stability/variability of sponge-associated bacterial communities in specimens transplanted from high light to low light habitats in temperate shallow-water rocky reefs, and the implications of our results to the local-scale adaptation of sponges

### **Taxonomy, biogeography and DNA barcodes of *Geodia* species (Demospongiae, Tetractinellida) in the Atlantic boreo-arctic region**

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Continental shelves and slopes of the cold-temperate North-Atlantic accommodate large accumulations of demosponges, so-called "ostur". In these sponge grounds, Geodiidae and Ancorinidae species (Demospongiae, Astrophorida) dominate in size and biomass. Geodiidae specimens can reach 80 cm in maximum dimension and weigh more than 38 kg. Boreo-arctic Geodiidae species from the North Atlantic appeared in the literature for the first time when J.S. Bowerbank in 1858 described *Geodia barretti* and *G. macandrewii* from Western Norway. Since then, a number of species have been based on material from various parts of the Northeast Atlantic, viz. *G. simplex* Schmidt, 1870 (W. Greenland), *Isops phlegraei* Sollas, 1880 (W. Norway), *I. pallida* Vosmaer, 1882, *I. sphaeroides* Vosmaer, 1882, *Synops pyriformis* Vosmaer, 1882 (all from N. Norway), *Geodia parva* Hansen, 1885 (Norwegian Sea, uncertain loc.), *G. normani* Sollas, 1888 (W. Norway), *G. atlantica* Stephens, 1915 (off Ireland), *Sidonops mesotriaena* Hentschel, 1929 (Spitzbergen) (now called *G. hentscheli* Cárdenas et al., 2010), and *Geodia simplicissima* Burton, 1931 (N. Norway). To these 12 nominal species are added four names of species described from elsewhere, but claimed to have been identified in material from the Northeast Atlantic, namely *G. cydonium* Jameson, 1811, *Cydonium muelleri* Fleming, 1828, *Geodia gigas* Schmidt, 1870, and *G. nodastrella* Carter, 1876. Almost 47 years after the review of Koltun (1966), we revise the boreo-arctic *Geodia* species with new morphological, molecular and biogeographical data.

Results show that there are six valid species of Atlantic boreo-arctic *Geodia*, the rest are synonyms or mis-identifications. *Geodia barretti*, *G. atlantica*, *G. macandrewii*, and *G. hentscheli* are well established and widely distributed. The



same goes for *Geodia phlegraei*, but the species shows a striking geographical and bathymetric variation, which led us to resurrect *G. parva* as the arctic sister-species of *G. phlegraei*. All six species have a deep-sea ampho-Atlantic distribution (they were identified in this work off Canada and in Western Greenland). Some are arctic species (*G. hentscheli*, *G. parva*), while others are typically boreal (*G. atlantica*, *G. barretti*, *G. phlegraei*); *G. macandrewii* is found in both boreal and arctic waters.

**A revision of boreo-arctic Atlantic Tetillidae (Demospongiae, Spirophorina):  
new records and new species**

Paco Cárdenas, Hans Tore Rapp, Megan Best, Francisco Javier Murillo, Elena Gerasimova, Mikael Thollesson, Ole Secher Tendal

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The worldwide distributed Tetillidae Sollas, 1886 comprises massive spherical sponges that can reach 20 cm in diameter. They are especially abundant in deep-waters of boreo-Arctic/Antarctic regions where they occasionally occur in large amounts creating impressive “sponge grounds”. Most of the Atlantic boreo-arctic species were poorly described in the late 18th or 19th century. Consequently, most of these species are poorly known (contemporary collectors tend to overuse the name *Craniella cranium*) and have never been revised. Furthermore, previous molecular phylogenetic data suggest that the Tetillidae should be revised since some genera (*Cinachyrella*, *Craniella*, *Tetilla*) are polyphyletic. We have collected new material during cruises along the Norwegian coast, in the Norwegian/Greenland Seas, and off Newfoundland/Labrador and Baffin Island. The material has been used for the partial sequencing of the cytochrome c oxidase subunit 1 (COI), including the Folmer and the I3-M11 partitions. Type material and museum collections are under study as well.

The WPD lists four valid species in the boreo-arctic Atlantic region (*Craniella cranium*, *C. polyura*, *C. zetlandica* and *Tetilla sibirica*) but the literature and our results indicate the region harbors at least 11-12 Tetillidae species. We are currently redescribing the six ‘historic’ species, all valid in our opinion: *Craniella abyssorum*, *C. cranium*, *C. infrequens*, *C. zetlandica*, *Tetilla polyura* and *T. sibirica*. Two of those species (*C. infrequens* and *C. abyssorum*) were usually identified as *C. cranium*. Further morphotypes and genospecies appeared in the course of this revision, and their status is also currently investigated: *Craniella* cf. *zetlandica* (Bergen, Norway), *Craniella* aff. *azorica* (Norway and Flemish Cap), *Craniella* aff. *sigmoancoratum* (Greenland Sea), *Craniella* sp. nov. 1 and 2 (Flemish Cap), *Craniella* sp. nov. 3 (Rockall Bank). Overall, there are 6 potential new species from the boreo-arctic Atlantic region. Three species have an ampho-Atlantic distribution in boreal (*C. abyssorum*, *C. aff. azorica*) or arctic waters (*C.*

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infrequens). We obtained COI for all of these taxa except *T. sibirica* (no new material was found). A molecular phylogenetic analysis was conducted integrating our boreo-arctic species sequences with Tetillidae sequences that we obtained from specimens from other regions and sequences from Genbank.

### **Phylogeny and biogeography of the genus *Metania* Gray, 1867 (Porifera, Haplosclerida, Metaniidae)**

Cristiana G.O. Castello-Branco, Adolfo Ricardo Calor, Carla Menegola  
*Universidade Federal da Bahia*

The genus *Metania* consists of freshwater sponges with a bulbous to massive body shape. Gemmules are present (asexual resistant body), with theca usually tri-layered and gemmoscleres that are pseudobiotules. *Metania* has eleven species with a circumtropical distribution: five showing a Neotropical distribution, three with an Afrotropical distribution, two displaying an Oriental distribution, and one with an Australian distribution. The aim of this study was to infer the phylogeny of the genus *Metania* and understand the processes that lead to the current biogeographic pattern using cladistic analysis. After analysis of species including scanning electron microscope, one matrix consisting of 26 morphological characters was analyzed in the TNT software, which resulted in two most parsimonious cladograms (equal weighting with score 59, CI = 0.44 and RI = 0.67) with strict consensus: ((Trochospongilla paulula ((Corvomeyenia everetti, Corvomeyenia epilithosa) (Oncosclera asiatica, Acalle recurvata) (Metania subtilis (M. godeauxi ((Drulia cristata (D. ctenosclera, D. conifera)), (M. pottsi (M. vesparium (M. reticulata ((D. brownii, D. uruguayensis), (M. ovogemmata (M. fittkau (Houssayella iguazuensis (M. vesparioides (M. kiliani (M. rhodesiana, M. spinata)))))))))))))). The monophyly of *Metania* was supported by two synapomorphies: the presence of acanthoxeas, an exclusive character of Metaniidae genera; and the presence of spines in the shaft of gemmoscleres. The species of the genera *Drulia* and *Houssayella* were suggested to be *Metania*, which now has sixteen valid species. BPA analysis of the genus *Metania* resulted in a single area cladogram showing a gondwanic pattern: (Neartic (Australian (Oriental (Afrotropical, Neotropical)))) explained for the breakup of Gondwana. The present study also resulted in the redefinition of the genus and proposition of a global identification key.

**Population genetics of the introduced sponge *Sycettusa hastifera* (Porifera, Calcarea) in the Brazilian coast.**

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Bioinvasion is considered one of the main threats to the marine ecosystem. In order to prevent and control invasions, molecular and ecological aspects of introduced organisms have been intensely studied. Therefore, it is possible to understand, for example, how populations can be found and maintained in areas where the species was not present before. The calcarean sponge *Sycettusa hastifera* is considered exotic in the Brazilian coast. It was first described to the Red Sea and in the 1980's it was first registered to Brazil. Nowadays, it is commonly found in ports and other areas with intense ship traffic. Larvae of *S. hastifera* are capable to recruit in habitats exposed to different environmental conditions, and it was recently suggested that these larvae have preference for artificial substrates. In the present work, we analysed the genetic differentiation and connectivity between populations of *S. hastifera* from two localities in Rio de Janeiro (Southeastern Brazil), based on five microsatellite polymorphic markers that we developed to this species. Heterozygote deficiency was found in most of these loci, probably due to inbreeding, self-fertilization or Wahlund Effect. The presence of null alleles was rejected because of a high success rate in the amplification step. Population differentiation was observed ( $F_{ST} = 0.037$ ;  $p < 0.001$ ), and a high number of private alleles was found in both populations, which may be related to different sources and/or number of introductions of *S. hastifera* in the Brazilian coast.

**First record of the “*Cliona viridis* complex” in the Southwestern Atlantic**

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*Cliona viridis* (Schmidt, 1862) is defined by having tylostyles and spirasters, a greenish brown color and strong excavating ability due to its association with symbiotic zooxanthellae. This species was originally described from the Adriatic Sea (Mediterranean). However, it comprises a species complex distributed in the Eastern Atlantic, Caribbean Sea and the Indian and Pacific Oceans. This is the first record of the “*C. viridis* complex” in Brazil, southwestern Atlantic. Three sponge specimens were collected on SCUBA at the Maricás Islands, Rio de Janeiro state, SE Brazil, on 26 September 2010. Specimens were photographed

in situ, fixed in 70% ethanol and deposited on the Porifera collection of the Museu Nacional, UFRJ, Brazil. *C. viridis* was one of the most common sponges at the Maricás Islands at about 12 m, covering and excavating calcareous algae. This species is thickly encrusting to massive. Ostial papillae are irregular to oval, numerous, 2 cm in average diameter, barely distinguishable after fixation. Oscular papillae are more pronounced than ostial ones, 3 cm in diameter, oval in shape and with a perioscular membrane. Color is moss green in life externally and internally, shifting to brownish green after fixation in ethanol. The surface is rough and undulated after fixation, consistency firm, only slightly compressible. The choanosomal skeleton formed by subtylostyles is disorganized in the inner parts of the body and in bundles or in a palisade arrangement next to the ectosome. Subtylostyles are slightly curved, with a rounded or slightly subterminal tyle, size: 182.5 – 543.8/ 2.5 – 12.5/ 6.3 – 15.0  $\mu\text{m}$  (length/ shaft width/ tyle width). Microscleres absent. Neighbor joining analysis of 28S rRNA (D2 region) sequences of *C. viridis* (AM293635, AM293636, AM293637 - Mediterranean), obtained from Genbank, and from *C. aprica* Pang, 1973 (Caribbean) and the specimens presently examined, revealed a high genetic similarity among Brazilian specimens and those from the Mediterranean Sea belonging to this species complex. Our preliminary results suggests that *C. viridis* is a cryptogenic species with occurrence at least on the Brazilian coast and in the Mediterranean/eastern Atlantic.

**Meeting the aliens: morphogenesis induction of the green alga *Ulva mutabilis* by sponge-associated bacteria**

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It is often assumed that symbiont microbial communities function as a fitness-enhancing factor to the benefit of their eukaryotic hosts. Regardless of their nature, the presumed benefits would suite the physiological and metabolic demands of the host organism, suggesting specialization as a key feature of fine-tuned, host-associated microbiomes. Here, we tested whether symbiont bacteria are capable of triggering physiological responses in an exogenous, non-corresponding host organism. This was achieved by exposing axenic gametes of the green alga *Ulva mutabilis* to marine sponge bacterial symbionts using a morphogenesis induction bioassay. The development of the gametes into germlings was monitored in the presence/absence of sponge symbionts and in co-cultivation with *Ulva*-specific bacteria after 18 and 42 days of incubation. We observed that several sponge-derived bacteria ? including, but not limited to, *Vibrio*, *Pseudovibrio* and *Aquimarina* strains - were capable of promoting the morphogenesis of *Ulva* at varying degrees. With few exceptions, *Ulva* developed into thalli composed of differentiated cells with characteristic deficiencies or even

into a callus-like morphotype without differentiated cells. Surprisingly, complete morphogenesis - a phenomenon known to be carried out exclusively by indigenous symbionts of the alga - was often observed when sponge-derived bacteria were co-inoculated with an *Ulva*-specific *Cellulophaga* mutualist. Phylogenetic analysis demonstrated that the sponge symbionts did not show taxonomic overlap with any of the typical *Ulva*-associated bacteria. We conclude that benefits derived from allochthonous bacterial metabolism might play a pivotal role in algal morphogenesis, highlighting generalist microbial traits as overriding factors structuring eukaryotic communities in the seas.

### **Diverse bacterial microbiome from the common shallow water Caribbean and Western Atlantic sponge *Cinachyrella***

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Marine sponges are vital components of benthic and coral reefs ecosystems, providing shelter and nutrition for many organisms. Because sponges can filter large quantities of seawater, they constitute an essential link between the pelagic and benthic environment. Many sponge species harbor high diversity and abundance of microbes (Archaea, Bacteria and eukaryotes) which can constitute up to 40% of the total sponge biomass. Bacteria abundance in sponge tissues can be two- to four- fold higher than concentration in the surrounding seawater. Furthermore, some bacteria permanently inhabit the sponge tissues while others are rapidly consumed. Here, we explore the bacterial and archaeal community diversity of the sponge *Cinachyrella* sp. using 16S rDNA tag pyrosequencing. *Cinachyrella* sp. are common shallow water Caribbean and Western Atlantic sponges that inhabit the reefs of south Florida and we collected specimens and seawater from the same reef at different times for one year. Over 14,000 rRNA amplicon sequences were analyzed from the sponges and 3,000 from the seawater. A total of 675 OTUs (> or= 97 % identity; 12 OTUs for archaea and 663 OTUs for bacteria) were detected in the sponge samples. OTUs belonged to 21 bacterial and 2 archaeal phyla and candidate phyla. Interestingly, our sponge samples appear to form two distinct groups, Group 2 with high Bacterial diversity (21 phyla) and Group 1 with lower diversity (12 phyla) and a diversity closer to the bacterial diversity of the seawater). These two sponge groups were observed independently of the time of year of sample collection. Both groups were dominated by Proteobacteria, but Group 1 had more Alphaproteobacteria and Group 2 had more Gammaproteobacteria. Abundant phyla also abundant in Group 1 included Actinobacteria, Bacteroidetes and Cyanobacteria. Group 2 had a few phyla that were present at very low abundance or absent in Group 1: Acidobacteria, Chloroflexi, Nitrospirae, PAUC34f, Poribacteria and Verrucomicrobia. Together with its ecology and concurrent transcriptome

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analysis of *Cinachyrella* sp in our laboratory, the microbial data can help establish the sponge as a useful experimental model.

### **First record of lithistid sponge from the submarine caves from the Nuku Hiva (Marquesas Islands) and Tahiti Iiti (Society Islands), French Polynesia.**

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Lithistid sponges inhabit usually deep water settings of the tropical and subtropical zones. However, they may also occur in shallow submarine caves. We report here for the first time new lithistid sponges from two shallow submarine caves (lavatubes) in French Polynesia: Ekamako in Nuku Hiva (Marquesas Islands) and Tepari on Tahiti-iti (Windward Islands, Society Islands). Only one species was found in Ekamako, a *Microscleroderma* sp., characterized by rhizoclones desmas and sigmaspire microscleres, but three species of lithistid sponges have been found in a longer submarine cave Tepari. One sponge belongs to *Microscleroderma* and is very similar to those sponges from Nuku Hiva, but a different species according to spicules and preliminary molecular analysis based on 28S rRNA (C1-D2 partition). The representatives of *Microscleroderma* are known mostly from Indian and Pacific oceans with 5 species, including one cave species. A *Microscleroderma* cave species is known from the eastern Mediterranean, and another one from shallow waters off tropical Atlantic coast of Africa. We report here that the new *Microscleroderma* species from Marquesas and Tahiti are most similar in morphology and spiculation to the Mediterranean cave species *Microscleroderma lamina*. The other lithistid species has also rhizoclone desmas, but exotylostyles as microscleres, and belongs to the genus *Gastrophanella*. This genus is relatively rare, but was reported from all oceans and here for the first time from Central Pacific. A very similar species was found in the submarine cave in Eastern Mediterranean. The third species belongs to sphaerocladine lithistids (desmas as sphaeroclones) and dichotriaenes with spinose/tuberculated surface of the caldome, and acanthorhabds and spinse styles as microscleres. It represents a new genus, and probably a new family as well. Its desmas are very similar to typical sphaeroclones known in some lithistids from the Mesozoic of Europe. A very similar assemblage of lithistids (*Microscleroderma*, *Gastrophanella* and the new spaherocladine) was reported from a submarine cave in Philippines. Considering all these features, the lithistids from the Marquesian and Tahitian caves may be considered as a relic of Mesozoic Tethyan fauna.

Extracts of the *Microscleroderma* from Marquesas displayed cytotoxicity and their chemical profile displayed known and new microsclerodermin type peptides.

## **Cytotoxic Alkaloids from Indonesian Marine Sponges**

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In a quest to discover novel secondary metabolites with anticancer activity, we screened methanolic extracts of a diverse range of marine sponges collected from Wakatobi Marine National Park in Southeast Sulawesi, Indonesia for their cytotoxicity. Two species of marine sponges that were identified as *Stylissa carteri* (order Halichondrida, family Dictyonellidae) and *Acanthostrongylophora ingens* (order Haploslerida, family Petrosiidae), were found to exhibit cytotoxicity toward HeLa (cervix) cancer cell lines at a concentration of 30 ppm. To identify their bioactive compounds, we employed flash chromatography and HPLC, as well as structure determination by using 2D NMR spectroscopic measurements. Herein, we report the isolation and structure determination of two classes of cytotoxic alkaloid compounds, bromopyrroles and 3-alkylpiperidines from *S. carteri* and *A. ingens*, respectively. Bromopyrroles have been considered as distinctive secondary metabolites from several sponge genera such as *Agelas*, *Axinella*, *Acanthella*, *Pseudaxynissa* and *Hymeniacion* and exhibit a range of biological activities including as antimicrobials,  $\alpha$ -adrenoreceptor blockers, antifeedants, and as antiserotonergic or anti-osteoarthritis agents [1]. Marine sponges of the order Haplosclerida are a source of bioactive polycyclic diamines, eg. halicyclamine B that shows selective antimicrobial activity, formed from a novel biogenetic process that may involve a Diels Alder cyclisation [2,3].

## **Astrophorid sponges grounds in the deep seabeds of the Joint Regime Area between Colombia and Jamaica, southwest Caribbean Sea**

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The investigation of previously unexplored areas of the ocean continues to yield unexpected records of sponges. In late 2011, trawling on hard and semi-hard bottoms were carried out in the Joint Regime Area between Colombia and Jamaica (JRA) near the Seaflower Biosphere Reserve in the southwest Caribbean Sea (Invemar-ANH campaign). The phyla Porifera was highly represented in the deep water fauna at sites Bajo Nuevo, Guyot Macondo and Serranilla Bank, even when it was only possible to collect a single sample in each one (390, 320 and 380 m of deep, respectively). Among the composition of

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sponges stand out Astrophorida with thirteen morphospecies identified inside the families Geodiidae, Calthropellidae, Pachastrellidae and Ancorinidae, some of them may correspond to new species for science and new records for the Caribbean Sea. The sponges were characterized by massive, spherical and cup growth forms, generally with hard consistency and quirky skeletal arrangements. Since astrophorids are poorly known in the deep waters of the southwest Caribbean sea, the present collection is an important contribution to the knowledge of the biodiversity and ecology of this oceanic area. Furthermore, it is worth noting the presence of some lithistids sponges found in association with the astrophorids. The results presented here suggest the presence of sponge grounds dominated by astrophorids where diverse invertebrate taxa, (echinoderms, bryozoans and crustaceans, also collected) are finding suitable habitats and building communities poorly known as well. These results are all the more relevant considering the importance of these JRA seabeds as places of future exploitation for energy and mineral resources.

### **Diversity of bacteria cultured from Irciniidae sponges**

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Several bioactive compounds originally isolated from marine sponges have been later ascribed or suggested to be synthesized by their symbionts. The cultivation of sponge-associated bacteria provides one possible route to the discovery of these metabolites. Here we determine the bacterial richness cultured from two irciniid sponge species, *Sarcotragus spinosulus* and *Ircinia variabilis*, and ascertain their biotechnological potential. A total of 279 isolates was identified from 13 sponge specimens. These were classified into 17 genera - with *Pseudovibrio*, *Ruegeria* and *Vibrio* as the most dominant – and 3 up to 10 putatively new bacterial species. While 16S rRNA gene sequencing identified 29 bacterial phylotypes at the “species” level (97% sequence similarity), whole-genome BOX-PCR fingerprinting uncovered 155 genotypes, unveiling patterns of specimen-dependent occurrence of prevailing bacterial genomes across sponge individuals. Among the BOX-PCR genotypes recovered, 49% and 23% showed the presence of polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) encoding genes, respectively, and 34% were active against clinically relevant strains. PKS genes were for the first time detected in isolates of the genus *Aquimarina* (Bacteroidetes), while *Vibrio* strains produced the most active antagonistic effect. Our results highlight great biotechnological potential and interest for the Irciniidae sponge family and their diversified bacterial genomes.



**Evidence of a conduction mechanism that stiffens the demosponge  
*Chondrosia reniformis* Nardo 1847 after localized mechanical stimulation.**

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Although sponges are still often considered to be simple, inactive animals, both larvae and adults of different species show clear coordination phenomena triggered by both extrinsic and intrinsic stimuli. *Chondrosia reniformis* (Nardo, 1847), a common Mediterranean demosponge, lacks both endogenous siliceous spicules and reinforcing spongin fibers and has a very conspicuous collagenous mesohyl. Although this species can stiffen its whole body in response to localized mechanical stimulation, no data are available in the literature on the propagation of this stiffening response. The present work was intended to demonstrate: 1) the ability of the whole body to react to localized stimulation; 2) the possible occurrence of a conduction mechanism; 3) the role of the exopinacoderm in the propagation of the stiffening responses. Data on mesohyl tensility obtained with mechanical tests confirmed the ability of the sponge to react to local stimulation by stiffening regions 2 cm away from the stimulated area, suggesting the presence of a conduction mechanism that propagates from the stimulated spot. Creating a discontinuity in the exopinacoderm, by removing 1 mm of the sponge surface in the area surrounding the stimulation spot, resulted in a strong decrease in the capability of the sponge to stiffen at 2 cm distance, which suggests that the exopinacoderm could play a role in the propagation of the stiffening response. We also provide further evidence (following Wilkie et al., 2006) for the presence of a chemical factor that alters the interaction between collagen fibrils, thereby stiffening the sponge mesohyl.

**Reconstruction of the oceanic silicon cycle using silicon isotopes**

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Occidentale*

Siliceous sponges, radiolarians and diatoms use dissolved silica (DSi) in the surrounding waters to build their opal skeleton. Few is known about the evolution of the oceanic silicon cycle through geological times. About 100-200 million years before present, diatoms started to colonize the ocean surface, reducing dramatically the DSi concentration in the surface ocean. Biomineralisation of opal by siliceous organisms results in a fractionation of silicon isotopes, leading to a relatively lighter isotopic composition of siliceous organisms compared to the ocean water. By measuring silicon isotopic composition in siliceous organisms from marine sediment cores, we can track the evolution of the silicon in the ocean. Whereas the fossil record of diatoms can be used only to reconstruct the

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silicon isotopic composition of the surface ocean for the last 100Ma, the fossil record of radiolarians and sponges allow us to reconstruct the isotopic composition of intermediate and deep water during the entire Phanerozoic.

Here, we present the silicon isotopic composition of sponge spicules and radiolarian tests from marine sediment core (Ocean Drilling Program) from the Paleogene (65.5 to 23Ma) allowing us to provide insight to the evolution of the silicon cycle, which could have influenced the evolution of siliceous organisms through time.

### **Deterrent function of a sponge-associated bacteria against a wide array of potential predators**

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*Hemimycale columella* is an Atlanto-Mediterranean sponge without known predators, despite it shares habitat with several species of sea urchins and fishes, which can feed on sponges. Uriz et al. (2012) reported that this sponge harbors high amounts of a calcifying bacteria (calcibacteria) without evident harmful effects for the sponge. The calcibacteria form a calcareous cortex at the sponge periphery, which may play a defensive role against potential predators. This purported physical defense may complement the chemical defenses already reported for the species (Becerro et al. 1997). The aim of this study was to assess whether the physical or chemical defenses, or both, account for the lack of predation observed. Deterrence experiments were conducted in both the laboratory and the field. We used as potential predators the sea urchin *Paracentrotus lividus* and the fish *Parablennius incognitus* (in the laboratory) and naturally occurring fish assemblages (*Oblada melanura*, *Chromis chromis*, *Diplodus vulgaris* and *Coris julis*) in the field. All the experiments consisted in offering either carrageenan food (sea urchins) or food pellets (fish) impregnated with ecologically relevant concentrations of the sponge crude extract or its physical component (mainly calcibacteria spherules). All the target species were significantly deterred by the physical treatment, but not by the chemical treatment. The sea urchins were similarly deterred by both the chemical and the physical sponge components. Fishes were differentially deterred according to the fish species. *Coris julis* and *Chromis chromis* were significantly deterred only by the physical treatment, while *Diplodus vulgaris* and *Oblada melanura* were equally deterred by the chemical and physical treatments. In conclusion, the calcibacteria protect *H. columella* from predation against an array of potential predators. This deterrent effect is even more relevant than that produced by the secondary metabolites. Thus, calcibacteria seem to exert a positive role on the sponge fitness, which points to a symbiotic nature of the bacteria-sponge association.

Uriz et al 2012. *Evolution*, 66:2993-2999

Becerro et al 1997. J. Chem. Ecol. 23:1527–1547

**Portuguese marine sponge extracts libraries for drug discovery: ASYN-TAU, TTR and IDO modulators**

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Ageing related diseases like cancer and neurological disorders constitute a major health problem worldwide, and their impact on public health and society is growing as life expectancy increases. The oceans with a myriad of unique microenvironments, many of which still unexploited, have already provided scaffolds for several important drugs, including AZT. In fact, for the past years, marine-derived natural products have been playing an increasingly significant role in drug discovery. Chemical investigations on marine sponges allowed the identification of a vast array of new bioactive compounds with unique structures. In this study we investigated the biomedical potential of sponges collected in Portugal in collaboration with BIOALVO, a Portuguese biotech company that explores natural products, converting them into innovative ingredients for several industrial applications.

The organic extracts (dichloromethane/methanol 1:1) of 182 sponge samples collected in several locations of the Portuguese shores (Berlengas, Azores and Gorringe bank) were screened for modulators of proteins involved in cancer and neurodegenerative diseases using BIOALVO's proprietary bioactive discovery technology based on the use of yeast as a model organism ? the Global Platform Screening for Drug Discovery (GPS D2). The three platforms used genetically modified *Saccharomyces cerevisiae* strains designed to express specific targets involved in diseases with a tremendous social and economic burden: (1) ASYN-TAU platform (?-synuclein and tau) for the neurofibrillary tangles and Lewy bodies characteristic of Alzheimer's and Parkinson's diseases; (2) TTR (transthyretin) platform for insoluble fibrous protein aggregates formed in Familial Amyloid Polyneuropathy disease; (3) IDO (indoleamine-2,3-dioxygenase) platform for cerebral malaria, neuroinflammation and cancer.

The screening assays (500?g/mL) allowed the identification of a total 17 extracts with potential therapeutic activity. 1% of the sponge extracts revealed to be IDO inhibitors based on the phenotypic assessment of enzyme inhibition, at a level of at least 75%; 5% are able to recuperate the toxicity caused by ASYN-TAU by 45%; and 3% are able to restore TTR toxicity at least 65%. These extracts are currently being further investigated for the activities under study.

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### **Lithistid as habitat: 3 dimensional sponge diversity in the Western Australian tropics.**

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Areas of tropical Western Australia are now known to support diverse sponge communities with high species richness and abundances. Sponges are critical habitats for other organisms in terms of providing refuges, food and attachment surfaces. A number of recent deeper water surveys off Western Australia have enabled the sponge fauna at depths greater than 30 metres (maximum SCUBA depth) to be documented. These surveys found that highly species-rich sponge assemblages dominated the megabenthic invertebrate biomass on hard substrates. We found that lithistids occur from Perth Canyon off Fremantle at 32°S latitude northwards, and have high biomass in some areas, for example in the Onslow region in the Pilbara. We report the presence of a new lithistid species of the genus *Microscleroderma* occurring from Kalbarri on the central coast to the Kimberley in the north of Western Australia. This new species acts as an attachment site for a suite of other sponge species, hosting up to six species of demosponges. *Microscleroderma* sp.nov. has a thick, flat, plate to tubular morphology and is overgrown by erect fans and branching sponges. The epifaunal sponges are halichondrid species of the genera *Axinella*, *Acanthella*, *Stylissa*, and *Ptilocaulis*. To our knowledge dense communities of epifaunal sponges have not been previously reported on lithistid species. We present the following findings: the geographical and depth distributions of the lithistid-epifauna association, the geographical and depth distributions of the epifaunal species and whether they also occur outside the association, and relative species abundances.

**New records of marine sponges (Porifera: Demospongiae) for the Gulf of Mexico**

Patricia Gómez, Diana Ugalde, Nuno Simões  
*Universidad Nacional Autónoma de México*

Sponge diversity is higher in tropical coral reefs than in other marine or freshwater habitats. The Caribbean coral reefs hold up to 640 sponge species whilst the Australian reefs add up to 2324 species. The sponge diversity of the southeastern Gulf of Mexico have not been thoroughly studied although this part of the basin presents the largest coral reef area in the entire Gulf of Mexico (GM), with five coral reefs emerged and, at least 15 coral bank reefs submerged. The main reefs are located along the East coast of Campeche Bank and at the north of the Yucatan Peninsula. The present work is the first comprehensive survey of Marine Sponge biodiversity from Alacranes, which is the largest coral reef in the GM, and the shallow submerged ?Bajos de Sisal? reefs, off the coast of Sisal (both in the northwest Yucatan). There were 22 new sponge species records for the Mexican coasts from which 12 of them are new records for the GM, these are: *Cliona amplicavata*, *C. flavifodina*, *Chondrilla caribensis*, *Strongylacidon bermuda*, *Dragnacidon lunaecharta*, *Hymeniacidon caerulea*, *Agelas tubulata*, *Amphimedon caribica*, *Verongula reiswigi*, *Aplysina alcornis*, *A. caissara*, *A. muricyana*. These species stand out from the common sponge community which inhabits along the Yucatan Coast and is comprised of the classical reef families such as: Geodiidae, Clionidae, Suberitidae, Chondrillidae, Chondropsidae, Halichondriidae, Axinellidae, Agelasidae, Chalinidae, Niphatidae, Spongiidae, Dictyodendrillidae, Dysideidae, Darwinellidae, Aplysinidae. The presence of *Erylus trisphareus* is particularly noteworthy as this new record for the Mexican coasts had not been reported since the original description from de Laubenfels, 1953, at the north of the GM, west side of Florida. These findings are contextualized with the broad geographic distribution of the sponge fauna previously known.

**Integrative approach to the taxonomy of the family Mycalidae Lundbeck, 1905, using morphological, chemical and molecular datasets**

Leesa Habener, Anthony Carroll, John Hooper, Kathryn Hall, Jenny Wilson,  
Jessica Worthington Wilmer, Merrick Ekins  
*Griffith University, School of Environment/ Queensland Museum*

Taxa belonging to Mycalidae (Poecilosclerida, Mycalina) are distinguished by both a tangential surface skeleton and the presence of palmate anisochelae. While spicule diversity may help differentiate species level taxa relatively well, support for genera and subgenera remains equivocal based solely on morphometrics. A number of classes of bioactive compounds have been characterised from Mycalidae, and of particular interest are the range of cytotoxic

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metabolites, such as the Mycalamides. The bioactive chemistry initially recognised, together with their high morphological diversity identify this group a good candidate for the application and interrogation of sponge chemotaxonomy. However, reports of sponge compounds in the current literature are biased towards the reporting of novel compounds. An integrative approach is needed to unequivocally delimit species boundaries and test traditional morphological characters where the inclusion of a molecular dataset would provide an additional line of inquiry. The expansion of the taxonomic analysis beyond morphological and chemical datasets will identify characters with more significant taxonomic implications. For this project access to frozen material from the Queensland Museum Great Barrier Reef Seabed Biodiversity collection, and freeze-dried material from the Eskitis Institute Nature Bank (Griffith University) was made available, with approximately 150 specimens of ~40 OTUs (operational taxonomic units) differentiated, of which only 7 OTUs are described species. Preliminary morphological analysis indicates that at least 10 (possibly up to 20) may be new species – but this might be even higher given the high incidence of cryptic speciation amongst Porifera. Through an integrative approach, all investigated OTUs will be allocated to a species taxon, with new species formally described based on morphological, molecular and chemical datasets. For sponges these integrative tools are still in their infancy, but those few studies that do exist, they have been pivotal in the discovery of cryptic species and in resolving phylogeny. Here the potential for an integrative approach is considered for a subset of these available samples. This incorporates morphological characters assessed using scanning electron microscopy (SEM), preliminary chemical analysis using conventional natural product techniques (eg. HPLC, NMR and MS) and a preliminary phylogenetic tree based on some molecular markers (mtDNA COI and rDNA 28S).

### **The diversity of sponges from French Polynesia**

Kathryn Hall, Merrick Ekins, Patricia Sutcliffe, John Hooper, Nicole de Voogd,  
Sylvain Petek, Eric Folcher, Cecile Debitus  
*Queensland Museum/ The University of Queensland/ Queensland Museum &  
Eskitis Institute for Cell & Molecular Therapies, Griffith University/ Naturalis  
Biodiversity Centre/ IRD, French Polynesia*

We report here on an extensive survey of sponges from French Polynesia, recording presence/absence and an estimate of local abundance on sites spread in the 5 group of islands of French Polynesia (viz. the Marquesas Islands, Windward and Leeward Islands (together making the Society Islands), Tuamotu and Australes islands. Sponge distributions within archipelagos were relatively homogeneous, showing some differential patterns in affinities between each of them and approximately one-third of the fauna apparently endemic to these archipelagos, but between-archipelago comparisons showed large heterogeneity.

The fauna of the Marquesas Islands (with sites consisting mostly of rocky slopes) was dominated by species in Order Poecilosclerida, and showed a similar range of taxonomic diversity to the remote fauna of the Hawaiian Islands. By comparison, the sponge fauna of the Society Islands, Tuamotu and Australes islands was dominated by Order Dictyoceratida, reflecting the predominance of coral reef and lagoon sites and associated phototrophic feeding strategies. Further investigation of Marquesas and Society islands using Parsimony and multivariate statistical analyses to compare French Polynesian sponge faunas with others in the southwestern Pacific showed closest nested faunal similarities between the (Marquesas Islands + Society Islands), (((Tonga + Fiji) + Vanuatu) + New Caledonia), and (North Great Barrier Reef + South Great Barrier Reef), but no or very low similarity between more geographically isolated faunas such as Palau and the collective Great Barrier Reef fauna.

**SpongeMaps: in-cloud taxonomic collaborations on Indo-West Pacific species of Theonella Gray, 1868**

Kathryn Hall, Merrick Ekins; Miranda Vidgen, Mary Kay Harper; Chris Ireland, Anthony Carroll; Leesa Habener, Dirk Erpenbeck; Gert Woerheide, John Hooper  
*Queensland Museum/ University of Queensland/ University of Utah/ Griffith University (Gold Coast Campus)/ Ludwig-Maximilians University, Munich*

2013 saw the launch of a new tool for collaboration in sponge taxonomy: SpongeMaps. We have used this web platform to share information on theonellid sponges, leading to the description of new species of Theonella Gray, 1868. With collaborators spanning 3 continents, SpongeMaps enabled viewing, editing, adding and commenting on observations by all members of the team in real-time. We used species of Theonella as a test case for exploring the utility of SpongeMaps as a collaborative tool. Recent studies are demonstrating that traditional morphological characters are inadequate to define species boundaries of theonellids and other astrophorids. Confoundingly, many species of theonellids (historically classified within lithistids and characterised by the possession of tetraclone desmas) completely lack or have greatly reduced complements of desmas, although DNA data strongly support their inclusion within theonellid lineages. The combination of chemotaxonomy, detailed morphological studies and DNA-based analysis can be used to discriminate among and within such groups. Here, SpongeMaps allowed us to integrate all potential sources of data and collaborate in real-time to test taxonomic hypotheses. Our study of a number of species of Theonella highlights, importantly, that SpongeMaps can and does bring together researchers with various skills and specialisations, allowing the integration of morphology, chemistry and DNA sequences in the taxonomic process. Using SpongeMaps to integrate, annotate, commentate, synthesise and organise data, taxonomic studies can be realised as truly multi-disciplinary, simultaneous, international collaborations.

**Fluid-Structure-Interaction: How cellular structures affect local flow in sponge canal systems**

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*Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum,  
Friedrich-Schiller-Universität Jena*

Sponges possess a highly efficient fluid transport system. As in other biological fluid transport systems the general architecture is supposed to be optimized for minimal flow resistance and consequently requires minimum work to move the fluid. Apart from the general architecture, especially small sized openings account for the largest contribution to flow resistance. Within the sponge canal system such structures are ostia, prosopylar and apopylar openings and the microvilli collars of choanocytes. In the demosponge *Tethya wilhelma* another cellular structure with only minute openings is present in the canal system. These are the so called reticuloapopycytes which span the apopylar opening with their sieve like cell morphology. Up to now, their function is unknown. Due to their location a role in flow regulation seems likely. In order to study their influence on local flow within the canal system I have developed two finite element models of a choanocyte chamber connected to a canal segment with and without reticuloapopycytes. By comparing local hydrodynamic parameters it is possible to test for fine-tuning local flow by changes in open/close states of reticuloapopycyte fenestra. The contribution of reticuloapopycytes to flow resistance could be extracted from comparative analysis of both models. Most prominent changes in flow occur in boundary layer thickness of the connected canal segment. As the present analysis is limited to a local scale the exact consequences of an altered boundary layer thickness in the excurrent canal system on transport processes on an organismic scale remain to be clarified. Further on I have observed changes in pressure distribution. Reticuloapopycytes seem to increase the static pressure within the entire canal system. This phenomenon might be related to body contraction expansion cycles and could play a role in the second phase of the expansion cycle. However the role of hydraulic pressure in body contraction expansion cycles remains to be experimentally proven.

**Degrees of host specificity, effects of sample handling, and extent of cultivation bias elucidated for bacterial communities in closely related marine sponges**

Cristiane Hardoim, Massimiliano Cardinale, Ana Cúcio, Joana R. Xavier, Cymon Cox, Rodrigo Costa

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Complex and distinct bacterial communities inhabit marine sponges and are believed to be essential to host survival. Our present-day inability to domesticate sponge symbionts in the laboratory hinders our access to the full metabolic potential of these microbial consortia. The extent and nature of this impairment has rarely been evaluated in a comprehensive fashion. Here, we address cultivation bias in marine sponges using a bacterial culturing procedure that circumvents the need to isolate single colonies during richness surveys, and instead enables direct comparison of symbiont compositional and diversity data with cultivation-independent methods. Bacterial community profiling of the sympatric and phylogenetically close species *Sarcotragus spinosulus* and *Ircinia variabilis* (Irciniidae) was performed by PCR-DGGE and 454-pyrosequencing of 16S rRNA genes amplified from sponge-derived metagenomic DNA samples. Ordination analyses of the data revealed largely contrasting community structures between cultured and uncultured sponge bacteria. Whereas cultivation-independent methods clearly unraveled species-specific community structures in these hosts, cultivation led to the detection of highly similar bacterial assemblages from both sponge species. Between 15 and 18 bacterial phyla were found in *S. spinosulus* and *I. variabilis* by cultivation-independent means, with prevalence of Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, PAUC34f, Poribacteria and Chloroflexi. Conversely, the cultured bacterial community was characterized by a massive dominance of Proteobacteria in the Alpha- and Gammaproteobacteria classes. Whereas cultivation-independent methods enabled the detection of c. 200 and 220 operational taxonomic units (OTUs, 97% gene similarity) in *S. spinosulus* and *I. variabilis*, respectively, only 33 and 39 OTUs were registered in these species via culturing. Surprisingly, around 50% of all OTUs recovered by cultivation was exclusive to this methodology, suggesting culturing is a means of enriching rare sponge-associated bacteria that escape detection by cultivation-independent, deep sequencing efforts. This study demonstrates that interpretation of ecological data on sponge microbiome diversity and composition is dependent on the choice of methodology. It further encourages the development of alternative culturing technologies needed to capture the dominant sponge symbiont fraction that remains recalcitrant to laboratory manipulation.

**Temporal maintenance of symbiont communities and ammonia oxidation potential in the marine sponge *Sarcotragus spinosulus***

Cristiane Hardoim, Rodrigo Costa  
*Microbial Ecology and Evolution Research Group, Centre of Marine Sciences  
(CCMar), University of Algarve*

Bacterial and archaeal communities compose a fundamental portion of the marine sponge holobiont. In spite of their putative relevance in host functioning, no surveys have so far inspected the stability of sponge symbiont communities over time with appropriate depth. This study tackles the extent of temporal variation in archaeal and bacterial assemblages in the marine sponge *Sarcotragus spinosulus*. Specimens (n=12, 4 individuals/year) were collected in three successive years off the coast of the Algarve, South Portugal. Prokaryotic community profiling was performed by PCR-DGGE and 454-pyrosequencing of 16S rRNA genes amplified from sponge metagenomic DNA. Both techniques showed that only a minor portion (c. 25%) in archaeal and bacterial 16S rRNA gene variation could be explained by sampling year effects. Prevailing bacterial phyla in *S. spinosulus* were Acidobacteria, Actinobacteria, AncK6, Bacteroidetes, Gemmatimonadetes, Chloroflexi, PAUC34f, Poribacteria, Proteobacteria and Spirochaetes, of which only Bacteroidetes and Chloroflexi presented different ( $p < 0.05$ ) relative abundances over the years. At the approximate species level (OTUs, operational taxonomic units defined at 97% gene similarity) no differences in bacterial richness (c. 80-90 OTUs per specimen) was found in *S. spinosulus* through time. Low within-year and larger between-year variation in bacterial OTU composition was observed. Nearly 50% of all detected bacterial symbionts (96 in 204 OTUs) were found in the three sampling years, whereas 136 OTUs (c. 67%) were detected in at least two sampling years. With the exception of one host specimen, archaeal communities were highly dominated by one single symbiont affiliated with the genus *Nitrosopumilus* (Thaumarchaeota; Nitrosopumilales), well known for its ability to aerobically oxidize ammonia to nitrite. Its remarkable prevalence and temporal persistence in *S. spinosulus* suggests an effective participation of this archaeon in host nutrient cycling and housekeeping functions. PCR-DGGE fingerprinting of bacterial ammonia oxidizers showed likewise that few phylotypes in these cohorts prevailed in *S. spinosulus* across the years. Our results reveal that *S. spinosulus* hosts a core and persistent community of prokaryotic symbionts through time and favor the view of temporal stability in the structure and function of the marine sponge holobiont.

### **Integrative taxonomy and phylogeny of family Cladorhizidae**

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*University of Bergen/ IMBE-UMR7263 AMU 2/ IMBE-UMR7263 CNRS*

The deep-sea demosponge family Cladorhizidae is known for its carnivorous feeding-mode, found to be ubiquitous within the family. This feature is generally regarded as an adaptation to the nutrient-poor conditions of the deep sea, where the cladorhizids constitute a large part of the sponge fauna, with a depth record of 8840 m.

Increased sampling activity in the deep sea as well as in special habitats such as hydrothermal vents and cold seeps has contributed to the description of a number of new cladorhizid species in recent years, and more than 120 species are now registered in the World Porifera Database. Compared to species described earlier, several new species display unusual combinations of spicules, adding to the morphological diversity and the complexity of the systematics of the group. The evolutionary history for the group is largely unknown, with only fragmentary molecular data available, and it is suspected that the current spicule-based systematics does not in all cases accurately reflect evolutionary relationships within the Cladorhizidae and allied groups.

We provide a review of the systematics of family Cladorhizidae based on currently available molecular and morphological data of a comprehensive set of species and genera of the group, using this integrative approach to present a phylogeny of the Cladorhizidae.

**Population density and structure of Indo-Pacific giant barrel sponge  
*Xestospongia testudinaria* (Lamarck, 1815) in the southern Penghu Island,  
Taiwan**

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Caribbean-Atlantic giant barrel sponges, *Xestospongia muta* had been considered as “Redwoods of the Reef” and estimated as the longest-lived animal aging more than 2000 years. Nevertheless, the relative studies of the counterpart species, *X. testudinaria* in the Indo-Pacific are scarce. In July 2013, population density and structure of the barrel sponges were investigated by SCUBA diving in FongGuei, ChingWan, and SuoGang within a 7 km range along the southern coastline of the Penghu Island, Taiwan. Surveys on three 50 x 4 m transect plots were conducted for each location. A total of 66 sponges within 10 m depth in the transect plots were measured. The population densities of the sponge ranged from 0 – 0.12 individuals.m<sup>-2</sup>. The population density in FG (0.08 ± 0.019 individuals.m<sup>-2</sup>) was significantly higher than that in CW (0.03 ± 0.004) and SG (0). Sponge volumes ranged from 116.57 to 68215.10 cm<sup>3</sup>. Larger sponges (>1077.13 cm<sup>3</sup>) were more frequent in FG. The largest sponge found outside of our transect plots (46 x 60 cm height x diameter) in FG was estimated to be 34 years using “X. muta Age Calculator”. Spongocoel-sponge-volume curves of *X. muta* and *X. testudinaria* demonstrated similar patterns in small and large size classes; however, expressed distinct patterns in middle size classes (Paired t-test, P < 0.001). The population densities and structures of *X. testudinaria* can vary across small spatial scale of 1.5 km. We hypothesized the growth model of *X. testudinaria* in depth shallower than 10 m is likely different from that of *X. muta* in deeper depths (15 and 30 m) resulting from varying magnitude of physical

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impacts such as wave and surge caused by tropical storms. The growth model of *X. testudinaria* in shallow water needs to be further reconstructed.

### **Characterising marine microbial diversity and function in sponges of Western Australia**

Megan Huggett, Jane Fromont  
*Edith Cowan University/ Western Australian Museum*

The importance of microorganisms in ecosystem health and function is now widely accepted. Despite this, nothing is known about marine microbes in the unique and relatively pristine habitats of the Western Australian coastline. This study aims to determine the diversity, specificity and function of microbes associated with marine sponges of a broad biogeographical area of the West Australian coastline using emerging molecular biology techniques. Data generated here will provide baseline information for key organisms of these vast areas that are now recognised both nationally and internationally as highly diverse and valuable ecosystems, allowing subsequent studies to investigate how specific assemblages respond to predicted future stressors.

### **Calcareous sponges from the Adriatic Sea**

Mirna Imešek, Michelle Klautau, Bruna Pleše, Vedran Nikolić, Masa Roller,  
Helena Četković  
*Ruđer Bošković Institute/ Universidade Federal do Rio de Janeiro/ Institute of Oceanography and Fisheries/ Bioinformatics Group, Department of Molecular Biology, Faculty of Science, University of Zagreb*

The first studies on the class Calcarea were mainly done by Schmidt and Haeckel in the XIX century, which means that the Adriatic Sea is the type locality of several species of calcareous sponges. As several of their original descriptions are much incomplete and some type species have disappeared, new collections of Adriatic calcareous species, with detailed morphological descriptions and DNA sequences are very welcome. Therefore, the aim of this work was to try to find and reexamine old Adriatic species and to possibly expand the number of known species. To accomplish this, 18 specimens of calcareous sponges were collected in the Adriatic (Island of Vis, Island of Ciovo and Selce) by SCUBA in 2010. We combined the traditional morphological approach (spicule size, shape and skeleton organisation) with DNA analyses (partial 18S, 28S ribosomal DNA sequences, and ITS1-5.8S-ITS2). We found a total of nine species of six different genera (*Arthuria*, *Borojevia*, *Clathrina*, *Leucandra*, *Paraleucilla* and *Sycon*). Four species are new to science. Our results point that the calcarean sponge fauna of the Adriatic Sea is still underestimated and new surveys should be done.

**Porifera collected by Hercules ROV in the NA017 Expedition (Gorringe Bank)**

Cristobo Javier, Manjon Eugenia, Rios Pilar  
*Instituto Español de Oceanografía/ Universidad de Malaga*

This study presents species of Porifera, collected in Gorringe Bank, Atlantic Waters off the Iberian Peninsula.

Specimens were collected on board R/V Nautilus (64.23 meters) by two ROV's: Hercules and Argus. They are state-of-the-art deep-sea robotic vehicle systems capable of exploring depths up to 4000 m. This exploration Vessel, of the Ocean Exploration Trust, is one of America's two ships of exploration supported by the US National Oceanic and Atmospheric Administration's (NOAA's) operate and equipped with control centres connected via a high-bandwidth satellite link to the Inner Space Centre at the University of Rhode Island's Graduate School of Oceanography.

Nautilus NA017 expedition (8-19th October 2011) was primarily designed for the study of active hydrothermal sites and volcanic activity in zones of Gorringe Bank of ultra-fast spreading ridges.

During the cruise we collected 84 specimens of Phylum Porifera in the four dives between 132 and 2287 m. Thanks to the collection of rock samples, where many of the organisms were attached, we had the opportunity to study species of small size that couldn't be collected in other ways.

Directly, with the ROV Hercules, we also had the opportunity to obtain 5 bigger samples of sponges with particular shapes and structures as *Asconema setubalense*, *Hyalonema* sp. and *Chondrocladia* sp. They are interesting to study by the depth because their morphology or ecology represents an important role or they are very abundant in some habitats.

About the Class distribution of species of this Phylum we had collected 3 samples belonging to the Class Hexactinellida and 81 of the Class Demospongiae. Within this class, orders better represented are Poecilosclerida, Astrophorida, Halichondrida, Lithistida and Verongida.

**Porifera collected in the Weddell Sea (Antarctica) on board R/V Polarstern by the Ecoquim Project**

Cristobo Javier, Rios Pilar, Taboada Sergi, Ballesteros Manuel, Ballesteros Laura, Avila Conxita  
*Instituto Español de Oceanografía/ Universidad de Barcelona/ Universidad de Oviedo*

The aim of the ECOQUIM projects was to obtain bioactive natural products from Antarctic benthic invertebrates that may provide information on the chemical

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ecology of the involved species, as well as may be useful because of their pharmacological potential. Based on our previous studies on the chemical ecology of Antarctic invertebrates, species of the following benthic invertebrate groups were selected: sponges, cnidarians, molluscs, nemerteans, polychaetes, bryozoans, echinoderms and tunicates. The samples of Porifera (963 specimens and about 55 species) were collected in the Weddell Sea (Cruise ANT-XXI/2.11 7 11-2003 / 01-2004) on board R/V Polarstern (Germany) by using different gears: Agassiz trawl, Rauscher dredge, Epibenthic sledge and Box Corer in depths between 82 and 1866 m.

One of the most obvious characteristics of the Antarctic benthos in the Weddell Sea is the exorbitant occurrence of Hexactinellid sponges all around the continent. Large glass sponges provide shelter, support, nursery ground and despite their low content of organic matter, they also provide food for a very diverse associated fauna comprising many phyla. Calcarea are rare in Antarctic and Demospongiae are well represented with more than 360 species.

In this expedition, the number of specimens by classes shows a result of 603 Demospongiae (84 % of Porifera samples), 107 Hexactinellida (15 %) and 4 Calcarea (1 %) collected in 37 stations.

Orders of Demospongiae best represented are Poecilosclerida (310 specimens, 47.91%), Spirophorida (122, 18.8%), Astrophorida, Hadromerida, , Halichondrida, Haplosclerida.

Among the Genera of this class the most abundant were Iophon, Tetilla, Rossella, Isodictya, Cinachya, Polymastia, Tedania, Lissodendoryx, Mycale, Stylocordyla, Myxilla, Clathria, Anoxycalyx, Latrunculia, Homaxinella, Inflatella.

In this work we will present the results obtained from the sponge communities found at these sampling stations.

### **Unexpected high abundance of carnivorous sponges in a deep canyon of the South West Atlantic Ocean**

Cristobo Javier, Muñoz Araceli, Gonzalez Daniel, Ríos Pilar  
*Instituto Español de Oceanografía/ TRAGSA-SGP*

During twelve research expeditions in 2007-2010, sponges other marine benthic invertebrate and fishes were documented and sampled between 44°30'S and 48°S in the framework of the Vulnerable Marine Ecosystems (VME) research of the High Seas of the South West Atlantic (ATLANTIS project) in the Patagonian Continental Margin between (depths between 120 and 1600 m).

The continental slope in the study area is cut by multiple submarine canyons and channels crossing the upper continental slope in a W–E direction organised in two submarine canyon systems, the Ameghino and the Patagonia (or Almirante Brown). All canyons and branches start between 610 and 740 m water depths, at the limit between the upper and middle slopes. The canyon targeted by this study belongs to the Patagonia submarine canyon system. This canyon starts at the

upper continental slope. In the interfluves between the canyons, erosive scours and sand waves have been identified demonstrating the role of bottom currents in the shaping of the slope.

The research zone is located around canyon #3, this canyon is composed by two main branches that join immediately down slope. These two branches have been both considered as main canyons and numbered as #3A and #3B.

The sample point DR5\_1208 is located exactly in the intersection between main canyon #3A and its branch #3AN.

Different gears were used to sample benthic fauna in the study area: a Lofoten bottom trawl fishing gear was used over soft bottoms and bathyal plains during fishing operations, whereas a rock dredge served to prospect particular bottom environments whose structures and composition had previously been geomorphologically characterized and identified.

In the sample point we have collected 540 specimens of carnivorous sponges. We discuss the observed distribution patterns by analysis of surrounding samples. The dense accumulations of carnivorous sponges occur in soft or semi-consolidated bottoms at 1300 m.

Sponge fauna composition in this bathyal habitat shows that carnivorous Family Cladorhizidae here is represented by 4 genus: Asbestopluma Topsent, 1901, Chondrocladia Thompson, 1873, Euchelipluma Topsent, 1909 and Cercicladia, Rios, Kelly and Vacelet 2011. Most of specimens (503) belongs to Euchelipluma, 8 Chondrocladia, 23 Asbestopluma and 6 Cercicladia.

### **Glass sponges reefs of British Columbia influence bottom water carbon and nitrogen by their record filtration capacity**

Amanda Kahn, Gitai Yahel, Verena Tunnicliffe, Sally Leys  
*University of Alberta/ Ruppin Academic Center/ University of Victoria*

Glass sponges form unique reef habitats, similar to coral reefs, in the Hecate Strait and the Strait of Georgia (SOG), a marginal sea near Seattle and Vancouver. Sponge reefs are a local silica sink, but whether they alter water properties such as carbon and nitrogen on a similar scale is still unknown. Reef sponges at Fraser Ridge Reef in the SOG filter over 5.5 million m<sup>3</sup> of water per day, removing bacteria while adding ammonium to the water. We used SIP samplers to compare ambient water near reef sponges with water exhaled from oscula of *Aphrocallistes vastus*, the dominant reef-forming species in the SOG. The average sponge at Fraser Ridge reef processes roughly 9 m<sup>3</sup> of water per hour if only active filtration is considered, but it nearly doubles that volume by passively taking advantage of ambient currents (17 m<sup>3</sup> h<sup>-1</sup>). Given the high density of sponges in the reef and their efficient removal of bacteria, the sponge reef at Fraser Ridge has the highest grazing rates of any other suspension feeding community measured to date (213 to 401 m<sup>3</sup> m<sup>-2</sup> d<sup>-1</sup>). While the substantial amounts of carbon removed and ammonia produced by this 1.4-ha reef are small in comparison to the terrestrial inputs from the overlying Fraser

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River, populations of sponges in fjords and shelf waters could have a substantial impact, especially if numbers present pre-trawling are taken into account. Sponge reefs would also have a large impact on water column properties in oligotrophic environments, such as exist in Antarctica, and which are expected to have existed in the Tethys Sea where huge sponge reefs covered hundreds of square kilometers of seafloor. Why sponge reefs exist on the Pacific coast of North America has much to do with particular water column characteristics and currents for growth required by their high processing rates; inversely, dense glass sponge communities are a carbon sink and nitrogen source for the water column locally and even regionally.

### **Victorian Marine Science Consortium**

Michael Keough, Liz McGrath

*The University of Melbourne/ Victorian Marine Science Consortium.*

VMSC is a consortium of five Victorian tertiary institutions, together with CSIRO, EPA Victoria, and the State Government's Fisheries Management & Science, of Fisheries Victoria. We operate excellent marine teaching and research laboratories at the historic fishing village Queenscliff, on the entrance to Port Phillip Bay, a large embayment on the southeast coast of Australia.

The location allows visitors easy access to the diverse habitat range of Port Phillip Bay, Swan Bay and Bass Strait ? including high-energy ocean beaches of Bass Strait; low-energy sheltered beaches of Port Phillip Bay; rocky intertidal platforms and subtidal reefs at Pt. Lonsdale and Barwon Heads; estuaries at the Barwon River; mudflats of Swan Bay and Barwon Heads; seagrass meadows in and around Swan Bay and Port Phillip Bay; mangroves at Barwon Heads; saltmarshes around Swan Bay and Barwon Heads; bay and shelf waters of Port Phillip Bay, Corio Bay, Swan Bay and Hobson's Bay; Marine Reserves at Pt Lonsdale, Swan Bay, Barwon Heads Bluff, Mud Islands and Pope's Eye. These habitats form a unique combination for marine research and teaching.

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- ? Office space available to visiting researchers and postgraduate students providing access to computers, printing, fax and photocopier services
- ? Dedicated ecotoxicology laboratory offering bench space, compressed air and seawater taps servicing bench-top aquaria
- ? Controlled light and temperature room supplied with seawater and compressed air
- ? Large preparation room adjacent to the ecotoxicology lab
- ? Aquarium equipped with dissecting table, single and double-tiered aquaria stands with seawater and compressed air supplied to each stand



? A high quality flow-through seawater system supplies all indoor, outdoor and benchtop aquaria

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### **Taxonomy and diversity of *Calcarea* (Porifera, *Calcarea*) from Western Australia**

Michelle Klautau, Pedro Leocorny, Bárbara Gomes, Eduardo Mendes, Tathyana Lamim, Aline Alencar, Jane Fromont Hans Rapp  
*Universidade Federal do Rio de Janeiro/ Universidade Federal do Rio de Janeiro/ Universidade Federal do Rio de Janeiro/ Western Australian Museum/ University of Bergen*

In several regions around the world, the knowledge on calcareous sponges diversity is still incipient. Australia has a large diversity of calcareous sponges, however, this knowledge is concentrated mainly in the south, east and north regions. The goal of the present work was to investigate the diversity of *Calcarea* in Western Australia. Specimens were collected by the Western Australian Museum in two surveys on RV Southern Surveyour in 2005 and 2007 using two epibenthic samplers with 25-mm stretched-mesh net cod ends and a demersal fish trawl, in 18 different localities at various depths along Australia's western continental margin, following the coastline from Bald Island to Ashmore Reef. All specimens are deposited in the Western Australian Museum. Spicules and sections preparations were made following standard procedures. Sixty eight specimens were analysed and 27 species were found in 11 genera: *Asclatis*, *Ascoleucetta*, *Clathrina*, *Dendya*, *Heteropia*, *Leucascus*, *Leucetta*, *Pericharax*, *Sycon*, *Synute* and *Teichonopsis*. The genus *Leucetta* was the most specious, with 12 species, and *Ascoleucetta ventricosa* was the most abundant species, with 16 specimens. *Ascoleucetta ventricosa* exhibited the widest distribution (nine localities). Eight species of *Leucetta*, one of *Clathrina* and one of *Teichonopsis* are new for science. This is the first occurrence of the genus *Clathrina* in Western Australia. Our results suggest that the diversity of *Calcarea* in Australia is even larger than expected. Also, although the western region shares species with the eastern, maybe some species are endemic of the former.

**Oogenesis and embryogenesis of a cryptogenic species of calcareous sponge (Calcaronea, Heteropiidae) in Southwestern Atlantic**

Michelle Klautau, Emilio Lanna

*Federal University of Rio de Janeiro/ Federal University of Bahia*

Reproduction in *Calcarea* seems to be a very conservative feature that diverged early in the separation of the subclasses *Calcinea* and *Calcaronea*. While *Calcinea* has the calciblastula larva, *Calcaronea* has the amphiblastula, a larva that is formed after a very curious process, the inversion of the embryo. Although these larvae have been shown to be very characteristic of each class, a better understanding of their formation via oogenesis and embryogenesis is still needed. *Sycettusa hastifera* is a calcaronean species considered to be cryptogenic in the Southwestern Atlantic. Therefore, knowledge on its development would help not only to improve our comprehension of *Calcaronea*'s reproduction, but would also help to understand the capacity of colonization of this putative exotic species. In the present work, we performed histological and electron microscopy analyses of individuals sampled monthly at Arraial do Cabo (Rio de Janeiro, Brazil) during eighteen months. To analyse the evolutionary pathways of some developmental aspects of *Calcaronea*, we performed phylogenetic character mapping (PMC) based on previously published phylogenies. Our results showed that the oogenesis of *S. hastifera* is long and nutrients are acquired by association with nurse cells and endocytosis of bacteria, yielding to a heterogeneous and complex yolk inclusion. Cleavages, inversion and amphiblastula larvae are similar to what has been observed in other calcaroneans, showing that reproduction aspects are conserved in *Calcaronea*. However, few differences were observed, such as the lack of amoeboid cells in the cavity and the relatively long swimming period of the amphiblastula. Our results indicate that: the vitellogenic mechanism characterized as mixed (a primitive characteristic of *Calcaronea*) is strongly influenced by autotrophy; embryo/larva cellular lineages seem to be determined during cleavages; and inversion of the amphiblastula larva directly into the choanocyte chamber is a primitive characteristic of *Calcaronea*. However, the absence of amoeboid cells in the larval cavity could be considered an evolutionary regression. Even without amoeboid cells or other structures to nourish the larvae, the long swimming period suggests that vitellogenesis is sufficient for the maintenance of the embryo and larvae until metamorphosis. This characteristic may influence this cryptogenic species to spread its populations.

**Bioinformatics analysis of the viral community within the sponge holobiont**

Patrick Laffy, Emmanuelle Botté, Karen Weynberg, Elisha Wood-Charlson,  
Thomas Rattei, Nicole Webster  
*Australian Institute of Marine Sciences/ University of Vienna*

Viruses are widely accepted to be the most abundant biological entities in the marine environment, with one litre of seawater estimated to contain 100 billion viruses. Many viruses form complex relationships with microbial organisms, influencing growth, abundance and nutrient cycling. Marine viruses are also found infecting eukaryotic hosts, including protists, algae, coral and sponges. The relationship between marine sponges and their bacterial symbionts is well documented, however very little is known about the role of viruses within the sponge holobiont. Our overall aim is to investigate the role of viruses within the sponge holobiont using metagenomic techniques and next generation sequencing technology. In order to do this, sequence analysis workflows need to be developed to adequately identify viral sequence composition. Illumina sequencing of the virus fractions extracted from two sponge spp. *Rhopaloides odorabile* and *Amphimedon queenslandica* is currently underway. Several different computational analyses will be performed in order to determine the best way of dealing with viral metagenomics data and an appropriate analysis pathway will be developed. Template biases, sequencing platform differences and reference database limitations are all factors that need to be considered when interpreting viral metagenome data. By optimising the analysis pathway for viral metagenomics analysis in sponges, we will be able to investigate the role that viruses play in many sponge processes, including stress responses and symbiont-host interactions.

**First record of *Phycopsis* and *Ciocalapata* (Demospongiae, Halichondrida) for Brazil**

Anaíra Lage, Mariana de S. Carvalho, Carla Menegola  
*Universidade Federal da Bahia/ Museu Nacional, UFRJ*

Halichondrida has nearly 690 described species allocated into six families with a wide geographical distribution and species found in all marine habitats ranging from the intertidal zone to the deeper regions. From that, 26 species are recorded for the Brazilian coast and this number is still lower for Bahia coast, with just eight species described. In this work the genera *Phycopsis* and *Ciocalapata* are recorded for the first time to shallow waters of the Brazilian coast with two new species described. The specimens were collected from Camamu Bay and surrounding areas (13° 02'S to 14°12'S and 38°54'W to 39°09'W) located at the southern coast of Bahia, Brazil. All specimens were found on rocky substrate by SCUBA diving from 14 to 23 meters depth and deposited in the Museu de Zoologia at Universidade Federal da Bahia (Porifera collection, UFBA-POR).

This is the first record of *Phycopsis* in the South Atlantic Ocean. *Phycopsis* sp. nov. is bushy-shape or cushion-shape and branches ending in rough ascending conules. The surface is microhispid and consistency is soft and compressible. The choanosomal skeleton is differentiated into axial and extra-axial regions and specialized ectosomal skeleton is absent. *Phycopsis* sp. nov. is the only species of the genus that presents three categories of spicules, two of styles (250-420 / 20-30 $\mu$ m and 205-350 / 7.5-12.5 $\mu$ m) and one of oxeas (320-600 / 20-30 $\mu$ m). *Ciocalapata* has its geographical and bathymetric distributions extended from the coast of Argentina to the northeastern coast of Brazil (state of Bahia) and from deep (1097 m) to shallow (14-20 m) waters. *Ciocalapata* sp. nov. has an irregular, massive or globose shape. The surface is irregular and microhispid, with depressions formed by a porous mesh and grooves near the opening of oscules. The consistency is firm and incompressible. The ectosomal skeleton is detachable and the choanosomal skeleton is confused with no distinction of bundles or reticules but exhibiting discrete beams of spicules in the subectosomal layer. *Ciocalapata* sp. nov. displays minor oxeas (I: 500-1060 / 20-50 $\mu$ m and II: 146.3-417.6 / 5.3-10.6 $\mu$ m) and styles (205.2-913.1 / 20.5-41 $\mu$ m) when compared to its congeneric *C. amorphosa* (Ridley & Dendy, 1886).

### **Unravelling the structure of the marine sponge microbiome across biogeographical gradients**

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Geographical isolation plays a crucial role in driving microbial evolution and community structure in nature. In this context, the study of microbial symbionts in animal hosts of restricted mobility is of particular interest given their contribution to host fitness and survival. Here, we inspect the extent to which the shape of the sponge-associated microbiome is driven by the host organism and its biogeographical background. To this end, specimens of the sponge genera *Ircinia*, *Sarcotragus* (Dictyoceratida, Irciniidae) and *Spongia* (Dictyoceratida, Spongiidae) were sampled at distinct biogeographical settings in the North Atlantic: the Algarve coast, the Madeira Island, and the Azores archipelago. Bacterial community profiling in marine sponges was performed by 454 pyrosequencing of 16S rRNA genes amplified from metagenomic DNA samples. Analysis of 133959 chimera-free sequences was performed using the QIIME pipeline. *Ircinia* spp. possessed a more variable microbiota than *Sarcotragus* spp. Surprisingly, bacterial communities from the former - and phylogenetically closer - species were less similar to one another than *Spongia* spp. and *Sarcotragus* spp. communities. The predominant taxonomic groups in *Sarcotragus* and *Spongia* were Acidobacteria and Actinobacteria (c. 20% relative

abundance), followed by Proteobacteria, Poribacteria, PAUC34f, Bacteroidetes and Chloroflexi (c. 10% relative abundance each group). The aforementioned abundances remained similar in these sponges regardless of the sampling locality. Conversely, phylum-level abundances shifted across localities in *Ircinia* spp. In these hosts, the Chloroflexi displayed dominance at the Madeira site (31%) but was found to be a minority taxon at the Algarve (7%) and Azores sites (3%), where Proteobacteria, Acidobacteria and Actinobacteria were dominant groups at varying degrees. *Ircinia* spp., the most variable host regarding symbiont community composition, maintained a common pool of symbionts (93 shared bacterial phylotypes in 312 detected) across all sample sites examined. Overall, our results suggest that the sponge host plays a pivotal role in shaping the structure of its associated microbiome across biogeographical gradients.

**Phylogenetic position and the time of origin of freshwater sponges: a mitochondrial DNA perspective**

Dennis Lavrov, Katrina Lutap  
*Iowa State University*

Although all animal phyla originated in the sea, only a few of them colonized fresh water. The transition to limnic ecosystems required multiple adaptations to cope with highly variable temperatures, salinities, oxygen concentrations, and other parameters that make freshwater environments hostile to animal life. Freshwater sponges are globally distributed and common members of limnic biotas. They are currently classified as six extant families in the suborder Spongillina within the class Demospongiae. However their monophyly, phylogenetic relationship to marine species, and the time of their origin remain controversial. The earliest freshwater sponge spicules are found from two disjoint time periods: from Permo-Carboniferous deposits of Europe and from Jurassic deposits of Europe and North America. It is unclear whether the observed gap in the fossil record is a paleontological artefact or a reflection of two independent transitions to fresh water. Similarly, although freshwater sponges have been traditionally grouped with marine haplosclerids, several recent molecular phylogenetic studies reject this relationship. Here we use complete mitochondrial genome sequences from key representatives of demosponges to explore the phylogenetic position of freshwater sponges and to compare molecular and paleontological estimates for the time of their origin.

**Taxonomy of *Crambe* s.l. (Crambeidae, Myxillina, Poecilosclerida) from south-western Australia, a morphological and molecular approach, with the description of four new species**

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*Crambe* Vosmaer, 1880 is currently defined by the presence of two categories of subtylostyles in combination with anchorate isochelae and eventually other microscleres as asterose desmas, sigmoid chelae or smooth/acanthose microrhabds. *Crambe clathrata* (Carter, 1883), type species of *Monanchora* (= *Crambe*), and *Crambe selachia* (Hooper, 1996), originally described as a *Clathria* (*Isociella*) (Suborder Microcionina) and transferred in this work to *Crambe*, were the only crambeid species known from Australia up to the present. The latter species and four new species of the genus from south-western Australia (SWA) are (re)described in this work. Specimens are deposited in the Porifera collections of The Natural History Museum (London, United Kingdom), Queensland Museum (South Brisbane) and Western Australian Museum (Perth), Australia. *Crambe clathrata* (from Fremantle) can be recognized by the presence of a single category of polydentate anchorate isochelae longer than 30 µm in average, and with long claws covering up to 34% of the shaft length, and microrhabds; sigmoid chelae are absent. *Crambe selachia* (from Shark Bay) is massive, with strongly spined ectosomal subtylostyles, polydentate anchorate isochelae with claws up to 25% of the shaft length, and acanthomicrorhabds; sigmoid chelae absent. *Crambe* sp. nov. 1 (from Escape Island) has scopiform branches, mucronate ectosomal subtylostyles, polydentate anchorate isochelae with short claws, up to 20% of the shaft length, and sigmoid chelae; microrhabds absent. *Crambe* sp. nov. 2 (from Dry Lump) is massive, with a honeycombed and hispid surface, spined and mucronate ectosomal subtylostyles, predominantly tridentate anchorate isochelae with short claws, up to 25% of the shaft length, acanthomicrorhabds are rare; sigmoid chelae absent. *Crambe* sp. nov. 3 (from Ningaloo) is flabellate, with strongly spined ectosomal subtylostyles and with polydentate anchorate isochelae with claws measuring up to 33% of the shaft length; sigmoid chelae and microrhabds absent. *Crambe* sp. nov. 4 (from Ningaloo) is arborescent and has polydentate anchorate isochelae with claws measuring up to 31% of the shaft length; sigmoid chelae and microrhabds are absent. Desmas were absent from all species. New sequences of 16S and 28S rDNA are being generated from some of the latter species for the Sponge Barcoding Project.

**Biodiversity of marine sponges from Ilha Grande, Rio de Janeiro state,  
southeastern Brazil**

Eduardo Leal Esteves, Gisele Lôbo-Hajdu, Humberto Fortunato, Anderson

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Ilha Grande Bay is a hotspot of marine biodiversity with ca. 350 km<sup>2</sup> and up to 40 m depth in the southeastern coast of Brazil. It comprehends 11 conservation units and its natural resources are used for many purposes, as for the production of thermal nuclear power and harbour activities. In the 2000 years there was a collaborative effort for a comprehensive characterization of the marine biodiversity in this area which led to the discovery of a high diversity of marine organisms, many of them were unknown to science. Nevertheless, there were only 36 marine sponge species registered from Ilha Grande Bay up to present. This is the purpose of the present study to make a biodiversity inventory of marine sponges from Ilha Grande, southern Rio de Janeiro state. Specimens were collected by scuba diving or snorkeling to a depth of about 12 metres. Collections were made in may and june, 2013 in six localities around Ilha Grande. Each collected specimen was photographed in situ, stored in plastic bags with corresponding field numbers and fixed in ethanol 92,8°gl. Specimens were deposited at the Porifera Collection of Rio de Janeiro State University (UERJPOR). Species were identified by the usual procedures described in the scientific literature. There were approximately 50 species at Ilha Grande belonging to Demospongiae and Calcarea. Our preliminary results suggests Poecilosclerida (18 spp.) as the most diverse group at Ilha Grande, followed by Hadromerida (4 spp. each), Calcarea, Dendroceratida and Haplosclerida (3 spp. each), Dictyoceratida, Halichondrida and Verongida (2 spp. each) and Chondrosida, Spirophorida and "Lithistida" (1 sp. each). 10 species remain undetermined. Seven genera represents new records to Ilha Grande: Amphimedon, Crambe, Guitarra, Hemimycale, Petromica, Polymastia, and Tethya. These numbers are certainly underestimated, as collections were restricted to a few localities at the eastern section of the island. Nevertheless, it can be assumed that the sponge fauna from Ilha Grande is one of the richest in the southeastern coast of Brazil and shows a remarkable "patchy" distribution. Hence, management plans for the marine conservation at Ilha Grande should consider wider areas, in order to an effective conservation of its natural resorces.

**Fungal community associated with marine sponges *Holoxea* sp. and *Xestospongia testudinaria* revealed by phylogenetic analysis at total DNA and RNA levels**

Zhiyong Li

*Shanghai Jiao Tong University*

Compared with bacteria associated with sponges, the diversity of fungi associated with sponges remains limited. In particular, no RNA-based investigation on the diversity of sponge-associated fungi has been carried out. In this study, the phylogenetic diversity of fungi associated with sponges *Holoxea* sp. and *Xestospongia testudinaria* from the South China Sea was explored based on the DNA and cDNA libraries. As a result, a total of 18 operative taxonomic units (OTUs) were obtained from four DNA and cDNA libraries of two species of sponges, representing 16 genera of Ascomycota, 1 genera of Basidiomycota, 1 genera of Blastocladiomycota. Phylogenetic analysis showed that some of these fungal genera were close related to the fungi isolated from other marine habitats, but almost half of the fungal genera were identified for the first time in marine sponges. The taxon *Aspergillus terreus* and *Alternaria alternata* were detected in all libraries. In addition, *Preussia* sp., *Septoria* sp., *Dactylella* sp., *Graphium* sp., *Chaetomium* sp., and *Catenomyces* sp. were detected in the cDNA library of *Holoxea* sp., and *Aureobasidium* sp., *Cladosporium* sp. and *Preussia* sp. were observed in the cDNA library of *X. testudinaria*. These results indicated that some of the fungi associated with these two sponges are transcriptionally active. This study represents the first report on the phylogenetic diversity at the total RNA level, extending our knowledge of sponge-associated fungi greatly.

**The 'lithistid' sponge fauna of Singapore**

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Two new species of 'lithistid' sponges, *Aciculites* sp. nov. and *Theonella* sp. nov., were dredged recently from shallow subtidal waters in the Singapore Strait. *Aciculites* sp. nov. is distinct from the congeners in having the largest strongyles recorded from this group (over 5000  $\mu\text{m}$  in length). The second species *Theonella* sp. nov. has an unusual encrusting habit of less than 5 mm in thickness and differs significantly in spicule sizes with congeners. Other 'lithistid' sponges recorded from Singapore waters are compared and presented in this study as well, and their distribution and species diversity discussed in relation to other similar species found elsewhere in the region.



**Prospects for a New “Global Invertebrate Genomics Alliance” (GIGA)**

Jose Lopez, GIGA Consortium of Scientists  
*Nova Southeastern University Ocean Center/ Multiple*

An organizational workshop intended to establish a new cooperative consortium named the “Global Invertebrate Genomics Alliance (GIGA)” was convened at Nova Southeastern University’s (NSU) new Center of Excellence in Coral Reef Ecosystems Research in Dania Beach FL, on March 1-3, 2013. This event was timely for several reasons: i) Marine invertebrate taxa are underrepresented in current tallies of whole genome projects ([www.genomesonline.com](http://www.genomesonline.com)), comprising less than 30% of currently completed genomes. Yet the marine realm holds representatives of all but two animal phyla, and many of these are not found in terrestrial or limnic ecosystems. Furthermore, >10 of these marine phyla have no current or planned representative genome projects (e.g., Brachiopoda, Bryozoa, Loricifera, Sipuncula, Nemertea, Kinorhycha, Priapulida, Tardigrada, Entoprocta, Phoronida, Xenoturbellida, Hemichordata, Cyclophora); ii) Marine habitats in which many of these taxa reside, such as tropical coral reefs, are increasingly threatened by multiple anthropogenic factors (e.g., pollution, climate change, overfishing). Deep-sea habitats are less threatened, but fairly inaccessible. iii) “Next Generation”, high-throughput DNA sequencing methods continue to advance quickly and dramatically, enabling lower costs and more accessible data. Roughly 41 leading experts in marine invertebrate biology, genomics and systematics and from as far away as China, Europe and California included institutions such as the Smithsonian and Scripps Oceanographic, regional invertebrate experts, industry reps (Life Technologies, PacBio and BioNanoGenomics), NSU graduate and undergraduate students. In depth topics were discussed in breakout groups such as which specific invertebrate organisms to sequence (coral, mollusks, crustacea, sponges worms etc) and prioritize, optimal types of DNA or RNA sequencing platforms, standards for collections, sample preparation, and an invertebrate taxon’s place on the Tree of Life. Porifera and other basal taxa were highlighted. Also discussed were policies on data submittal and sharing for a GIGA consortium. The workshop was primarily sponsored by the American Genetics Association, and the proceedings have been submitted for publication in the AGA’s Journal of Heredity. The workshop website can be viewed at [www.nova.edu/ocean/giga](http://www.nova.edu/ocean/giga).

**What is the cost of filtration? Sponges use behavioural responses to take advantage of ambient currents**

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Sponges are suspension feeders that process up to 900x their body volume in water daily, and extract bacteria with up to 98% efficiency. The energy required

## **Ninth World Sponge Conference 2013**

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for filtration is thought to be less than 1% of metabolism, yet sponges have long been considered to take advantage of passive flow to reduce the cost of pumping. If filtration is inexpensive, why would passive flow be beneficial? Recent work on deep-sea glass sponges suggests pumping may be more costly, at nearly 30% of metabolism, and the expense is reduced by taking advantage of current induced flow. But the fine structure of sponge tissues suggests there is substantial resistance to movement of water through the fine canals and passages of the choanocyte filter. Glass sponges live in food-poor deep water habitats; they also have large canals and oscula which should reduce resistance to flow. In contrast demosponges generally live in food-rich shallower habitats and have up to 5X narrower canals and choanocyte chambers. Based on numerical calculations using dimensions from the literature and previously published flow models, we predict that passive flow does not occur in demosponges; instead their food-rich habitats provide enough energy to sustain maintenance and growth despite the high cost of pumping. To test this we studied excurrent filtration rates and oxygen consumption during ambient currents of 0-20cm/s in three temperate and two tropical demosponges using particle imaging velocimetry, profiling acoustic Doppler velocimeters, and oxygen optodes. We found that despite different excurrent velocities, oxygen drawdown was similar for all sponges. In all 5 species (temperate and tropical), increased ambient flow induced a behavioral response – contraction of the osculum - that increased excurrent velocity, but not the volume processed. There was no increase in oxygen consumption with increasing ambient currents; therefore no additional energy was expended to pump during increased ambient flow. Morphometric analysis of the aquiferous system will now be used to confirm by modelling whether increased ambient currents can overcome resistance to induce excurrent flow through these sponges.

### **Elevated nitrogen effects the sponge-microbe symbiosis**

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*1NAMRA and the Research Institute for the Environment & Livelihoods, Charles Darwin University/ Museum and Art Gallery of the Northern Territory/ Research Institute for the Environment & Livelihoods, Charles Darwin University/ Australian Institute of Marine Science*

Nitrogen is essential to all life and whilst it is often limiting in marine systems, levels in coastal areas have been rapidly increasing due to inputs of sewage and terrigenous sediments carrying fertilizers. Because sponges filter such large volumes of water (1000s of litres each day), they are likely to be particularly susceptible to increased exposure to nitrogen. Sponges host a wide diversity of microbes involved in nitrogen metabolism, yet little is known about the effects of nitrogen loading on these symbiotic partnerships. Manipulative experiments were conducted to address whether elevated nitrogen alters the innate microbial

community of a common intertidal sponge, *Cymbastela stipitata*. Sponges were exposed to 3 levels of total nitrogen: 300  $\mu\text{mol/l}$ , 500  $\mu\text{mol/l}$  and 1000  $\mu\text{mol/l}$  for a two-day period, mimicking a sewage discharge event. In addition, individuals from across the tidal flat were collected to establish the baseline bacterial community of *C. stipitata*. Sponges were not visibly affected by any treatment, yet microbial profiling revealed a distinct community shift in all sponges exposed to elevated nitrogen. In particular, individuals exposed to the highest nitrogen treatment (1000  $\mu\text{mol/l}$ ) had a markedly different community than those in the lower two treatments. While the composition of the bacterial community altered, chlorophyll *a* values were similar between sponges in all treatments, suggesting that the photosynthetic symbionts were relatively unaffected by the nitrogen pulse. Future research aims to identify the microbes involved in the sponge nitrogen cycle using a novel microarray and will assess the implications of any changes in symbiotic structure and function for sponge health.

**Deep-shelf sponge communities of the Alboran Island (Western Mediterranean): new, rare, and migrating species**

Manuel Maldonado, Cèlia Sitjà, María López-Acosta  
*Center for Advanced Studies of Blanes (CEAB-CSIC)*

In the frame of the LIFE+ INDEMARES EC Project, we investigated the deep-shelf (70-200m) of sponge communities of the Alboran Island (Alboran Sea, Western Mediterranean,) using ROV monitoring along with dredging and trawling sampling devices. This tiny Mediterranean Island appears to function as a marine biogeographical ecotone, as its continental shelf and upper slope are bathed by a complex pattern of North-Atlantic waters coming into this western Mediterranean area through the Gibraltar Strait. In the deep shelf of the island, we found 4 major habitats: 1) deep beds of alive rodoliths abundantly covered by encrusting sponges; 2) moderately slanted, rocky bottoms, characterized by dense sponge fields of diverse, small lollipop-shaped and digit-like species along with *Poecillastra-Phakellia* mixed populations; 3) overhangs and vertical walls typically occupied by submassive and massive astrophorids intermingled with precious coral and gorgonians; and 4) sand and gravel bottoms showing low density of sponges, but at high taxonomic diversity. Out of the samplings and videos, we identified a total of 329 individuals belonging to 87 species, three of which are new to science (*Axinella alborani* sp. nov., *Axinella spatula* sp. nov., and *Endectyon (Endectyon) acanthoclavus* sp. nov.). Three other species (*Jaspis eudermis*, *Hemiasterella elongata* and *Axinella vellerea*), originally known from the North Atlantic, are reported for the first time in the Mediterranean, what was surprising given the dense populations that we discovered for some of these species.

Whether some of these are recent Mediterranean invasions remains unclear. We also collected the second known individual of the rare, ill-known Mediterranean species *Rhadobaris (not Cerbaris) implicatus*, providing material for a neotype,

since the original description was merely based on a spicule preparation. These faunal findings help to support the growing view that the deep shelf and upper slope of the Alboran Island harbour singular Mediterranean communities that need to be protected from trawling activities, receiving a conservation status similar to the one already enforced for the bottoms of the shallow shelf.

**The spicule-collecting protozoan who wanted to be a carnivorous sponge:  
*Spiculosiphon oceana* sp. nov. (Foraminifera, Astrorhizida)**

Manuel Maldonado, María López-Acosta, Celia Sitjà, Ricardo Aguilar, Silvia García, Jean Vacelet

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The foraminifer *Spiculosiphon oceana* sp. nov. is a giant (>4 cm) agglutinated astrorhizid, which makes the second known species of this unusual genus and its first Mediterranean record. It has a peculiar stalked, capitate, monothalamous test. Bleach digestion and X-ray microanalysis indicated the test to be made exclusively of siliceous sponge spicules agglutinated in organic cement. The organism stands on a hollow, 4cm long, 0.5 cm thick stalk built with highly selected, long and thin spicule fragments, tightly cemented together in parallel to the main axis of the stalk. The proximal end of the stalk is closed and slightly expanded into a bulb-like structure, designed to penetrate between the sand grains and maintaining the test upright while avoiding a permanent attachment to the substratum. The distal stalk end becomes a hollow, globe-like structure that contains the main protoplasm. The globelike region is built with loosely agglutinated and irregularly-shaped spicules, allowing extrusion of the pseudopodia through the cavities between the spicules. The globelike structure also serves as an anchoring structure, from which long and thin, solid tracts protrude radially to make a spherical crown that attains about 4 mm in total diameter. These radiating tracts are built with highly selected aciculate spicule fragments held together with a translucent organic cement that recalls the poriferan nodal spongin. The spicule tracts provide skeletal support for the extension of a crown of pseudopodia into the water column. This arrangement is thought to enhance the chances of the pseudopodia to contact demersal planktonic prey. In summary, *Spiculosiphon* collects sponge spicules from the bottom with high selectivity and arranges them to recreate a body morphology that strongly converges to that of some carnivorous sponges. This architectural design allows these predatory foraminifera to exploit a prey capturing strategy similar to that of the carnivorous sponges. This idea is also consistent with our finding of an additional, yet undetermined, *Spiculosiphon* species occurring in the same sublittoral Mediterranean cave where carnivorous sponges were first discovered.

**A second species of the rare genus *Thymosia* (Demospongiae,  
Chondrosida, Chondrillidae).**

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The order Chondrosida consists of 30 species contained in a single family (Chondrillidae) with four genera (Chondrosia: 10 spp; Chondrilla: 17 spp; Thymosiopsis: 2 spp; Thymosia: 1 spp). Despite being small, this taxonomic order displays the largest internal skeletal diversity in Demospongiae. The genera Chondrosia and Thymosiopsis lack any skeleton, either spicules or spongin fibers; Chondrilla has aster-like spicules, but no spongin; Thymosia elaborates a poorly-developed network of spongin fibers, but lacks spicules. The skeleton of nodulose fibers characterizing the only known species of Thymosia (*T. guernei*) is perceived as quite different from those found in the remaining "horny" sponges (orders Dendroceratida, Dictyoceratida, and Verongida), but the monotypy of Thymosia hinders comparative assessments. Here we are describing a second species of Thymosia, represented by a population discovered at the Chafarinas Islands (Southwestern Mediterranean). Sponges are up to 0.5 m across, thickly encrusting to submassive, with a lumpy, irregularly conulose surface. They occur in two colors, white and purplish red. Partial CO1 and 28S sequences are identical for the color varieties, suggesting that they represent conspecifics and are closely related to, but in 28S distinguishable from, *T. guernei*. Additional differences include a thicker growth habit, a denser fiber skeleton, and the ability to become colored by incorporating cyanobacteria. The mesohyl includes abundant detritus, but fibers are clean from inclusions. Fibers are nodulose, with laminated bark and a foamy pith with irregular voids in the spongin matrix. TEM confirmed extracellular bacterial abundance (i.e., HMA sponge). Choanocyte chambers are scarce and small (<40 µm across). Choanocytes are irregularly shaped, with lateral-basal pseudopodia, and a cilium with a patent radial vane-like glycoalyx. The mesohyl contains spherulous cells charged with few, large, electron-dense vesicles, as typically in other chondrillids. Amoebocytes, collencytes, vacuolar cells, microgranular cells and other cell types also occurred. As in *T. guernei*, granulose cells were absent. The exopinacoderm is absent at some surface zones, being the underlying dense collagenic mesohyl exposed (pseudocortex?). This new species is hypothesized to have derived from the North-Atlantic *T. guernei* after reproductive isolation triggered by fragmentation of a wider Atlantic-Mediterranean distribution in the past.

**Sponge spicules in marine sediments: a significant silicon sink**

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Siliceous sponges produce a skeleton of silica spicules. Upon sponge death, spicules are released to the sediment, but little is known about the fate of those free spicules, their spatial patterns of distribution, and their role in the silica cycle. In this study, we examined sediments obtained from piston and gravity cores collected from a variety of sedimentary marine environments, including continental shelves, slopes and abyssal basins from various seas and oceans (Antarctic, Western and Eastern Atlantic, Western Mediterranean, Western and Eastern Central Pacific, and Arctic). In each core, the sediment sequence extended from Recent Time to about Mid and Late Holocene (i.e., 4,000 to 10,000 years). Through light microscopy inspection and alkaline digestions of sediment subsamples, we estimated the amount of sponge biogenic silica (mg) relative to the total biogenic silica per gram of sediment at various burial depths within each core. We discovered that sponge silica mass may vary over an order of magnitude, with much variation depending on the particular deposition environment but little between burial depths within the core, what suggests no major changes in local sponge abundance during the Holocene. The relative importance (%) of sponge biogenic silica versus total biogenic silica substantially increased with burial depth within the cores, indicating higher preservation of spicules relative to diatoms. Scanning electron microscopy confirmed spicules buried longer than 4,000 years to have only very incipient dissolution scars. These findings revealed that preservation of sponge-produced silica in continental-margin sediments is notable, being responsible for much silicon export from the marine biological cycle to the geological cycle. To date, most silicon burial in the oceans has been attributed to planktonic diatoms and directly connected to levels of planktonic primary production in the overlying photic waters. Our findings strongly disrupt this traditional view and introduce the role of benthic sponges as significant exporters of silicon, through burial of biogenic silica that has been produced following processes decoupled from primary production. These findings have important implications to improve the current understanding of processes controlling silicon turnovers in several ocean compartments used to model the global silicon cycle.

**Bioaccumulation and speciation of europium in the Mediterranean sponge  
*Aplysina cavernicola***

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The fate of natural and anthropogenic radionuclides in the environment remains a major concern in our modern societies[1]. Within the environmental compartments, the hydrosphere is ubiquitous and transports organic compounds or inorganic elements over long distances. Among the radionuclides of concern, actinides are the heaviest elements involved in nuclear activities. Surprisingly, very little is known about the speciation of actinides in seawater[2] and their accumulation in marine organisms. Improving knowledge on the interaction between actinides and marine organisms is therefore essential to better understand the transfer mechanisms from the hydrosphere to the marine biosphere and to evaluate their global impact on the environment. Marine sponges have already been identified as hyper-accumulators of several trace elements and are proposed in this case as model biomonitor organisms[3].

To address this question, we focused the present study on americium (III), an actinide with a relatively simple RedOx chemistry compared to plutonium or uranium. Because of the relatively high specific activity of americium, the chemical analogue europium (a stable lanthanide with chemical properties very close to that of americium) was used. As a first step, the accumulation and speciation of europium (III) was investigated in the Mediterranean sponge *Aplysina cavernicola*. This sponge is commonly found in the caves off the coasts of the Northwestern Mediterranean and previous laboratory studies have shown that it has a substantial capacity for metal accumulation[3]. The amount of europium integrated in sponges exposed to the radiotracer <sup>152</sup>Eu was measured using a high-purity Ge gamma spectrometer system. Sponges were analysed by X-ray Absorption Spectroscopy (XAS) at the europium LII edge, which is one of the best techniques to determine the speciation of europium within biological tissues. Then, Scanning Transmission X-ray Microscopy (STXM) was used to locate europium in sponge.

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**Sponge and cyanobacteria: benefits and risks of a long established symbiosis**

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There is an increasing awareness of beneficial interactions between microbes and animals. For example, gut microbes aid in digestion of complex organic matter (e.g. termites and ruminants) and photosynthetic symbionts harness the power of the sun to fuel growth of corals and sponges. Sponge species hosting cyanobacterial symbionts represent up to 50% of the sponge community, but can amount to nearly 90% of the communities in some tropical reef areas. Environmental factors, such as temperature and nutrients, have been shown to affect the host and its microbiome, and ultimately sponge ecology and function with broader implications for ecosystem health. For several decades, the changing nutrient status in reef systems, e.g. eutrophication through anthropogenic input, has been linked to an increase in the heterotrophic sponge population, at the expense of phototrophic types, with impact on the nutrient balance of the ecosystem as a whole. We are just beginning to explore the role the sponge microbiome plays in the fitness of holobiont in relation to environmental factors. My research seeks to dissect out the biochemical and molecular basis of the ancient symbiosis between marine sponges and cyanobacteria. I have undertaken targeted metagenomic studies of cyanobacterial symbionts from two sponge species from the class Demospongiae dwelling in contrasting habitats. One species is an encrusting photosynthetic sponge widely found in shallow habitats from coastal temperate zones around Australia (*Chondrilla australiensis*), with closely related species worldwide, the sponge specimen analysed was sampled from the temperate zone off Sydney. The other sponge species is a foliose dominant and widespread throughout the Indo-Pacific and Great Barrier Reef, and was sampled from tropical waters off Townsville (*Carteriospongia foliascens*). I have isolated the photosynthetic prokaryote symbiont of those sponges through flow cytometry sorting, then amplified the whole genetic material of those symbionts for subsequent next generation paired-end Illumina sequencing. The genomic assembly of the symbiont provides insights into how the symbiosis is maintained and provides a better understanding of the genetic mechanisms underlying the biochemical fluxes between the host and symbiont.



### **Kimberley Marine Research Station**

Erin McGinty, James Brown  
*Kimberley Marine Research Station*

The aim of KMRS is to provide critical support services to research scientists towards bridging the existing knowledge gaps on this remarkable yet largely under-studied marine region. Located at Cygnet Bay Pearl Farm on the tip of the Dampier Peninsula 200km north of Broome, KMRS operates year-round and is home to a complete team of personnel representing over 65 years of local marine experience and expertise. On-site research support includes accommodation and catering, vessel charter, transport and logistical assistance, a new custom-built aquaculture facility with wet and dry marine laboratories, qualified skippers and resident scientific staff experienced in assisting research teams in the field and with at-distance monitoring capabilities ([www.kmrs.com.au](http://www.kmrs.com.au)). KMRS is the first and only fully operational marine research facility along the 13,500km contours of the Kimberley coastline.

The Kimberley marine environment is the planet's last great tropical marine wilderness and, alongside the polar Arctic and Antarctic, falls within the remaining 3.7% of the world's oceans least impacted by humans. The marine environment of the region supports high biodiversity and highly dynamic ecosystems strongly influenced by extreme physical processes, including large-amplitude tides of 10 m. The marine environment in the Kimberley's is vast, remote and, to modern science, remains relatively unstudied with largely undescribed marine fauna and flora. Traditionally, coastal and ocean-based operations in the Kimberley have been defined and restricted by isolation, lack of services and infrastructure and natural extremes in climate and tides. Since its inception three years ago, the KMRS team has worked towards developing and diversifying a more accessible research profile for the Kimberley region, working hand-in-hand with the indigenous people of the area.

### **Propagating rubble-binding sponges for use in reef restoration**

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A common result of vessel groundings on coral reefs is the generation of coral rubble whose movement may result in reef erosion, damage to surviving organisms, and impaired recruitment and recovery of benthic organisms. Several species of branching sponges bind rubble in reef ecosystems, and show promise as stabilizing agents in restoration applications due to their low mortality, rapid growth rates, and ability to quickly form attachment points to various substrates. The potential use of sponges as a restoration tool would require a continually growing, sustainable source of fragments. In this study, we evaluated the feasibility of propagating four species of branching sponges collected from native populations in Biscayne National Park, FL. Fragments of *Aplysina fulva*, *Amphimedon compressa*, *Aplysina cauliformis*, and *Iotrochota birotulata*, were

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harvested and attached to propagation structures constructed from PVC rods and cement paver tiles. Fragment retention (persistence throughout the study), growth, and new branch count were recorded monthly for approximately one year. Donor sponges were monitored to assess recovery after harvesting, and intact sponges in the natural population were monitored as controls. The abundance of these species in the ambient reef community was also assessed. After 328 days, propagated fragments had high retention (92-100%) and produced substantial biomass relative to initial volume (105% to 424% across species). Donor sponges healed harvest wounds within one month and continued to grow by 280% to 645%. Our study demonstrates that propagating branching sponges is an effective, practical, and inexpensive method of obtaining sponge fragments for use in restoration, with little negative impact on donor populations.

### **Selective isolation of actinobacteria associated with four South Australian marine sponges by surface sterilization, drying, pre-treatments and media modification**

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Selective isolation of many actinobacteria was achieved by surface sterilization, overnight drying the sponges under laminar flow and pre-heating the dissociated sponge dilution series. The isolation was further improved by the media modification by including sea water, potassium nitrate, sponge extracts and antibiotic supplements. A total of 123 actinobacteria isolates were obtained from four South Australia Sponges and six different media. Nineteen of these isolates were selected to evaluate their antimicrobial potential, based on different colony morphology and spore formation. Phylogenetic analysis of the partial 16S rRNA sequences showed that these strains mainly belong to the genera of *Streptomyces* and *Micromonospora*. Three of these strains identified as potential new species of *Streptomyces*. The 19 isolates were grown by solid state fermentation on various substrates including orange peel, coffee waste, oat bran, sea grass, long grain rice and ISP-2 media. Crude extracts from ISP-2 media showed the best inhibitory activity against *Staphylococcus aureus* and *Micrococcus luteus* followed by oat bran and rice. Among the 19 isolates, 9 isolates showed activity against *Staphylococcus aureus*; 11 isolates showed antifungal activity against *Rhizoctonia solani* or *Fusarium*. Profiling of secondary metabolites in the crude fermentation extracts showed that two isolates possibly produce interesting new compounds, however not these isolates showing highest antimicrobial activity. TLC and Bioautogram also revealed similar results. The experiments are ongoing to elucidate the structures of potential new compounds.

## **An approach to zoogeography of shallow-water sponges from Western Atlantic**

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Zoogeographical studies on neritic sponges in the western Atlantic have been scarce; in great extent due to the persistent immaturity of their systematics and insufficient knowledge of the sponge fauna in various geographical areas, particularly in Brazil. The theme in the Western Atlantic (Massachusetts to Argentina) is still insufficiently addressed. The detailed analysis of pair-wise similarity using Simpson's index (1960) showed that: The vast territory of Brazil (provisional endemism of 37%) revealed, from south to north, the existence of a remarkable similarity (68%) between the sections Parana-Rio Grande do Sul (Pn-RGS) and Rio de Janeiro-São Paulo (RJ-SP). In turn, this high level of endemism separates them, as a whole, from the Tropical Northwest Atlantic. Because of their joint provisional endemism (16%), Pn-RGS + RJ-SP could form a northern part of the Warm Tempered South West Atlantic Province (Paulista Province as Hajdu et al., 2011), covering both the Southeastern Brazil and Rio Grande Marine Ecoregions. The second group, Pernambuco- Espírito Santo together with Trindade Island (Pe-ES + ITr; provisional endemism of 35%) would correspond to the Tropical West Atlantic Province, covering several eco-regions. The stretch Amapá-Pará (Am-PA) shows a slight intermediate position but more related to tropical Brazil. Uruguay-Argentina departs drastically. In the Northern Hemisphere, the continental and insular Caribbean unite into one area, and marginally do the same Florida, Northern Gulf of Mexico (NGM) and Bermuda, forming the Caribbean Province (66.6% endemism). The section Georgia + eastern North Carolina U.S. (provisional endemism of 26%) suggests the Warm Temperate Northwest Atlantic Province, and marginally excludes the NGM. The Virginia-Massachusetts stretch, (calculated raw endemism of 41%) separates as the called Virginian Province. All this is corroborated by the Sorensen's (1948) and Simpson (1960) similarity indices, which show an enormous cohesion between areas within the continental and insular Caribbean. Of this group gradually deviate the Northern Gulf of Mexico and Bermuda, and in a greater extent Georgia-North Carolina and Virginia-Massachusetts, consecutively. Pn-RGS and RJ-SP are grouped, but in a lesser extent than the areas of the Caribbean. The Brazilian tropical areas also appear associated but in a lesser degree than the Caribbean.

**Sponges from the North coast of Bahia, Brazil (Demospongiae, Calcarea, Homoscleromorpha)**

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The state of Bahia has a high diversity of coastal ecosystems with mangroves, coral reefs and seagrasses, with one of the richest Brazilian sponge fauna of shallow water exhibiting strong tropical affinity. Prior to this study, there were 140 species of Demospongiae and Calcarea registered for the state of Bahia. Studies with sponges are traditionally concentrated in central and southern regions. This work shows for the first time the scenario of species richness of the North Coast of the state, in order to generate data for biogeographic analysis of shallow water sponges of the western Atlantic. The sponges were collected on rocky substrate of Guarajuba (12°39'1"S /38°4'27"W) and Praia-do-Forte (12°33'50"S /37°59'36"W) beaches, between 0 and 5 m, and in soft bottoms in around two outfalls (12°44'12.6" - 12°53'34.1"S / 38°04'05.5" - 38°16'06.6"W) between 22 and 50 m, where predominates respectively, gravel and sand. Of the three classes detected in the North Coast of Bahia, Demospongiae constitutes 86% of the fauna. Poecilosclerida is the order with the highest number of families (11) and species (18). Verongida is the second most diverse order, with 10 species in a single family, Aplysinidae, abundant in shallow waters of the coast of Bahia. We detected four new species: *Ecionemia* sp., *Rhabdastrella* sp., *Geodia* sp. and *Oceanapia* sp., all provisionally endemic to the state of Bahia. The species *Geodia gibberosa*, *Geodia* sp. and *Yucatania sphaeroidocladus* are rare, but sponges typical of soft bottoms, as *Tribrachium schmidtii*, *Cinachyrella apion* and *Tetilla pentatriaena*, are abundant in the study area, adding dozens of samples deposited at the collection UFBA-POR. The class Calcarea was the second in species richness (16), with 11 of the order Leucosolenida and 5 of Clathrinida. Homoscleromorpha has its first record for the state of Bahia, represented only by *Oscarella* sp. The 121 taxa detected are distributed in three classes, 14 orders and 40 families, of which 35 have their first record for the coast of Bahia, one for Brazilian waters (*Smenospongia aurea*) and three for the Southwest Atlantic (*Spongionella* sp., *Stelletta* aff. *hispidus* and *Chondrilla caribensis* f. *hermatypica*)

**Five new species of Plakinidae (Porifera: Homoscleromorpha) from Brazil**

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Seven species of Plakinidae are currently known from Brazil, belonging to genera *Plakina* (*P. trilopha*), *Plakinastrella* (*P. microspiculifera*, *P. onkodes*), and *Plakortis* (*P. angulospiculatus*, *P. halichondrioides*, *P. insularis*, *P. microrhabdifera*). In this study we describe five new species belonging to *Plakinastrella* and *Plakortis*, increasing to 12 the number of plakinid species in Brazil.

Plakortis petrupaulensis sp. nov. is beige, and its skeleton shows few traces of reticulation; lacunae are absent; the spicules are diods and characteristic tuberculate microrhabds; spheres are common and triods are absent. Plakortis spinalis sp. nov. is distinguished by the spines in part of the diods and triods; microrhabds are tuberculate and smooth spheres are common. The sponge is encrusting, gray, cartilaginous, and has a reticulated ectosomal skeleton. Plakortis potiguar sp. nov. is encrusting, brown with gray tinges, cartilaginous; the skeleton is disorganized and scarce, composed of diods and triods thin, irregular and relatively large microrhabds. Plakortis bahiense sp. nov. is massive with rugose surface, dark brown; the tangential skeleton has a double reticulation; the spicules are diods, triods and microrhabds; spheres were not observed. Plakinastrella globular sp. nov. has a globular shape and dark blue color; the ectosomal skeleton has a double tangential reticulation and the coanosomal skeleton is reticulated. The spicules are diods, triods, calthrops and microrhabds; spheres are common. The new species have some exclusive characters previously unknown in their respective genera: the spines in the diods and triods of Plakortis spinalis sp. nov. and the blue color and microrhabds of Plakinastrella globular sp. nov. The presence of microrhabds in Plakinastrella is another evidence of a close relationship of this genus with Plakortis. Other three species of Plakinidae from Brazil are currently under description, indicating that the biodiversity of Homoscleromorpha is still highly underestimated.

**Effect of the neuroactive compounds, epinephrine, dopamine and L-dopa on larvae settlement and metamorphosis of red sponge, Mycale microsigmatosa**

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Neuroactive compounds factors with potential effects on the settlement and metamorphosis of many invertebrate larvae have been studied, but no research of neuroactive compounds on sponge larvae settlement and metamorphosis. The aim of the present study was to elucidate the effects of neuroactive compounds, epinephrine, dopamine and L-dopa on settlement and metamorphosis of the sponge larvae, to provide insight into appropriate methods for the sponge's sexual reproduction. This study deals with the effects of epinephrine, dopamine and L-dopa on larvae settlement and metamorphosis of the red sponge, *Mycale microsigmatosa*. Three neuroactive compounds were used at three different concentrations ( $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M). All the compounds at concentration of  $10^{-4}$  M did not promote the larvae settlement but showed toxic and settlement inhibition effects. The mortality of larvae reached 100% at  $10^{-4}$  M for L-dopa group. There were no significant different of settlement rate between  $10^{-5}$  and  $10^{-6}$  M experimental group and control sets of three neuroactive compound. The role of three neuroactive compounds on sponge larvae settlement and mortality were also discussed.

**Population and individual dynamics of *Clathrina aurea* (Calcarea)**

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Sponge populations may be maintained by sexual and asexual reproduction. While the first contributes to the genetic diversity of the species, the second generates clonal individuals. *Clathrina aurea* is a calcareous sponge widespread in the Brazilian coast. Previous studies have accompanied this species in Rio de Janeiro and, during 18 months, have found no embryos and larvae, but only a few percentage of individuals were clones. The aim of this study was to follow individuals of *C. aurea* in Papagaios Island, Rio de Janeiro. Over 10 months, we quantified recruitment, death, growth, fusion and fragmentation events in an area of 400cm<sup>2</sup> using photographs. Also, small fragments of the individuals were collected and analyzed with microsatellite loci. Until now, 57 individuals were accompanied. Both the recruitment ( $p=0.002$ ) and the occupied substrate area ( $p=0.0001$ ) were significantly correlated with the number of individuals of each month. The highest recruitment was observed in June, period that was followed by the highest abundance of individuals (July). The highest mortality occurred in August, followed by the lowest number of individuals in September. Individuals lived 2.8 (?1.6) months in average, but some lived less than one month and others more than nine months. The size of the individuals varied considerably. While some decreased up to 92% (-0.11 cm<sup>2</sup>/month), others increased up to 1400 times (0.09cm<sup>2</sup>/month) of its original size. Clonality was observed in a very small scale (with individuals less than 1 cm apart). Few events of fragmentation occurred and were confirmed by their genotypes. Fusions also occurred and the resulting genotypes are being accompanied. The *C. aurea* population in Cabo Frio seems to be very dynamic, with the individuals living for a few months and the constant recruitment and death of individuals shaping its dynamic. Events like fragmentation and fusion were observed in *C. aurea*, just like in other species of this genus. Although it was not yet described for *C. aurea*, the sexual reproduction may be the main responsible for the maintenance of its population, as asexually events seems to be rare.

### **Calcareous sponges are fashion**

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Sponges may present a wide variety of associations with a great sort of marine invertebrate organisms. These associations range from mutualism to parasitism, being obligatory or not. One of the taxonomic groups mostly described in association with sponges is Crustacea and one of the most famous associations between these two taxa is represented by the decorator crab (infraorder Brachyura, superfamily Majoidea). In this association, the crab uses sponges (or other sessile marine organisms) as body camouflage. But there are other representatives of brachyuran families that also use sessile marine organisms (including sponges) to cover their carapace. Species of the family Dromiidae (also known as sponge crabs) use specially sponges and colonial ascidians to cover their body. *Moreiradromia antillensis* (Stimpson, 1858) is a dromiid species with a wide distribution in the Western Atlantic: from North Carolina (Southeastern USA) to Rio Grande do Sul (South Brazil). We found one individual of *M. antillensis* in Arraial do Cabo, Rio de Janeiro, Southeastern Brazil ?wearing? a specimen of *Leucandra rudifera* (Poléjaeff, 1883). The body of the sponge was well adapted to the crab, who took half of the sponge to cover its carapace. Therefore, the atrium of the sponge was in contact with the carapace and its osculum was surrounding the rostrum of the crab like a crown. In the parts where the atrium was touching the carapace, canals were closed with the growing of pinacoderm. To our knowledge, this is the first report of a calcareous sponge being used as camouflage by a sponge crab. This is interesting because calcareous sponges are commonly found in cryptic habitats and one of the suggested reasons for this is that they would not have good defences against predators.

### **Connectivity among populations of *Clathrina aurea* (Porifera, Calcarea) in South-Southeastern Brazil.**

André Padua, Haydée Cunha, Michelle Klautau  
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Sponges have lecithotrophic, short-lived larvae and usually present restricted distributions with strong population structure due to their allegedly low dispersal capabilities, phylopatry and inbreeding. Therefore, it is surprising to observe species with wide distributions and to imagine that distant populations are capable of maintaining gene flow through larval dispersal. *Clathrina aurea* is a calcareous sponge with wide distribution across the Brazilian coast. Its populations have been reported from the states of Rio Grande do Norte to Santa Catarina (more than 3,000km apart). In the present work we analyzed the morphology and genetic connectivity of four populations in Southeastern (Cabo Frio, Cagaras and Ilhabela) and Southern (Arvoredo) Brazil, using about 30 specimens per site and seven specifically developed microsatellite loci. The external morphology of the analyzed specimens was

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conservative, but spicule measurements were very variable within and among populations, possibly due to environmental factors. A small percentage of clonality (two individuals, 6.6% in Cabo Frio) was found. *Clathrina aurea* presented high average of allelic diversity (20.4 alleles/locus), with populations presenting high numbers of total alleles and of private alleles compared to other sponge species studied so far. All populations presented departures from Hardy-Weinberg Equilibrium with two loci showing homozygote excess, suggesting the presence of null alleles. Therefore, analyses were done with all loci but also excluding the two loci that could show null alleles. Both set of loci (seven and five) generated the same results. While FST values indicated the presence of four populations, the Bayesian clustering analysis pointed to the presence of three structured clusters: two in the Southeastern (Cabo Frio and Cagarras + Ilhabela) and one in the Southern (Arvoredo) regions. Isolation by distance was detected among populations, and combined with the larval biology, coastal currents and the presence/absence of intermediate subpopulations between them could explain the observed results.

### **Sponge assemblages of the Condor Seamount (Azores) characterized from underwater imagery**

Raquel Pereira, José Nuno Gomes Pereira, Fernando Tempera, Filipe Porteiro, Joana R Xavier

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Sponges constitute an important and diverse group in deep-sea communities. In some areas, large specimens (i.e. >5 cm) are found in high densities, forming highly structured habitats known as sponge aggregations or sponge grounds that play key roles in ecosystem functioning. However, due to biological and ecological features these aggregations are vulnerable to the impact of human activities such as fisheries and have therefore become classified as Vulnerable Marine Ecosystems (VMEs).

The Azores archipelago is known to harbour a diverse deep-sea sponge fauna and a few sponge-dominated biotopes are known to occur associated to both Island slopes and neighbouring seamounts. In this study we characterized the megasponge fauna of



the Condor, a large seamount located 17km southwest of Faial Island, from the annotation and analysis of 12h of underwater videos acquired by the ROV Luso. A total of 4350 megasponges were recorded, of which 49% corresponded to the birds' nest sponge *Pheronema carpenteri* and 11% corresponded to 14 identified taxa. The remainder 40% of the records corresponded to unidentified sponges. Six biotopes that include megasponges were identified, of which three were considered dominated by this group: i) an area of mixed substrate found between 430-1100 m where 14 taxa were identified, was the most taxonomically diverse; ii) an area located at 700-825 m characterised by the presence of a multispecific assemblage of sponges strongly dominated by *P. carpenteri*; and iii) an area at 810-850 m depth where only *P. carpenteri* and some *Stylocordyla pellita* specimens were found. Based on their bathymetric distribution two main groups of species were distinguished: i) species within the families Axinellidae and Petrosiidae along with *Neophrissospongia nolitangere* were found on the shallower areas, from the summit at 180 m down to 500 m depth; and ii) species within the Azoricidae, Macandrewiidae and the species *Xestospongia variabilis* and *S. pellita* were found on the deeper areas of this seamount, between 450-1100 m depth.

This study contributed to the ongoing detailed characterization of the benthic assemblages of this seamount and highlights the potential and pitfalls of video imagery analyses in deep-sea research.

**Antitumor potential of bacteria associated to the Mediterranean sponges *Crambe crambe* and *Sarcotragus spinosulus* (Demospongiae)**

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Sponges are known to harbor a vast array of microbes, some of them being strongly associated and in some cases living in symbiosis with the host. The biomass of the associated microbes can represent up to 40% in High Microbial Abundance Sponge. In the same time, a high interest has emerged for sponges due to the chemical diversity they produce which raised the issue of the origin of the targeted bioactive compounds. Even if intense work has been carried out to cultivate sponge associated bacteria in order to respond the supply issue of these high value added biomolecules, the expectations are far from being reached.

Another way to take advantage of the association between sponges and their microbiota is to valorize, not only the main compounds produced by the sponges, but also the bioactive molecules that are largely produced by the cultivable associated microbes (BAMMBO UE FP7, [www.bammbo.eu](http://www.bammbo.eu)). In this contest we decided to inspect the cultivable microbes associated to the NW Mediterranean demosponges *Crambe crambe* and *Sarcotragus spinosulus*, both sponges being well known for the

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biosynthesis of bioactive alkaloids and terpenoids respectively. The associated bacteria were grown to obtain enough biomass for biological screening and we assess the anti-tumor potential of the resulting microbial extracts against neuronal cell line (SH-S5Y5 cells). 40 bacterial extracts were tested and three of them, cultured from *S. spinosulus*, showed promising anti-proliferative activities at subtoxic concentration. Further analysis is required to identify the molecules responsible for the activity and at meanwhile identify the strain responsible for the production.

### **Coral boring Porifera (Phloeodictyidae) from Galicia Bank (Atlantic Ocean)**

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The INDEMARES project began in 2009, it is co-financed by the EU (LIFE+) and its objective is to study ten possible new areas that fulfil the necessary requirements for being established as MPAs. One of the areas proposed by the scientific community is Galicia Bank.

Data were collected during the four INDEMARES Galicia bank surveys carried out on board the R/V *Thalassa* (IFREMER-IEO) in July 2010 and R/V *Miguel Oliver* (SGP) in July 2011.

The Galicia continental margin can be defined as a starved, non-volcanic passive margin and its complex structure has resulted from Mesozoic rifting that has caused the development of a complex extensional system and Cenozoic compressive phase.

The Galicia Bank is a structural high in the western Galicia continental margin (Atlantic NW Iberian) and the region is bounded to the north and to the west by the Biscay and Iberian Abyssal Plains respectively. This bank is located about 120 km away from the coast, and shows a trapezoidal geometry of 220 km wide in E–W direction and 300 km length in N–S direction. The literature indicates that the Galicia continental margin shows a great complexity in its structural evolution.

Sponges of the family Phloeodictyidae Carter, 1882 are known for their ability to penetrate and to dissolve calcium carbonate substrates through a chemical process. They are encrusting, massive, lobate or more frequently spherical or tubular growth forms, buried in the substrate, with fistules on the surface, occasionally excavating coralline substrates, shells or stones. As the sponge penetrates the coral, the substrate is gradually destroyed as a result of the sponge hollowing out an extensive system of cavities and tunnels.

In this work we have collected 38 specimens of boring sponges at depths between 807 and 1414 m. Most of them mainly bore dead coral (rubbles) of *Lophelia pertusa* and *Madrepora oculata* but we found also several specimens eroding carbonate rocks. We will describe 4 species of Phloeodictyidae in this area.

**First results on sponges from the Mozambique Channel collected by the  
MAINBAZA cruise**

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The coastal and marine zone of Mozambique presents ecosystems with high biodiversity and many endemic species. Most of the available information on the marine biodiversity in this area is limited to exploitable fish and crustacean species or species in danger of extinction

The first objective of the MAINBAZA cruise was the study of benthic biodiversity at the continental margin (outer continental shelf, continental slope) and upper part of the abyssal plane of Mozambique.

The expedition was developed in April 2009 on board R/V Vizconde de Eza, belonging to Secretary General for Fisheries of the Spanish Ministry of Agriculture, Food and Environment, as part of a joint project between Muséum National d'Histoire Naturelle (MNHN) and the Instituto Español de Oceanografía (IEO).

Forty-six hauls were conducted at depths between 100 and 1800 meters on four transects off Maputo, Inhambane, Bazaruto and the mouth of the Zambeze.

Benthic sampling was carried out by two types of trawl: a beam trawl, a drezen type and one rock dredge for hard bottoms.

As part of the results of this expedition we collected more than 400 specimens that were preserved on board in 70% ethanol.

In this preliminary results, the most of the samples belong to Class Demospongiae Sollas, 1886 (84%): Spirophorida Bergquist & Hogg, 1969 (6%), Astrophorida Sollas, 1887 (35%), Lithistida (11%), Hadromerida Topsent, 1894 (17%), Poecilosclerida Topsent, 1928 (19%), Halichondrida Gray, 1867 (5%) and Haplosclerida Topsent, 1928 (8%).

Some of these samples are endemic of Indian Ocean or South African waters like Genus Tentorina Burton, 1959 or Genus Fangophilina Schmidt.

The Hexactinellida Schmidt (16% of the samples) are Amphidiscosida Schrammen, 1924 (80%) principally Hyalonema sp. and Platylistrum platessa Schulze, 1904, Lyssacinosida Zittel, 1877 (19%) and Hexactinosida Schrammen, 1912 (1%).

**Preliminary assessment of sponge responses to dredging-related pressures**

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Dredging activity poses an environmental risk to local filter feeder communities through turbidity and light reduction, impediments to filtration organs and smothering by sedimentation. Within the WAMSI (Western Australia Marine Science Institution) Dredging Science Node, we aim to test pressure/response effects on sponges in controlled aquarium experiments. To determine relevant sponge species and morphologies for dredging experiments and to understand how dredge sediments would

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behave within experimental tanks, we undertook a preliminary sediment-pulse experiment. Ten different sponge species were selected in order to encompass the four main morphologies (i.e. encrusting, massive, erect and cups). Sponges were collected from Broadhurst Reef (GBR) and transported to the AIMS (Australian Institute of Marine Science) aquarium facilities in Townsville (Queensland). Three sediment dosing levels were tested: 0 mg L<sup>-1</sup> (control), 250 mg L<sup>-1</sup> (medium), and 500 mg L<sup>-1</sup> (high). Two to four replicates of each species and 5 sediment collectors (sedpods) were randomly placed in each treatment tank and left for two weeks after addition of the initial sediment pulse. Total Suspended Solids (TSS) and Sedimentation Rates (SR) were recorded every 8 hours for the first 48 h followed by weekly measurements. Underwater pictures of each sponge individual were taken prior to commencing the experiment, after 48 h, after 7 d and at the completion of the experiment. Image analysis software (Image J) was used to measure the percentage of surface covered by sediments for each sponge during the experiment. At the end of the experiment, the sediment remaining on the sponges was quantified. Mortality and areas of necrotic tissue were recorded and tissue samples were frozen in liquid nitrogen for subsequent analysis of chlorophyll and microbial symbionts. Initial results indicated a rapid deposition of sediments onto sponges after the pulse and a concomitant drop in TSS in the water column within the first 48 h. Image analyses showed higher deposition rates in horizontal cups and some massive species compared to more erect morphologies. Moreover, a differential ability for cleaning of tissue surfaces through pumping was observed among the sponge species. These initial results will inform the experimental design of subsequent dose-response experiments aimed at determining sponge thresholds to dredging related pressures.

### **A new species of *Spongia* (Dictyoceratida, Spongiidae) from the Azores**

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The Azores shallow-water sponge fauna is relatively well studied; however new records are not uncommon. In the course of a project aimed at investigating the microbial communities associated with marine sponges along a biogeographic and phylogenetic gradient, several dictyoceratid sponges were collected throughout the island of São Miguel.

Of the obtained samples, four were at a first glance identified as *Spongia officinalis* Linnaeus, 1759, a species widely distributed in the Mediterranean Sea. However, a closer examination of these specimens' skeleton revealed consistent morphological differences in regards to what was reported in the literature, namely the diameter of both primary and secondary fibres.

Phylogenetic reconstructions based on the mtDNA COI gene further confirmed our assignment and showed that the same species occurs on the South coast of Portugal

(Algarve) and in Gibraltar (Ceuta). The remarkable external similarity between this species and *S. officinalis* is likely the factor that has made it go unnoticed until now. This species adds to *S. officinalis*, *S. zimmocca* Schmidt, 1862, *S. nitens* (Schmidt, 1862), *S. virgultosa* (Schmidt, 1868) and *S. lamella* Schulze, 1879 raising the number of species within this genus in the Atlanto-Mediterranean region to six.

### **The culturable heterotrophic bacteria associated with *Erylus* spp. (Astrophorida, Geodiidae)**

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Sponges are a reservoir of environmental microorganisms due to their filter-feeding life style. Some of these microorganisms evolved along with the sponges for millennia and became exclusively associated to Porifera. The heterotrophic microbial community associated with six specimens belonging to the genus *Erylus* was studied by culture dependent methods. All specimens were collected by scuba diving in Portuguese waters: three specimens of *Erylus* cf. *deficiens* Topsent, 1927 were collected in the Gorringe Bank (Gettysburg and Ormonde peaks), a large seamount located 150 nm from the southwest coast of Portugal; two specimens of *Erylus discophorus* (Schmidt, 1862) were collected in Berlengas islands, near the coast of Peniche; and one specimen of *Erylus mamillaris* (Schmidt, 1862) was collected in São Miguel island on the archipelago of the Azores. For the isolation of bacteria sponge extracts were serially diluted and incubated in different agar media, which favored the growth of heterotrophic bacteria. The taxonomy of the isolated bacteria was based on the analysis of the 16S rRNA gene sequence. In total 354 isolates were identified of which the majority belong to Alphaproteobacteria (39.9 %) followed by Gammaproteobacteria (28.5 %), Actinobacteria (17.5 %) and Firmicutes (14.1 %). However, the most diverse groups regarding genera were Gammaproteobacteria and Actinobacteria. *Pseudovibrio*, *Ruegeria* and *Labrenzia* were the most abundant genera among the Alphaproteobacteria isolates especially present in *E. discophorus*. *Vibrio* and *Pseudoalteromonas* were the two genera with higher numbers of isolates affiliated to the Gammaproteobacteria. *Vibrio* was found associated with *Erylus discophorus* and *E. mamillaris*, and the *Pseudoalteromonas* to the *Erylus* cf. *deficiens*. Actinobacteria affiliated to *Microbacterium*, *Dietzia*, *Gordonia* and *Rhodococcus* were the most abundant genera in this phylum but only *Microbacterium* was present in all three host-species. Regarding the Firmicutes the most abundant genus was the *Bacillus* present in samples from all the locations. Analysis of the 16S rRNA gene sequence similarities suggests the presence of potential new taxa in Alpha-, Gammaproteobacteria and Actinobacteria. The heterotrophic culturable analysis of the microbiome of *Erylus* spp. allowed the isolation of a substantial bacterial diversity.

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**Till death do us apart: Stable sponge-bacteria associations under thermal and food shortage stresses**

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The Mediterranean sponges *Ircinia fasciculata* and *I. oros* harbor a distinct, species-specific mix of bacterial symbionts that are highly stable over time and space. However, sporadic mass mortality events of *Ircinia* spp. following periods of anomalously high seawater temperatures suggest that these sponges are sensitive to thermal stress, which may alter the structure of the resident bacterial community or disrupt well-established sponge-bacteria interactions. Here, we monitored the bacterial symbiotic communities of *I. fasciculata* and *I. oros* maintained in controlled aquaria under 4 treatments: control conditions (14°C, unfiltered seawater), low food availability (0.1 µm filtered seawater), elevated temperatures (25°C), and a combination of the 2 stressors (25°C, 0.1 µm filtered seawater). Seawater and sponge samples were collected from 3 individuals per species and treatment following an acclimation period (1 wk) and at the end of the assay (4 wk). Bacterial community structure was assessed using transmission electron microscopy (TEM) and terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA gene sequences. In addition, as *I. fasciculata* is known to harbor cyanobacteria, we also measured chlorophyll a (chl a) levels for this species. TEM observations did not reveal any discernible change in the bacterial composition or abundance for any of the samples. Similarly, multivariate analysis (PERMANOVA and PERMDISP) and nMDS plots revealed no significant differences in T-RFLP profiles among treatments. For *I. fasciculata*, chl a concentrations did not significantly differ among treatments. Our results suggest a tight link between host and symbionts, probably originated after a long co-evolution process. As observed in the field, sponge-bacteria associations are very stable and able to resist stressful conditions, including elevated temperatures and low food availability, for a period lasting several weeks. These conditions mimicked realistic stress pressures that have been purported as responsible for mass mortalities events. Our results indicate that these stressful situations per se may not be the ultimate cause of mortality episodes, though they may favor them indirectly or in combination with other factors that compromise holobiont health, perhaps reducing resistance to pathogens that, in turn, proliferate and kill the sponges.

**Composition and diversity of Archaea in seawater, sediment and sponges in the Kepulauan Seribu reef system, Java, Indonesia**

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Coral reefs are among the most diverse and productive ecosystems in the world. Most research has, however, focused on eukaryotic species including taxa such as corals and fishes. Recently, there has been increased interest in the diversity and composition of prokaryotes, particularly those inhabiting corals and sponges, but these have mainly focused on bacteria. There have been very few studies of coral reef Archaea, despite the fact that Archaea have been shown to play crucial roles in nutrient dynamics, including nitrification and methanogenesis, of oligotrophic environments such as coral reefs. Here, we present the first study to assess archaeal composition in four different microbial biotopes (seawater, sediment and two sponge species, *Stylissa massa* and *Xestospongia testudinaria*) in a coral reef environment, namely the Kepulauan Seribu reef system, Indonesia. The archaeal community of both sponge species and sediment was dominated by Crenarchaeota, while the seawater community was dominated by Euryarchaeota. The biotope explained almost 70% of the variation in archaeal composition. The number of OTUs was highest in sediment and seawater biotopes and substantially lower in both sponge hosts. Dominant taxa ( $\geq 300$  sequences) in both sponge hosts belonged to phylogenetically distinct lineages. No 'sponge-specific' archaeal OTUs were found, i.e., OTUs found in both sponge species but absent from abiotic biotopes. In summary, our study shows that each biotope hosts highly distinct archaeal communities with the greatest species and phylogenetic diversity found in sediment and the least in sponge hosts.

**Immobilization of Sponge Hybridomas in Gel Microdroplets: a Novel Approach to Development of a Sponge Cell Culture Model**

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Sponges are dominant components of many benthic marine ecosystems, and they produce thousands of bioactive compounds with potential biomedical and therapeutic applications. The ecological and physiological roles of these chemicals and the benefits they provide to the sponge are not well understood. In vitro cell culture models are one approach to study how and why these compounds are produced, however, no cell lines with an extended life span have been established for any marine invertebrate. We hypothesized that: (1) a sponge cell line could be developed by creating a sponge hybridoma, i.e., by fusing sponge cells of one species with those of another, or by fusing sponge cells with rapidly dividing, marine-derived, non-sponge cells; and (2) sponge cell culture conditions could be optimized by immobilizing and culturing cells and hybridomas in a 3-dimensional gel microdroplet (GMD). We have previously demonstrated that cells from individuals of the same sponge species, as well as cells

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from individuals of two different sponge species can be fused (Pomponi et al., 2013, *Integr. Comp. Biol.* doi: 10.1093/icb/ict032), and that sponge cells can be immobilized in 3-D gel layers (Wijffels et al., 2001, in Cabral et al., eds. *Multiphase Bioreactor Design*, Taylor & Francis, London and New York). GMDs are an improvement over gel layers; they provide a more easily controlled microenvironment for cell culture. In this study, we evaluated and compared viability, cell division, and differentiation of sponge cells immobilized in GMDs using three polymers: one synthetic (polyethylene glycol diacrylate) and two natural (sodium alginate, ultra-low temperature agarose), and we optimized methods for verifying cell fusion using fluorescent markers for DNA (Hoeschst 33342) and metabolic activity (CellTracker™ Green CMFDA). Although individual and fused sponge cells were viable in all three polymers, ultra-low temperature agarose resulted in the most transparent and consistently-sized GMDs. In addition to the potential use of this technology for in vitro production of sponge-derived bioproducts, sponge cells immobilized in GMDs may also be used as models to test hypotheses related to naturally-occurring sponge chimeras, hybridomas, and hybrids.

### **Species diversity of marine sponges along the western coast of the Gulf of Thailand**

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Species diversity of marine sponges was investigated along the western coast of Gulf of Thailand. Collections were conducted from 26 collection sites of three Islands including Samui Islands, Chumporn Sea Islands and Tao Islands during the period of January 2010, March 2011, and March, 2012. Most of the collections were carried out by SCUBA diving during daytime and the observations were randomly collected throughout all collection sites. Three hundred and fifty sponge specimens were collected. The results showed 89 marine sponge species from 12 orders, 34 families and 51 genera. Order Haploaclerida (27 species) was the most abundance sponge group, follow by order Poecilosclerida (21 species). Out of these, four species were the new records in Thai Waters, namely *Corticium niger* Pulitzer-Finali, 1996, *Higginsia massalis* Cater, 1885, *Lissodendoryx (Ectyodoryx) sp.* "orange" and *Cladocroce sp.* "blue". *Gelliodes petrosioides* Dendy, 1905, *Neopetrosia sp.* "blue", and *Hyrtilos erecta* (Keller, 1889) were the common species of the study area.



**Are poriferan biomarkers preserved in the geological record?**

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Sponges are considered to be the most ancient metazoans, but their emergence is as yet not well documented. In order to test the utility of biomarkers as a tool to elucidate the origin and evolution of sponges, the preservation potential of three classes of sponge-derived lipids in the bitumen fractions of different fossilized sponges and sediments (Recent-Lower Cambrian) containing abundant sponge remains were tested: (i) demospongiac acids, (ii) mid-chain branched fatty acids and (iii) 24-isopropyl sterols. Recent and subrecent sponges and sponge microbial carbonate crusts were used as reference materials. All modern sponge samples contained “demospongiac acids” in variable abundance and a high abundance of branched fatty acids, including terminally branched fatty acids. The mid-chain branched fatty acids (MBFA) were characterized by a great variety of structural isomers and showed m-C16, m-C18 and m-C24 as the most prominent homologues. In the sub-recent samples, demospongiac acids had decreased below detection limit, but MBFA were still detected in low abundance and still revealed the characteristic variety of structural isomers. One subrecent sponge microbial crust also contained a 24-isopropyl bearing sponge sterol, namely 24-isopropylcholesta-5,22-dien-3 $\beta$ -ol. Mid-chain branched alkanes (MBA), possible diagenetic products of MBFA, occurred in small amounts in some of the ancient samples. Although the fossil MBA displayed a great variety of structural isomers in the C16–C20 range, their distribution patterns suggest molecules other than sponge-derived MBFA as biological precursor compounds. Likewise, neither plausible geological derivatives of 24-isopropylsterols nor of demospongiac acids were detected in the ancient materials. The results indicate that the in-situ preservation of common sponge biomarkers of ancient samples, if occurring at all, can be considered as a rare exception.

**First record of Demosponge spicules in a late Devonian stromatoporoid basal skeleton (Frasnian, Belgium)**

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We show the first record of demosponge spicule framework in a single specimen of a Devonian stromatoporoid from the Frasnian of southern Belgium (La Boverie Quarry 30 km east of Dinant). The stromatoporoid basal skeleton is similar to the genus *Stromatopora*. The spicules are arranged in the calcified basal skeleton, but not in the gallery space, and are recrystallised as multicrystalline calcite. The spicules fall into two size ranges: 10 - 20  $\mu$ m diameter and 500 - 2000  $\mu$ m long for the large ones and between 5 to 15  $\mu$ m and 50 to 100  $\mu$ m for the small ones. In tangential section, the

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spicules are circular, they have a simple structure and no axial canal has been preserved. The large spicules are always monaxons, straight or slightly curved styles or strongyles. The spicules and spicule arrangement most closely resemble halichondrid/axinellid demosponge spicules, and are important rare evidence of the existence of spicules in Palaeozoic stromatoporoids, reinforcing the interpretation that stromatoporoids were sponges. The basal skeleton may have had an aragonitic spherulitic mineralogy. Furthermore, the spicules indicate that this stromatoporoïd sample is a demosponge, and indicates that the class Stromatoporoidea may not be a valid taxonomic instrument.

### **Reproduction in cold waters: embryonic development and vitellogenesis in Antarctic demospönges**

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Vitellogenesis is a crucial process during the embryogenesis of species possessing lecithotrophic larvae, given that their survival will depend upon their own nutrient reserves entirely. Most demospönges produce lecithotrophic larvae whose yolk could have been produced by either autösynthesis or heterösynthesis (transferred by nurse cells). Embryos with heterösynthesis usually complete the embryonic development at a faster rate and possess more yolk platelets than those in charge of their own yolk formation process. In Antarctica, invertebrates tune their embryonic processes to adapt to cold waters, increasing the duration of the embryonic development and the amount of yolk in the larvae to ensure survival upon hatching. Even though sponges are one of the main components of the Antarctic benthos, their reproduction has been rarely addressed given mainly to collection constraints. Reproduction has been described only for the demosponge *Stylocordyla borealis*, but still the vitellogenesis was not the main point of the study, since the sponge is a direct developer. Here we describe the embryonic development of five species of common sublittoral demospönges inhabiting the rocky coast of Deception Island (South Shetland's Archipelago, Antarctica) of the orders Dendroceratida (*Dendrilla antarctica*), Haplosclerida (an unidentified species), and Poecilosclerida (*Mycale acerata*, *Phorbas areolatus*, and an unidentified species), and we provide details of their vitellogenesis comparing the strategies across the orders. All five sponges are viviparous, brooding their larvae inside the body in large follicles. In the unidentified haplosclerid, large brooding chambers containing 10-15 embryos were observed. The follicle was complex in the poecilosclerids, showing fusion of the follicle cells, and a wide concentration of collagen lining the follicle in all cases. Poecilosclerids showed greater amounts of glycogen in the blastomeres and nurse cells. Vertical transmission of bacteria was observed only in *Mycale acerata* and *Phorbas areolatus*. Yolk was created via both autösynthesis and heterösynthesis of yolk, observing introgression of nurse cells through the follicle to be phagocytosed by the blastomeres. Embryogenesis in all these selected viviparous species proceeded similarly as in the rest of the members of the orders.

**Inferring the ancestral sexuality and reproductive condition in sponges (Porifera) using phylogenetic inference**

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Centro de Estudios Avanzados de Blanes/ Harvard University*

Considerable diversity abounds among sponges with respect to reproductive and developmental biology. Their ancestral sexual mode (gonochorism vs. hermaphroditism) and reproductive condition (oviparity vs. viviparity) however remain unclear and these traits appear to have undergone correlated evolution in the phylum. To infer ancestral traits and investigate this putative correlation, we used DNA sequence data from two loci (18S ribosomal RNA and cytochrome c oxidase subunit I) to explore the phylogenetic relationships of 62 sponges whose reproductive traits have been previously documented. Although the inferred tree topologies, using the limited data available, favoured paraphyly of sponges, we also investigated ancestral character-state reconstruction on a phylogeny with constrained sponge monophyly. Both parsimony- and likelihood-based ancestral state reconstructions indicate that viviparity (brooding) was the likely reproductive mode of the ancestral sponge. Hermaphroditism is favoured over gonochorism as the sexual condition of the sponge ancestor under parsimony, but the reconstruction is ambiguous under likelihood, rendering the ancestry of sexuality unresolved in our study. These results are insensitive to the constraint of sponge monophyly when tracing the reproductive characters using parsimony methods. However, the maximum likelihood analysis of the monophyletic hypothetical tree rendered gonochorism as ancestral for the phylum. A test of trait correlation unambiguously favours the concerted evolution of sexuality and reproductive mode in sponges (hermaphroditism/viviparity, gonochorism/oviparity). Although testing ecological hypotheses for the pattern of sponge reproduction is beyond the scope of our analyses, we postulate that certain physiological constraints might be key causes for the correlation of reproductive characters.

**Tools for conservation of marine species subjected to population decimation in the Mediterranean: Optimization of microsatellite loci in the sponge *Ircinia fasciculata***

Ana Riesgo, Gema Blasco, Patrick Erwin, Rocío Pérez-Portela, Susanna López-Legentil  
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Periodic episodes of massive die-offs are creating extensive gaps in the distribution of many species, including sponges. Reports of sponge disease have increased

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dramatically in recent years throughout the Mediterranean and Caribbean. In the Mediterranean, sponge populations of the genus *Ircinia* have been reported to be decreasing because of abnormally high seawater temperatures favoring the colonization of bacterial pathogens. The recovery of decimated populations following such mass mortality events will largely hinge on their connectivity with conspecifics in unaffected locations. Here, we report on the optimization of microsatellite markers for further estimation of the degree of isolation and population connectivity among populations of the sponge *Ircinia fasciculata* from the Mediterranean, including areas where populations are known to be affected by disease. A genomic pyrosequencing run on a 454 GS-FLX Titanium System (Roche) was performed to screen for microsatellite loci. We obtained a total of 220876 sequences that were then analyzed for repeat motives using Phobos v 3.3.12. Preliminary allelic variation of twelve loci was assessed in 39 individuals collected from five populations in the Mediterranean Sea (Caials (NW), Blanes (NW), Alicante (SW), Cabrera (NE), Corsica (NE)). The number of alleles per locus ranged from five to eighteen, observed heterozygosity from 0.536 to 0.675, and expected heterozygosity from 0.539 to 0.667. Significant linkage disequilibrium between pairs of loci was not detected in any of the microsatellite loci. Inbreeding coefficient values (FIS) were not significant for any of the twelve loci, indicating that the populations were in Hardy-Weinberg equilibrium. Further studies applying the microsatellite markers developed herein to additional *I. fasciculata* populations will allow for the assessment of this species recovery potential following disease episodes and population decimation.

### **Sequencing of the *Ephydatia muelleri* genome: preliminary results**

Masa Roller, Mirna Imesek, Matija Harcet, Gert Wörheide, April Hill, Helena Cetkovic, Sally Leys Kristian Vlahovicek

*Bioinformatics Group, Department of Molecular Biology, Faculty of Science, University of Zagreb/ Department of Molecular Biology, Rudjer Boskovic Institute/ Department of Earth and Environmental Sciences & GeoBio-Center, Ludwig-Maximilians-Universität München/ University of Richmond/ Department of Biological Sciences, University of Alberta/ Department of Informatics, University of Oslo*

With their unique evolutionary position of arguably the simplest extant and earliest branching metazoans, sponges are rapidly gaining focus in genomic research. Only one draft genome sequence of a sponge has been published to date, that of *Amphimedon queenslandica*. Genomic information from additional taxa is required for comparative genomic approaches within the phylum Porifera to describe their genomic makeup, gene architecture and structure, eventually leading to elucidation of early metazoan gene regulation and development. We sequenced and assembled a preliminary draft of the genome of the freshwater sponge *Ephydatia muelleri* using next-generation sequencing technology. Paired-end Illumina libraries yielded contigs covering at least 85% of the genome. Addition of SOLiD mate-pair libraries produced scaffolds covering the entire genome. We identified a preliminary gene set through automated annotation and functionally classified them using the STRING Clusters of Orthologous Groups

(COG) database. Further sequencing is currently underway to improve coverage and complete the genome.

### **Freshwater and marine sponges as biomonitors of heavy metal accumulation in Thai waters**

Nisit Ruengsawang, Alongkorn Yoosamran, Thamarong Arpamasakul, Supaluck Tujumroon, Jamrearn Buaruang, Chutima Hanjavanit, Narumon Sangpradub  
*Rajamangala University of Technology Krungthep/ Khon Kaen University*

This study was designed to monitor the status and trend of heavy metal accumulation in Thai waters by using freshwater and marine sponges as biomonitors or sentinel organisms. The project has been launched a pilot study in May 2013. A common species of freshwater sponge, *Corvospongilla siamensis* Manconi & Ruengsawang 2012, and marine species, *Xestospongia* sp., were chosen as model organisms. Samples of *C. siamensis* were collected by wading, snorkeling from various locations along the Pong River (Lower Mekong Basin) while *Xestospongia* sp. were collected by SCUBA diving from the Gulf of Thailand (Mu Koh Chang and Mo Koh Lan) and the Andaman Sea (Mu Koh Phi Phi and Mu Koh Lanta). Both pristine and polluted sites were included for this study. Collected samples were kept cool in icebox in the field and were immediately transported to the laboratory and frozen at -20 °C until analysis. Concentrations of heavy metals (Cd, Zn, Cu and Pb) were determined by atomic absorption spectrophotometer (AAS). Comparisons of accumulated heavy metal concentrations in both freshwater and marine sponges samples were provided for the first time in Thailand. Suitability of these biomonitors for long-term monitoring of heavy metal contamination in Thai waters was discussed.

### **The perks of being endolithic**

Christine Schönberg, Max Wisshak  
*Australian Institute of Marine Science/ Senckenberg at the Sea*

Bioeroding sponges are macroendoliths living and eroding in calcareous hard substrates, but we do not know why they developed this life style. Two common Great Barrier Reef bioeroding sponges were sampled in May 2011: *Aka mucosa* (Bergquist, 1965) and *Cliothosa aurivillii* (Lindgren, 1898). Despite being cavernous eroders with soft internal tissue exposed after sampling, they initially healed very quickly, showing scarring and recovery after only 3-4 days. However, endolithic tissue of *A. mucosa* exposed to direct sunlight became putrid and rotted, while shaded tissue healed, toughened and turned brown-black like the external fistules. About two weeks after sampling, some *C. aurivillii* began to rot and had patches of orange sediment underneath them. Their undersides were teeming with small organisms that were identified as copepods feeding on the sponge tissue. Hence, being endolithic has multiple sheltering functions beyond the obvious escape from grazers and predators,

very likely including shading and shielding from parasites and disease, and savings on maintenance costs.

### **Siphonodictyon type material: lost in history**

Christine Schönberg

*Australian Institute of Marine Science*

From the presently 29 described species of *Siphonodictyon* five are fossils, and three were transferred to other genera. Almost half of the remaining 21 species are very difficult to work with, because type material has been lost or is inaccessible. The Hancock (1849) specimens for *S. labyrinthicum* and *S. nodosum* were destroyed during WW2, and only slide preparations and simple line drawings of spicules remain. Photos of spicules on the slides became available through K. Rützler in Schönberg & Beuck (2007), but especially *S. labyrinthicum* has caused much confusion. Schmidt's (1870) specimens for *S. densum* and *S. terebrans* could not be located, but bad spicule preparations were accessed that provide more detailed information than the short original descriptions. All of Johnson's (1899) material for *S. infestum*, *S. insidiosum* and *S. rodens* was lost during a fire in the Lisbon University Museum. A sponge recently sampled from the Mediterranean Sea was tentatively identified as *S. infestum* and redescribed in detail, but otherwise only short original descriptions and insufficient drawings are available for these three species. *S. rodens* is presently considered to be synonymous with *S. insidiosum* (World Porifera Database), but this is not based on investigations of the type material, but a simple assumption. Thomas' species *S. diagonoxeum* (1968) and *S. minutum* (1972) are poorly described, but the type material still exists at the Indian Museum in Calcutta (Thomas, pers. comm.). Repeated attempts to get a loan of Thomas' species remained unanswered. When members of the Museum were contacted directly, they claimed that the material could not be found, which matched reports from other colleagues. Apart from *S. infestum*, the above species remain thus unavailable to the scientific community. However, *Siphonodictyon* is a very important genus for bioerosion studies, and redescriptions as in Schönberg & Beuck (2007) should be attempted, including the establishment of neotypes. We should thus scrutinise shallow-water, Indo-Pacific mollusc shells for *S. labyrinthicum* and *S. nodosum*, try to obtain fresh material for *S. densum* and *S. terebrans* from Florida and St. Thomas, ask fisherpeople for their bycatch from deep waters of coastal Madeira for Johnson's species and keep insisting on loans from the Indian Museum.

### **Storm damage after cyclone Yasi – bioeroding sponges survived**

Christine Schönberg, Haley Burgess

*Australian Institute of Marine Science/ Orpheus Island Research Station, James Cook University*

On 3.2.2011 category 5 cyclone Yasi crossed the coast into Queensland only about 100 km north of the Palm Island Group on the central Great Barrier Reef. At the Research Station at Orpheus Island mostly southeasterly winds above 100 km/h were recorded, peaking at 170 km/h just before the weather station was damaged. The windward side of the Island was exposed to enormous wave energy, and vegetation and structures at the Research Station on the leeward side still received significant damage, but scientific experiments in the sheltered Little Pioneer Bay survived. In June 2011 a rapid survey was undertaken to obtain a rough estimate of the storm damage inflicted on the fringing reefs at Orpheus Island. Leeward bays were largely unchanged or showed only patchy damage, mostly of branching and tabulate coral. Another bay on the exposed, eastern coast appeared also mostly unharmed, but it is dominated by encrusting and mound-shaped corals that withstood the breaking waves. However, at the site most exposed during the storm, the NE corner of the Island, the formerly diverse and highly structured reef was eradicated and replaced by barren ground. Over large areas branching and tabulate corals were reduced to fields of rubble, while large bommies were turned over. Surfaces were smoothed out, only just recolonizing with weedy invertebrates such as soft corals. Seeing this widespread devastation, we visually investigated the state of bioeroding sponges and found their occurrence and distribution ?normal? at all sites, with *C. orientalis* dominating and forming large, healthy colonies. The NE corner proved to be a diverse site for bioeroding sponges that are sheltered during storms, as long as they invaded massive substrates. They likely lost part of their surface structures when superficial layers of the reef were grated away by moving debris, but all specimens looked healthy and intact 4.5 months after the storm. With virtually no competing live tissue in their vicinity their lateral growth and larval settlement will be comparatively unhindered and their abundances are expected to increase. However, no before-the-event data exist for comparison, demonstrating the importance of regular surveys on bioeroding sponges.

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### **Biodiversity of demosponge species in shelf waters off South Australia, including Commonwealth waters to the shelf edge**

Shirley Sorokin, Thierry Laperousaz, David Currie, Jane Fromont, Lisa Goudie  
*SARDI Aquatic Sciences/ South Australian Museum/ GHD, Doha, Qatar/ Western Australian Museum/ Sponge consultant*

Distinctive physical and biological features divide the waters of South Australia into eight marine bioregions that host a diverse range of marine fauna. Many of these bioregions are dominated in biomass and richness by sponges. South Australia has the longest stretch of south-facing coastline in the Southern Hemisphere, a large continental shelf, and two sheltered gulfs forming inverse estuaries. The waters are low in nutrients and typically warm in the west (influenced by the Leeuwin current) and cool in the south east (influenced by upwelling and easterly coastal currents). The waters of the gulfs become very warm in summer (>30 °C) and support some species usually encountered in the tropics. The Australian Biological Resources Study lists 90 named species of demosponge from South Australia. Work done in the last decade, including studies on marine park biodiversity, and trawl fishery bycatch shows the sponge biodiversity to be much higher than this number. We have used the data from several studies as well as an examination of sponges in the South Australian Museum to show that the sponge fauna of South Australia may be closer in number to the species listed as occurring in Bass Strait (293 species) and off the coast of Victoria (279 species).

### **Evenings at the microfiche - Researches among Swedish spongiologists and sponge collections in Sweden**

Mikael Thollesson, Paco Cardenas  
*Uppsala University*

Including Linnaeus, major contributions to sponge systematics have historically been made by several Swedish taxonomists, who have been working with significant collections and expedition material hosted in Swedish museums. In particular, extra attention is due for the contributions, and collections worked upon, by Carl Aurivillius, Conrad Fristedt, Nils Gustaf Lindgren (all working in Uppsala) and Harald Alander. We present an overview of these scientists and the related sponge collections hosted in Swedish museums.



**Deep-water calcareous sponges (Calcarea: Porifera) from the Norwegian, Greenland and Iceland Seas (GIN) – from abyssal plains to seamounts and hydrothermal vents**

Hans Tore Rapp, Mari Heggernes Eilertsen, Henning Flørenes, Adriana Alvizu, Ole Secher Tendal

*Department of Biology and Centre for Geobiology, University of Bergen/ Zoological Museum, SNM, University of Copenhagen, Denmark*

Calcarea has by and large been reported from all over the world as a shallow water group (<1000 m depth), with most species having been described from temperate seas, followed by certain tropical hard substrate areas. Investigations in the deeper parts of the Nordic Seas have revealed a very diverse calcareous sponge fauna where one can see very clear distribution patterns according to depth, substrate and between basins. It is a striking and peculiar situation that quite a number of species live in the abyssal and lower bathyal areas of the GIN Seas at negative temperatures, and some occur in high abundance. The abyssal (>2000 m depth) zones of the GIN Seas are parts of the Arctic deep-sea region. While depths of more than 2000 m are found in only a small part of the Iceland Sea, depths to more than 4500 m occupy considerable areas in the Greenland- and Norwegian Seas. Characteristics of the sponge fauna are a high proportion of endemic species, the generally low diversity as compared to the Atlantic abyssal fauna, and that some species are very abundant. More than 6000 specimens are examined, and we recognize 7 species representing 6 different genera, and describe 4 species and 1 genus new to science.

Living both under high hydrostatic pressure and at negative temperatures is a challenge to animals relying on a calcium carbonate skeleton protected against dissolution by only thin membranes. Calcareous sponges from the CO<sub>2</sub>-rich Arctic hydrothermal vents face another but similar challenge, the locally low pH. However, calcareous sponges appear to be highly diverse and abundant component of the fauna of these vents, with an undescribed species of *Grantia* as the most common species.

By far the number of species is highest at bathyal depths. However, hardly accessible deep-sea habitats are heavily under-sampled, and the use of modern sampling techniques as ROV supported by high-resolution mapping of the seabed have revealed that calcareous sponges are very well represented both on abyssal plains, new basalts and pillow lavas along the mid-ocean ridge as well as at hydrothermal vents in the Nordic Seas.

**Diversity of hexactinellid sponges on the Arctic Mid-Ocean Ridge**

Marte Torkildsen, Ole Secher Tendal, Hans Tore Rapp

*Department of Biology and Centre for Geobiology, University of Bergen/ Zoological Museum, SNM, University of Copenhagen, Denmark*

The Arctic seamount, the Schulze Massive, located on the Arctic Mid-Ocean Ridge (AMOR), has been found to host a highly diverse community of hexactinellid sponges. The seamount extends from 500 m and down to 3000 m depth, and hexactinellids are present at all investigated depths. Mass occurrences of sponges have previously been reported from seamounts, and at the top of the Schulze Massive there are dense aggregations of sponges in general, and hexactinellids in particular. This mass occurrence of sponges rests on an up to 1 m thick spicule mat, a stable and porous substrate that is perfect for settlement of sponge larvae and buds. Species identification and community structure investigation was made possible by direct sampling supported by ROV HD photage. The glass sponges dominating at the seamounts top are the previously well-known arctic species: *Schaudinnia rosea*, *Trichasterina borealis*, *Scyphidium septentrionale*, and *Asconema foliata*. The most common species of these are *S. rosea*, and this is also the most frequently reported species from the whole arctic area. The hexactinellid community changes with depth, and *S.rosea* extend down to the mid-depth areas of the seamount (ca. 1400 m - 1800 m), where it is joined by individuals of species *Amphidiscella monai*. At the deepest part of the seamount the hexactinellids *A. monai*, *Caulophacus arcticus*, and *Asconema megaatrialia*, are all present. *A. megaatrialia*, described as late as in 2007, is surprisingly the most common species below ca. 2000 m. ROV photage show that there are still unidentified hexactinellids present at the Schulze Massive.

**Phylogenomics reveals polyphyly of haploscleromorph clades and provides insight into the early evolution of sponges**

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With the recent focus on the monophyly of sponges, the internal relationships between the four classes of sponges have attracted less attention. For the last two decades sponges have been divided into two groups based on either the nature of their skeleton, Calcarea + Silicea (hexactinellids + demosponges including homoscleromorphs), or based on the syncytial organization of the hexactinellids, Symplasma (hexactinellids) + Cellularia (sponges with cellular organization: Calcarea + demosponges + homoscleromorphs). More recently Homoscleromorpha was erected as the fourth class of sponges, but its phylogenetic affiliations with other sponge groups remain contentious, in part because the intra-class relationships of sponges have been

addressed using mostly single gene phylogenies or combinations of few genes. Here, a phylogenomic approach using protein-encoding genes has been applied to examine deep relationships within sponges. This analysis included 22 sponges (twelve of them sequenced de novo with Illumina RNAseq technology) resulting in two different matrices based on different orthologous gene sets, one including 78 genes (SCPD; Torruella et al., 2012) and the other containing 128 genes (P127; Philippe et al., 2009). The SCPD matrix provided higher resolution for major sponge clades, although calcareous sponges suffered from systematic artifacts when using the P127 matrix. The analysis of the SCPD matrix showed a monophyletic Porifera with a deep split between a homoscleromorph + calcareous sponge clade and a hexactinellid + demosponge clade. Within demosponges, the clades G1 to G4 were recovered. A long suspected placement of Spongillidae within the G4 group instead of within G3 (Haploscleromorpha) is supported by our analyses. Our results provide insight into the early evolution of sponges and one of our data sets supports monophyly of Porifera.

### **Stress protein HSP70 from marine sponge *T. muricata***

Kerli Vallmann, Carmen Kivisild, Annika Lopp  
*Tallinn University of Technology*

The cellular response to stress involves activation of a number of specific signal transduction pathways in order to preserve functions which are vital to cells. Heat shock response is one of the most common stress responses, in which the heat shock proteins act as molecular chaperones. This response is directed to retain or restore cellular homeostasis of proteins.

The members of the HSP70 protein family are among the most conserved and widely studied stress proteins. The expression levels of HSP70 proteins have widely been used as molecular markers for various types of environmental stress – from elevated temperatures to different pollutants. The sequence of the protein has also been used in phylogenetic analysis of prokaryotes and eukaryotes.

In the present study the HSP70 expression was characterized in a series of samples from the marine sponge *Thenea muricata* which were collected in the framework of the SPONGRAM (Sponge Risk Assessment and Monitoring) project. The main goal of the SPONGRAM project was to assess the potential negative impact on deep water sponge communities caused by offshore oil and gas drilling activity. The results of the project indicated stress conditions for the sponges kept in sea water and sediment culture. In this study the expression of HSP70 proteins was studied on the protein and mRNA levels. As there were few molecular data about *T. muricata* available, it was the first time that HSP70 was characterized in this sponge. Several fragments from *T. muricata* encoding HSP70 family proteins were described. The obtained sequences were compared to those from other organisms.

**One Tree Island Research Station**

Ana Vila-Concejo, Maria Byrne, Maddie Rosenthal  
*One Tree Island Research Station/ The University of Sydney*

One Tree Island Research Station is located on the southern part of the Great Barrier Reef, approximately 100 kilometres off the Queensland. It is a scientific research zone (SRZ) which means that it can only be visited by researchers under strict permit conditions. This is the only entire reef system on the barrier reef with this status and has been a SRZ for decades. The research station is on an outer reef island ? 7 km from the continental shelf where, aside from the sheltered lagoon, the reefs are exposed to ocean waves from the South Pacific. The sheltered lagoon system provides easy access to study sites. The research station is efficiently managed by two station managers. OTIRS embraces sustainability running on solar power majority of the time. Fresh water is collected from rainfall, is UV sterilised and filtered to 1 micron.

OTIRS can accommodate up to 26 persons. There are several laboratories: the dry laboratory has 12 linear metres of bench space, a small bench top centrifuge and 3 to 6 microscopes; the aquarium-wet laboratory is a sheltered outdoor space bordering the shore with running filtered seawater; finally, there is an air-conditioned laboratory to run temperature and light controlled experiments.

The station has two main vessels (up to 11 m) which are skippered by the station managers and can be used for research. There are also several smaller vessels which are available to visitors for undertaking their research; use of the smaller boats is included in the benchfees.

Come and work with us! We will help you with the permits!

**Molecular synapomorphies as diagnostic characters for separating cryptic species in the *Leucetta chagosensis* species complex**

Oliver Voigt, Miriam Küstner, Andrea Murillo, Gert Wörheide  
*LMU Munich, Department of Earth and Environment Sciences, Palaeontology & Geobiology*

*Leucetta chagosensis* (Calcarea, Calcinea, Clathrinida, Leucettidae) is distributed from the Red Sea to the central Pacific Ocean. Previous studies suggested that the species comprises a complex of morphologically more-or-less undistinguishable cryptic species. We extended an elaborated genetic dataset of nuclear genes (ITS region, partial 28S, atps beta-intron II) with two mitochondrial markers (partial cox2 and partial cox3) and performed phylogenetic and phylogeographic analyses. Our results corroborate the deep genetic splits between different clades of sponges that fit the morphological definition of *Leucetta chagosensis*. The overlapping distribution of some clades suggests the existence of reproductive barriers between some clades. We present molecular synapomorphies that can distinguish the different clades and discuss the possibility to define species based upon these characters.

## **SPECIAL - Sponge Enzymes and Cells for Innovative Applications**

Joana R. Xavier, SPECIAL Consortium

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Running from December 2010 to November 2013, the SPECIAL project aims at delivering breakthrough technologies for the biotechnological production of cellular metabolites and extracellular biomaterials from marine sponges. These include a platform technology to produce secondary metabolites from a wide range of sponge species, a novel in vitro method for the production of biosilica and recombinant technology for the production of marine collagen. Alongside this research, the project is focused on applications, such as the identification and characterization of potential anticancer drugs and novel biomedical/industrial applications of biosilica and collagen, hereby taking advantage of the unique physico-chemical properties of these extracellular sponge products.

Among the results achieved so far, a large variety of sponges from different habitats, namely Mediterranean Sea, Red Sea, Azores and Caribbean Sea (Curaçao) have been collected. Sponge extracts have been screened for anti-tumor activity. Several candidate compounds have been revealed, for which antitumor mechanisms have been characterized and shown to involve both impaired cell cycle progression and prominent induction of apoptotic cell death. In vivo studies for these promising anti-tumor compounds in mouse tumor models are ongoing. Furthermore, protocols for the in vitro production of secondary metabolites have been developed; the successful mariculture of two species has been established; sponge-derived collagen has been characterised along with the genes regulating its biosynthesis, then successfully transferred in a yeast-model for in vitro production; nature-made scaffolds for tissue engineering approaches have been investigated; the in vitro culture of sponge cells with a primmorph development state has been achieved. SPECIAL newsletters are available through the project website (<http://www.project-special.eu/>), while videos on project topics are available through the project SPECIAL YouTube channel (<http://www.youtube.com/user/projectSPECIALeu>).

**Molecular Taxonomy of Seventeen South Australian Sponges by Analyses of 28S rDNA, COI mtDNA and ITS2 rDNA Sequences**

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The molecular taxonomy of 17 South Australian sponge specimens, representing 12 sponge species belonging to seven different orders Dendroceratida, Poecilosclerida, Hadromerida, Verongida, Astrophorida, Haplosclerida and Dictyoceratida, were inferred from combined analyses of 28S rDNA, cytochrome oxidase subunit I (COI) mtDNA, and internal transcribed spacer 2 (ITS2) rDNA sequences. Among them there are five species with the cluster similarity lower than 97%. The selected three sets of primers are suitable and effective to amplify most of the three different target genes. All the BLAST results using three sequences match each other exactly at order level. Only seven sponge species belonging to seven different families could be inferred according to 28S rDNA sequencing results. Combining 28S rDNA and COI mtDNA data, three additional species belonging to three different families were inferred. The ITS2 region is commonly used as a high resolution marker to study intra- and inter-specific evolution in sponges. Based on ITS2 rDNA sequencing, two more species belonging to two different families can be inferred. Using all three group sequencing results, a combination of the COI mtDNA with 28S and ITS2 rDNA markers for sponge molecular taxonomy is a more reliable approach than using one DNA marker alone. This work is the first molecular study of South Australian sponges using a combination of three DNA markers for identification.

### **Distribution of a barrel sponge in the Eastern Gulf of Thailand**

Thamasak Yeemin, Watchara Samsuvan, Mathinee Yucharoen, Paulwatt Nuclear,  
Makamas Sutthacheep  
*Ramkhamhaeng University/ ajamangala University of Technology Krungthep*

Sponges are sessile filter feeders comprising an important component of the high biodiversity on coral reefs. Species richness, abundance and biomass of sponges are comparable to hard and soft corals. Sponges, especially barrel sponges, can provide refuge for many small organisms which are critical to benthic-pelagic coupling. Moreover, sponges can produce secondary metabolites with important ecological and pharmaceutical roles. A barrel sponge *Xestospongia testudinaria* is a common sponge found on coral communities in the Gulf of Thailand. This species is an attractive invertebrate for divers. The focus on this study was to examine the abundance of the barrel sponge at five study sites, i.e. Ko Salak, Ko Jan, Hin Bai Dung, Ko Loa Nok and Ko Phrao Nok, in Mu Ko Chang, Trat Province, the Eastern Gulf of Thailand. Belt transects were used to estimate the abundance of the sponge and the cover of live and dead corals. The average density of the barrel sponge found on the five reefs varied from 0.7 colony per 100 m<sup>2</sup> at Ko Phrao Nok to 6 colonies per 100 m<sup>2</sup> at Hin Bai Dung. There were significantly more colonies of the barrel sponge at Hin Bai Dung and Ko Jan. These study sites are in relatively deep water and high percentage cover of live corals, compared with other reef sites. The low abundance of the barrel sponge at Ko Phrao Nok was related with shallow water and the low percentage cover of live corals. As economics of Mu Ko Chang depends on incomes from coral reef-based tourism, conservation of coastal living resources for tourism, like sponges, is essential. Clearly, the condition of sponges on coral reefs in the Gulf of Thailand needs to be monitored regularly to understand long-term trends and relation with environmental factors. Future work is recommended to further investigate the population ecology of marine sponges in the Gulf of Thailand.

**SPONGE CULTURE IN TUNISIA (SOUTH OF MEDETIRRENEAN SEA):  
PRELIMINARY RESULTS Tunisia is the major country in Mediterranean Sea  
harvesting bath sponges. Hippospongia communis is the main bath sponge  
species exploited in Tunisia. Hippospongia communis, pre**

Souad Zarrouk,

*Institut des Sciences et Technologie de la Mer*

Tunisia is the major country in Mediterranean Sea harvesting bath sponges. Hippospongia communis is the main bath sponge species exploited in Tunisia. Hippospongia communis, previously abundant around the Kerkennah Islands and offshore benches of Zarzis (Tunisia South eastern littoral), is subjected to an overexploitation in these traditional fisheries. Sponges have a relatively fast growth rates in the Tunisian tempered waters (Golfe de Gabès). This suggests the possibility of a special sponge's aquaculture potential in this area. One of commercial sponges preservation strategies is to develop its culture in situ to reduce the human exploitation pressure by fishing. Within a sustainable development context, Hippospongia communis aquaculture experiments were conducted in Kerkennah Islands and Zarzis offshore waters. In this paper, we describe these experiments and the preliminary results.