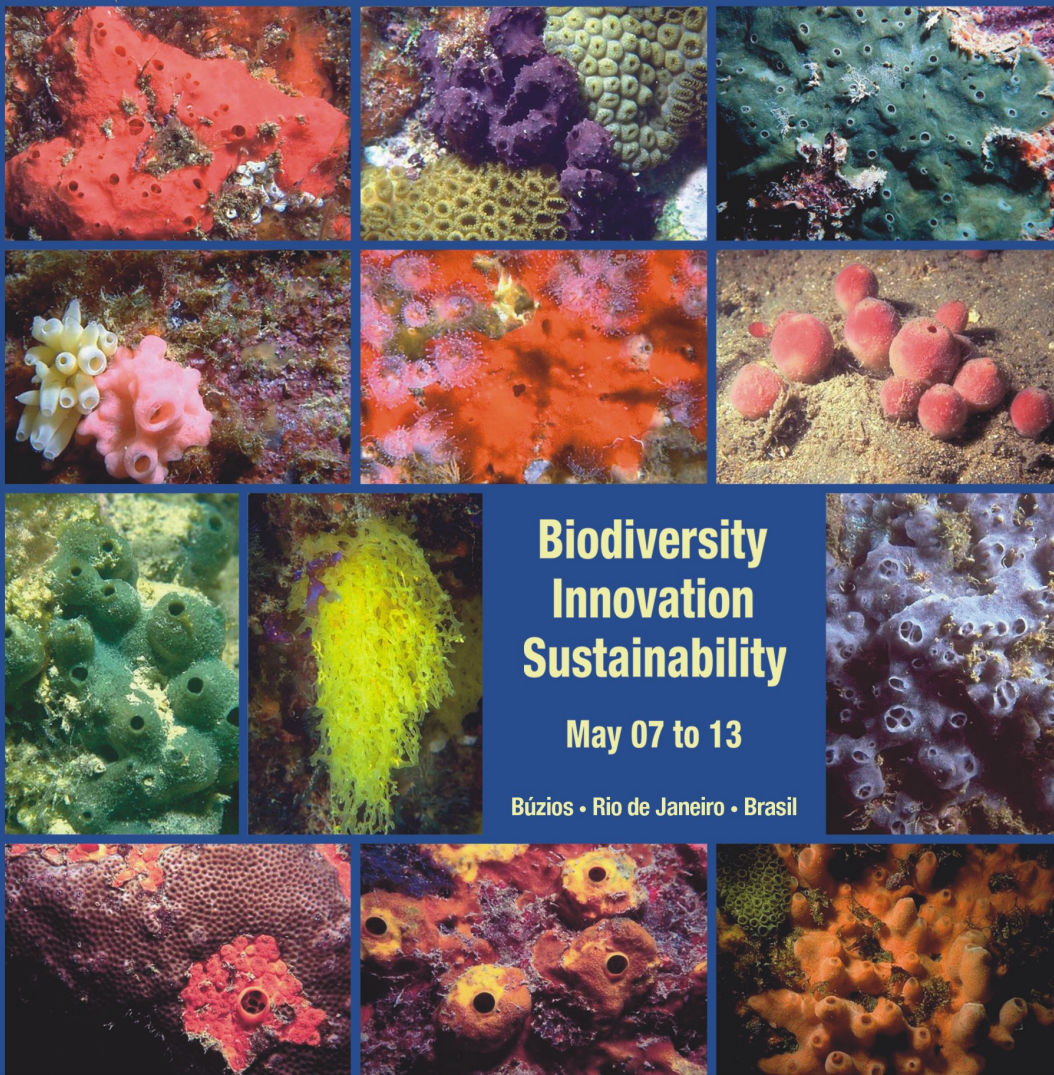


7TH INTERNATIONAL SPONGE SYMPOSIUM

2006



**Biodiversity
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May 07 to 13

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Book of Abstracts

7TH INTERNATIONAL SPONGE SYMPOSIUM

BIODIVERSITY, INNOVATION, SUSTAINABILITY

BOOK OF ABSTRACTS

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7th INTERNATIONAL SPONGE SYMPOSIUM
BIODIVERSITY, INNOVATION, SUSTAINABILITY

BOOK OF ABSTRACTS

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Márcio Reis Custódio
Gisele Lôbo-Hajdu
Eduardo Hajdu
Guilherme Muricy

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PREFACE

The International Sponge Symposium (ISS) is the main meeting about Porifera in the world, with a history almost 40 years long. From May 7 to 13, 2006, in Rio de Janeiro State, Brazil, the city of Búzios will be joining London (1968), Paris (1978), Woods Hole (1985), Amsterdam (1993), Brisbane (1998), and Rapallo (2002), which have already hosted this symposium in earlier editions. The Museu Nacional of the Universidade Federal do Rio de Janeiro (MN-UFRJ) and the Sociedade dos Amigos do Museu Nacional (SAMN) are honored to organize the seventh edition of the sponge symposium. The Museu Nacional, which is the oldest research center in Brazil, has one of the largest sponge collections in Latin America, with nearly 15.000 specimens.

The 7th ISS is the first international meeting of this series happening in Latin America and the largest so far, with nearly 300 participants registered, and over 300 abstracts distributed in 14 sessions: Developmental Biology, Cell Biology and Physiology, Molecular Biology and Biochemistry, Ecology, Systematics and Biogeography, Population and Ecological Genetics, Paleontology, Natural Products Chemistry, Porifera Collections, Human Health and Education, Special Habitats, Phylogeny and Evolution, Biotechnology and Pharmacology, and Microbiology. In this book, the abstracts are arranged by sessions and in alphabetical order of authors in each session.

It would have been impossible to organize the 7th ISS and this Book of Abstracts without the help of many people and sponsors. Our deepest acknowledgements go to the authors of the abstracts published along these pages for their scientific contribution, which is the heart of the symposium. Next, we owe much to the members of the Scientific Committee and the Chairmen of each scheduled session in the Symposium for a smooth handling of the large amount of abstracts received and all the accompanying electronic correspondence. Finally, the early positive responses by some sponsors made us confident that many more people on the different decision-taking levels of Brazil's system for the support of scientific and technological endeavours believed on us, and of course, on sponges. We are grateful to all for their contribution to the success of the 7th ISS.

Welcome to all, and have a nice and productive symposium!

Márcio Reis Custódio, Eduardo Hajdu,
Gisele Lôbo-Hajdu & Guilherme Muricy

Editors

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Tissue homeostasis, repair and regeneration: The concept of stem cells

Radovan Borojevic{ XE "Borojevic, Radovan" }

Instituto de Ciências Biomédicas. Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde,
Av. Pau Brasil s/n. CEP 21.940-590, Rio de Janeiro, Brazil

All the tissues are derived from a single cell, the zygote. The progressive differentiation of cells for specific functions and positions in tissues is frequently understood as a restrictive phenomenon. The Haeckel's gastraea theory claims the initial segregation of ectoderm and endoderm, with a posterior generation of mesoderm, involving a subsequent restriction of cell destinies following the basal pattern of the three embryonic leaflets. A similar chronology in higher animals is frequently observed. Albeit transitions between the differentiation patterns of gene expression and behaviour, such as epithelio-mesenchymal transition, are often observed both in embryogenesis and in adult tissues, a concept of restrictions imposed by initial cell commitment and differentiation corresponding to embryonic leaflets is deeply rooted. Recent studies have widely shown that the tissue homeostasis as well as tissue capacity of growth, repair and regeneration depend upon stem cells. Resident tissue stem cells can generate various subtypes of cells belonging to their own tissue. Several recent reports have claimed transdifferentiation of tissue stem cells across the embryologic borders, but the presence of blood vessels and circulating bone-marrow-derived cells in all tissues has given alternative proposals. The bone marrow apparently harbours multipotent stem cells, able to give origin to all the other tissue cells, including those of the germinal lineage. These cells regularly circulate in blood vessels, they may be physically associated to the vessel wall structure, and they may transmigrate into the tissues involved by repair and regeneration. They may be understood thus as a systemic reserve of multipotent cells, equivalent to embryonic stem cells prior to differentiative restrictions. When necessary, they can be mobilised from bone marrow to circulation by tissue injury. They can give support to the tissues in which chronic injury, degeneration or senescence exhausted the capacity of local resident stem cells, and which require a systemic supply of new cells. Bone-marrow stem cells have thus their own function in producing all the blood cell lineages, but may be simultaneously the systemic regulators of the tissue capacity of regeneration. Such a concept has been already proposed for archaeocytes in sponges. They act normally as circulating tissue macrophages, but can function also as systemic stem cells that can grant regeneration of all the other sponge tissues, when required. Admitting that the evolutionary step from Protista to Porifera represents crossing the border between unicellular and multicellular organisms, the hierarchical organisation of the systemic and tissue stem cells systems may be understood as fundamental for understanding the maintenance of tissue homeostasis and granting the capacity for tissue repair and regeneration.

South American continental sponges: State of the art of the research

Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecilia" }

Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul. Rua Dr. Salvador França, 1427.
Bairro Jardim Botânico. Cep. 90.690-000. Porto Alegre, RS. Brazil, cvolkmer@fzb.rs.gov.br

The first descriptions of South American freshwater sponge species were produced in the 19th century and were based on a few specimens gathered in rivers Orinoco, Amazon and Uruguay by foreign expeditioners. The next taxonomic efforts date of the middle of the last century when Bonetto & Ezcurra started the surveying of Argentinian continental sponges and Volkmer-Ribeiro that of the Brazilian ones. At that time the authors faced a confusing situation in what respected the taxonomy of the world freshwater sponges long ago splitted by Carter's taxonomic proposals. The problem was however overcome due to the remarkable differences that the new materials presented when compared with the descriptions available for Nearctic species, in particular. That way an outstanding number of new species was described which resisted the continued studies and surveyings being carried up to present time. The appearance of Penney & Racek's 1968 comprehensive revision of the world freshwater sponges offered next a sound basis for revisional studies. The first concern at this point was to attain the best possible understanding of which characteristics appeared as trustworthy in dried preserved materials to work with. Following that purpose a genus with distribution in several continents was chosen for deep revisive study. Genus *Metania* Gray, 1867 was then the one elected. An array of characteristics came to light which allowed a confirmation of Gray's feeling of the value of gemmoscleres and microscleres into genus definition and species characterization. Also out of this revisional effort the time elapsed to attain speciation was for the first time glimpsed for a group of continental sponges i. e. the Cretaceous drifting apart of the Gondwanan plates, not to speak of the resilience value of some generic aspects of the spicules of sponges and the new evidences favoring passive mechanisms of invasion of the continental habitats. Upon this settled paradigm a continued revisive effort was extended to all genera of South America continental sponges, allowing for a more confident redefinition of monospecific genera and the definition of new genera as well as description of new species. A quite different picture stands now of the diversity of this South American fauna as one of the richer if not the richest of the world. The species and genera are now subjected to constant checking offered by continued and extended surveyings and habitat descriptions in the continent. Such procedures are at the same time enabling and enhancing confidence in a series of applications of this taxonomic knowledge, such as the use of sponge species and assemblages into biomonitoring and habitat preservation strategies, and of spicules captured in quaternary sediments into paleoenvironmental and climatic interpretations, or of spicules present in archeological ceramics into archeological studies.

Porifera collections around the world. A round-table status report for year 2005

Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(1*); Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }⁽²⁾; John N.A. Hooper{ XE "Hooper, John N.A." }⁽³⁾; Henry M. Reiswig{ XE "Reiswig, Henry M." }⁽⁴⁾; Klaus Rützler{ XE "Rützler, Klaus" }⁽⁵⁾; Toufiek Samaai{ XE "Samaai, Toufiek" }⁽⁶⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽⁷⁾; Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }⁽⁸⁾; Wei Zhang{ XE "Zhang, Wei" }⁽⁹⁾

(1) Dept. Invertebrados. Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil, hajdu@acd.ufrj.br; (2) Dept. of Embryology, Biological and Soil Sciences Faculty, St. Petersburg State University, Russia, aereskovsky@mail.ru; (3) Centre for Biodiversity, Queensland Museum, Australia, JohnH@qm.qld.gov.au; (4) Dept. of Biology, University of Victoria, and Natural History Section, Royal British Columbia Museum, Canada, hmreiswig@shaw.ca; (5) Dept. of Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, Ruetzler.Klaus@NMNH.SI.EDU; (6) CSIR, Environmentek, Coastal programme, South Africa, TSamaai@csir.co.za; (7) Zoologisch Museum van de Universiteit van Amsterdam, The Netherlands, soest@science.uva.nl; (8) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Brazil, cvolkmer@fzbr.rs.gov.br; (9) Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Weizhang@ms.dicp.ac.cn

A pioneering appraisal of Porifera collections was made by Shirley Stone in 1986, curator of the sponge collection housed in The Natural History Museum, London. Her efforts were primarily focused on old historical collections. Ever since, in spite of global efforts to convey biodiversity-based studies, such as on the systematics of extant species, no light has been shed on an updated database on the care status and localization of poriferan collections. Given the extensive bibliography on sponge biodiversity produced along the last decades, coupled to the fact that a vast majority of sponges needs to be worked on in the laboratory in order to be identified, one can infer that new collections have certainly been assembled, and old collections became much larger. It is the objective of this contribution to summarize the status of sponge collections around the world by the end of year 2005, by assembling a database to include the following pieces of information: 1, official name of institution; 2, abbreviation or acronym; 3, official mailing address; 3, mailing address of Porifera collection manager; 4, name and e-mail of curator in charge of Porifera by end of 2005; 5, additional staff currently working with Porifera; 6, size of collection; 7, approximate proportion of collection data already entered on a database; 8, geographical coverage (political or geographic origin of collections as a percentage of the whole collection); 9, type series housed (and major authors & any expeditions of significance); 10, fire security and climate control; 11, availability to outsiders (both physical access and potential to borrow specimens by mail); and 12, prospect of expansion (including acceptance of collections made by others). Data will be presented on a national/continental size frame. The MNRJ Brazilian collection is offered here as an example. 1, Museu Nacional; 2, MNRJ; 3, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil; 4, E. Hajdu (hajdu@acd.ufrj.br) & G. Muricy (muricy@acd.ufrj.br); 5, one temporary technician; 6, 14000 catalogued specimens, 0 uncatalogued, and corresponding slide collection - nearly 70% databased; 7, 90% Brazil, 10% elsewhere (Caribbean, NE Atlantic, Mediterranean, SE Asia, SE Pacific); 8, 96 primary type specimens (24 holotypes and 72 paratypes, mainly from the work by Hajdu, Muricy et coll.), 83 schizotypes (mainly Boury-Esnault, Hentschel, Ridley & Dendy, Sollas, Van Soest), 3 syntypes and 160 microscopic preparations from type specimens, major expeditions: N.O. "Almirante Saldanha", REVIZEE; 9, manually operated fire extinguishers, large sized air conditioners to be installed in the near future; 10, available upon contact with curators, DNA access upon obtention of special permits; 11, the collection is growing nearly exponentially, construction of new building planned for the near future; and 12, possibly double in a decade, usually accepts any collections made by others.

An evaluation of the financial feasibility of small-scale commercial sponge culture in Pohnpei, Federated States of Micronesia

Charles Adams{ XE "Adams, Charles" }^(1*); John Stevely{ XE "Stevely, John" }⁽²⁾; Donald Sweat{ XE "Sweat, Donald" }⁽³⁾

(1) University of Florida, Gainesville, Florida, PO Box 110240, 32611, USA; (2) Florida Sea Grant, 1303 17th St. West, Palmetto, FL 34221, USA; (3) Florida Sea Grant, 830 First Street South, St. Petersburg, FL 33701, USA

The commercial culture of native sponge has received increased attention in the Federated States of Micronesia and the surrounding areas. This historical analysis examines the financial feasibility of small-scale culture by family units on the island of Pohnpei, FSM. The scale of sponge farming activities chosen for this analysis is not intended to be the sole source of family income, but rather a source of supplemental income. A farm with one-acre of water-surface area was chosen for this analysis. The current technology for culturing sponges in a lagoon setting was also applied. In addition, unit prices for supplies, labor, etc. that were current at the time of the study were utilized. Based on the prices and assumptions utilized, a one-acre commercial sponge farm can generate a positive annual net return of \$1.744 to the owner's capital, labor and risk. The return per hour of labor expended on the farm is approximately \$2.30. Returns remained positive (\$784) when foregone wages were considered. The estimated production cost of farmed sponges is \$0.27 per sponge. A sensitivity analysis is performed on several key management and production variables and the effects of these variable changes to returns, cost per sponge, and break-even survival rate are presented. Profitability was found to be most sensitive to changes in opportunity wage rate, survival rate, and market price. The continuing interest in sponge farming in the FSM region and the continually growing market for high quality, natural sponges warrants this study be revisited and potentially revised to investigate any changes in technology and financial feasibility.

Relative temporal gene regulation in cultured *Axinella corrugata* cells upon exogenous perturbation by phytohemagglutinin M form

Jay J. Bottesch{ XE "Bottesch, Jay J." }; Jane H. Thompson{ XE "Thompson, Jane H." }; Shirley A. Pomponi{ XE "Pomponi, Shirley A." }^(*)

Division of Biomedical Marine Research Harbor Branch Oceanographic Institution, Inc. 5600 U.S. 1 North
Fort Pierce, FL 34946 USA

The production of a continuously dividing sponge cell line has been unsuccessful to date. In this study, we elucidate some of the genetic machinery involved in actively growing cultured sponge cells to identify targets for gene regulation to create an immortalized cell line or a normal cell line with extended life span. Cryopreserved *Axinella corrugata* cells were thawed and grown in nutrient medium with phytohemagglutinin M form (PHA-M) for five days. Upon confirmation of increased cell number, total RNA was isolated for cDNA production and amplified with 28 different primer sets encompassing DNA replication machinery, interleukins, and cell response. Several genes known to be involved in cell proliferation were significantly upregulated with PHA-M stimulation [nuclear protein -AT (NPAT), chromatin assembly complex-2 (CAC-2), calmodulin (CMD), and interleukin 15 (IL-15RA)] while others were downregulated [interleukin 5 (IL-5) and interleukin 10 (IL-10)]. Results were confirmed with continuous real-time RT-PCR labeled with SYBR Green I.

Effect of ingenamine G isolated from the marine sponge *Pachychalina* sp. on *in vitro* chromosomal aberrations and DNA strand breaks in human lymphocytes

Bruno Coêlho Cavalcanti{ XE "Cavalcanti, Bruno Coêlho" }⁽¹⁾; Carla Maria Lima Sombra{ XE "Sombra, Carla Maria Lima" }⁽¹⁾; Jaine H.H.L. De Oliveira{ XE "Oliveira, Jaine H.H.L. De" }⁽²⁾; Roberto Gomes de Souza Berlinck{ XE "Berlinck, Roberto Gomes de Souza" }⁽²⁾; Manoel O. de Moraes{ XE "Moraes, Manoel O. de" }⁽¹⁾; Claudia O. Pessoa{ XE "Pessoa, Claudia O." }^(1*)

(1) Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Coronel Nunes de Melo Street 1127, 60430-270 Fortaleza, CE, Brazil; (2) Chemistry Institute of São Carlos, University of São Paulo, CP 780, 13560-970, São Carlos, SP, Brazil

Marine organisms have been attracting attention as potential sources of bioactive substances and continue to provide novel lead compounds for the pharmaceutical industry. Many of these substances have the ability to induce many types of DNA lesions. The consequences of this effect could potentially lead to carcinogenesis. The present study evaluated the *in vitro* genotoxic effects of ingenamine G isolated from the new Brazilian marine sponge species *Pachychalina* sp. in human lymphocytes assessed by chromosomal aberrations test and alkaline comet assay (SCGE). Isolated human peripheral lymphocytes were obtained by Ficoll-Histopaque gradient. About 2×10^5 cells were treated for 3 hr without S9-mix at 5, 10, 15, 20 and 40 $\mu\text{g/mL}$ of ingenamine G for both assays. The mitotic index was determined for 3,000 cells and given as the number of mitoses per 100 cells (%) and 100 well-spread metaphases per culture were analyzed for the presence of chromosomal aberrations. For SCGE, 100 randomly selected cells (50 cells from each of the two replicate slides) were analyzed for each concentration of the test substance. Cells were scored visually into five classes, according to tail size (from undamaged - 0, to maximally damaged - 4) and a value (damage index) was assigned to each comet according to its class. Damage index thus ranged from 0 (completely undamaged: 100 cells \times 0) to 400 (with maximum damage: 100 cells \times 4). The damage frequency (%) was calculated based on the number of cells with tails versus those without. The vehicle was used as negative control and doxorubicin (0.3 $\mu\text{g/mL}$) was used as positive control in both assays. Ingenamine G significantly reduced the mitotic index at all concentrations ($3.6 \pm 0.21^*$, $2.8 \pm 0.2^*$, $2.5 \pm 0.2^*$, $2.1 \pm 0.2^*$ and $1.6 \pm 0.26^*$ at 5, 10, 15, 20 and 40 $\mu\text{g/mL}$) and clearly showed an increase in the number of aberrations at the three highest concentrations, inducing chromosome and chromatid breaks. The SCGE showed a significant increase of the DNA damage index ($102.0 \pm 6.53^*$, $143.3 \pm 11.22^*$ and $183.6 \pm 9.0^*$ at 15, 20 and 40 $\mu\text{g/mL}$, respectively) and damage frequency ($32.33 \pm 4.5^{**}$, $47.3 \pm 8.7^*$, $64.0 \pm 4.5^*$ and $76.3 \pm 7.5^*$ at 10, 15, 20 and 40 $\mu\text{g/mL}$, respectively) for ingenamine G in human lymphocytes (* $p < 0.001$; ** $p < 0.05$). It was observed evidences for the genotoxicity of ingenamine G and confirms the usefulness of the chromosomal aberration test and SCGE for evaluation the balance between the genotoxic proprieties of the drug and its therapeutics effects.

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Comparison of growth performances of *Spongia officinalis* var. *adriatica* off the Southern Italian coast (Central Mediterranean Sea) in relation to different rearing techniques

Giuseppe Corriero{ XE "Corriero, Giuseppe" }^(*); Caterina Longo{ XE "Longo, Caterina" };
Maria Mercurio{ XE "Mercurio, Maria" }; Carlotta Nonnis Marzano{ XE "Marzano, Carlotta
Nonnis" }

Zoology Department - Bari University - Via Orabona, 4 - 70125 Bari, Italy, g.corriero@biologia.uniba.it

Mediterranean bath sponges of the genus *Spongia* are considered of commercial value for their absorption properties. In the last decades their availability has been reduced by the depletion of natural banks due to the fishing pressure together with repeated epidemics. At present, the commercial supply is below the demand. Sponge farming is a low-technology activity which could play a role in the Mediterranean aquaculture, where the diversification of species and rearing systems is considered a challenge for the future. Since 1996 the Zoology Department of Bari University has been studying the rearing of *S. officinalis* var. *adriatica*. We report here the rearing performances of this sponge in three culture systems: two suspended along the water column and another placed on the bottom. The trials were carried out off the Ionian coast of SE Italy (Central Mediterranean). Two types of suspended structures were prepared: in the former nylon ropes were sewn through the sponge cuttings; the ropes were then tied up to a plastic frame vertically assembled along the water column and anchored to the bottom, with floaters on the upper end. The latter consisted of tubular nylon nets similar to those used for mussel culture, divided into compartments each containing a sponge cutting, and tied along a horizontal rope. The third system was constituted by cement bearings placed on the bottom, with stainless steel needles to support sponges. Wild specimens of sponge were cut into pieces. Fifty grams proved to be the most suitable initial weight for cultivation purposes since it allowed to limit the waste of wild sponges. In general most of the cuttings reached the commercial size after three years of rearing. The final shape of the cuttings reared on suspended systems was mainly rounded, while on steel needles, close to the cement, the adhesion to the substrate caused the development of lobes similar to those of natural specimens. The mortality rate resulted comparable (about 25%) for the suspended systems, while it was lower (<10%) on cement bearings. Generally, a peak of mortality was observed during the first year, when two main factors were acting on the recovery capability of the sponges: manipulation (collection, cutting and sewing) and cicatrisation of tissue. In suspended structures additional mortality occurred in the last two years as a result of competition with fouling organisms. In nylon nets the dominance of large filtering organisms in competition with sponges caused inadequate growth in 17% of the sample. In contrast, on cement bearings the growth of fouling organisms was lower than that observed on suspended structures allowing to limit the need for underwater maintenance. Among the experimented rearing systems, the latter was the easiest to carry out and the most time-saving. Moreover cement bearings could be displaced on shallower bottoms, thus resulting the most suitable system for cultivating *S. officinalis* var. *adriatica* in the study site.

Developing methods for farming bath sponges

Alan R. Duckworth{ XE "Duckworth, Alan R." }^(*); Carsten Wolff{ XE "Wolff, Carsten" };
Elizabeth Evans-Illidge{ XE "Evans-Illidge, Elizabeth" }

Australian Institute of Marine Science, PMB No 3 Townsville, QLD 4810, Phone: +61 7 4753 4444.
Australia, a.duckworth@aims.gov.au

For sponge aquaculture to supply sufficient and sustainable quantities of bath sponges, suitable farming methods need to be developed. In this study, we developed farming methods for *Coscinoderma* sp. and *Rhopaloeides odorabile*, two Australian species with commercial importance. In one experiment we tested 11 methods, grouped into four farming categories: threaded line, cut-explant (explants cut half way through then rope placed in the middle), spike (hard plastic pushed through) and mesh. For each farming category, we trialled different material types (e.g. nylon and polyethylene) or compartment sizes. After 1 year, most *Coscinoderma* farmed in mesh survived, probably because these methods are less damaging to the explant. In contrast, survival in the other farming categories was poor with many explants dying within six months. Unlike survival, growth of *Coscinoderma* was similar among the farming methods with explants doubling in size. Survival of *R. odorabile* also varied between farming methods, being highest (90-100%) when farmed in mesh or on threaded lines. Growth of *R. odorabile* was also highest using these methods, with most explants doubling or tripling in size over one year. In a separate experiment, we examined whether survival of *Coscinoderma* and *R. odorabile* farmed on threaded lines could be improved by letting the cut explants heal first inside mesh for several months (nursery period) before being threaded. For both sponge species, the nursery period did not improve final explant growth or survival. Overall, *Coscinoderma* and *R. odorabile* grew and survived well when farmed in mesh. This method is now being modified to commercially farm both bath sponge species in Australia.

Sponges as natural composites: From biomimetic potential to development of new biomaterials

Hermann Ehrlich{ XE "Ehrlich, Hermann" }^(*); Hartmut Worch{ XE "Worch, Hartmut" }

Max Bergmann Center of Biomaterials, Institute of Materials Science, Dresden University of Technology,
Budapester Str. 27, D-01069 Dresden, Germany, Hermann.ehrlich@mailbox.tu-dresden.de

Investigations of the compositions and the microstructures of the sponge skeletons (spicules, fibers, filaments) as examples for natural structural biomaterials are of fundamental scientific relevance. Here we present a study of two discoveries related to glass sponges (Hexactinellida) and to keratose sponges (Verongida: Demospongia) which we made recently. The first is the finding of collagen within basal spicules of *Hyalonema sieboldi*. To test our hypothesis that an organic matrix within the *H. sieboldi* spicules is responsible for the extraordinary mechanical properties, structural (SEM, TEM, AFM, LSM, XRD, FTIR) and biochemical (HPLC, MALDI, LTQ) analyses of these glassy composites and extracted proteinaceous components were performed. We applied a new approach for the desilicification of glass sponge spicules based on a slow etching procedure using alkali solutions at 37°C during 14 days. As a result of the application of this method, data was obtained unequivocally confirming the presence of a collagen which acts as template for the biomineralization of silica. To verify whether the collagen scaffold shapes the morphology of the spicules, we carried out *in vitro* experiments in which we exposed collagen isolated from the spicule of *H. sieboldi* to silicic acid solution. We obtained rod-like structures of several mm diameter of similar shape to the sponge spicules. The ultrastructural analysis of these self-assembled, collagen-silica composites demonstrates that amorphous silica is deposited on the surface of collagen fibrils in the form of nano-pearl necklets, closely resembling the nanoparticulate structure of natural spicules. The second discovery is based on the results of a detailed study of the structural and physico-chemical properties of skeletal fibers of some keratose sponges (*Verongula gigantea*, *Aiolochoira crassa*) with the aim of confirming our hypothesis that chitin must be an essential component of their fibrous skeletons. The main practical approach we used for chitin isolation was based on alkali treatment of corresponding exoskeletal sponge fibres with the aim of obtaining alkali-resistant material for detailed analysis. The structural similarity of chitin derived from *V. gigantea* and *A. crassa* exoskeletal fibres to invertebrate alpha-chitin has been confirmed by us unambiguously using FTIR, Raman, XRD and specific staining-based LSM techniques. From this point of view, investigations of the chitin estimation in sponge exoskeletons are of great scientific importance especially for evolutionary research and sponge systematics. A comprehensive understanding of chitin-covered sponge fibres with respect to chemical composition and structure may prove to be a novel model for biomimetic synthesis of sponge-like chitin-based composites with specific elastomeric and bioactive properties for applications in different modern technologies including material sciences and biomedicine.

**Geographic and temporal variation of manoalide analogues in the sponge
Luffariella sp.**

Piers Ettinger-Epstein{ XE "Ettinger-Epstein, Piers" }^(1*); Rocky de Nys{ XE "Nys, Rocky de"
}⁽¹⁾; Dianne M. Tapiolas{ XE "Tapiolas, Dianne M." }⁽²⁾

(1) School of Marine Biology & Aquaculture, James Cook University, Townsville, QLD, Australia; (2)
Australian Institute of Marine Science, Townsville, QLD, Australia.

The manoalide group of compounds are secondary metabolites of the sponge *Luffariella* sp. They are potent inhibitors of the enzyme Phospholipase A2 (PLA2) which is used as a molecular tool in the study of cancer in humans. Manoalide also has applications as an anti-inflammatory/anti-allergy compound. Patterns of quantitative variation in the commercially valuable compound, manoalide and its analogues, secomanoalide and luffariellin-A, and an additional compound manoalide monoacetate were monitored over one year in the sponge *Luffariella* sp. (Demospongiae; Dictyoceratida; Irciniidae) at three locations (separated by kilometres to 10s of kilometres) in the Palm Islands, Central Great Barrier Reef, Queensland, Australia. This was to determine the potential yield from wild harvest or aquaculture. A baseline study quantifying the four above compounds was made using HPLC for individuals over time. There was little variation in chemistry within or between individuals throughout the year at all locations. Mean levels of manoalide were ~15-20 mg/g dry weight of sponge and levels of manoalide monoacetate ~35-50 mg/g dry weight of sponge. The rank abundance of the four compounds was consistent throughout the study with manoalide and manoalide monoacetate being the most abundant. Studies have been done to determine the effect of biological parameters on the production of manoalide and its analogues.

Antimitotic effect on sea-urchin eggs of marine sponges collected at the Pedra da Risca do Meio state park, Ceará, Brazil

Elthon G. Ferreira{ XE "Ferreira, Elthon G." }⁽¹⁾; Paula C. Jimenez{ XE "Jimenez, Paula C." }⁽¹⁾; Diego V. Wilke{ XE "Wilke, Diego V." }⁽¹⁾; Thiago A. Portela{ XE "Portela, Thiago A." }⁽²⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽³⁾; Claudia O. Pessoa{ XE "Pessoa, Claudia O." }⁽¹⁾; Manoel O. de Moraes{ XE "Moraes, Manoel O. de" }⁽¹⁾; Edilberto R. Silveira{ XE "Silveira, Edilberto R." }⁽²⁾; Letícia V. Costa-Lotufo{ XE "Costa-Lotufo, Letícia V." }^(1,4)

(1) Depto. de Fisiologia e Farmacologia - UFC; (2) Depto. de Química Orgânica e Inorgânica - UFC; (3) Museu Nacional - UFRJ; (4) Laboratório de Ciências do Mar - UFC

Marine sponges are well-know as a rich source of bioactive substances. This study aimed on screening 21 sponge species colleted at the Pedra da Risca do Meio State Park, Ceará, off the Northeast coast of Brazil for antimitotic activity in the *Lytechinus variegatus* sea-urchin eggs. The collection took place at the Pedra da Risca do Meio State Park, located at A, 3°33'800" S e 38°26'000" O; B, 3°36'000" S e 38°26'000" O; C, 3°36'000" S e 38°21'600" O; D, 3°33'800" S e 38°21'600" O, and depth raging from 17 to 30m. A small sample of each species was separated for identification and the rest was immersed in 100% ethanol and stored at low temperatures. The solvent was removed and the solid residues were assayed for anti-mitotic activity. The sea-urchins were stored in a water tank under controlled conditions of salinity and temperature. The gametes liberation was induced by injection of KCl 0,5 M in the coelomic cavity, washed properly and mixed. Fertilization was insured by the observation of the fertilization membrane around the egg. The extracts derived from the 21 species were tested at 500 µg/mL and the effects were observed after the 1st cleavage of the egg and at the blastulae phase. Most of the sponge derived extracts showed some kind of effect on the sea-urchin eggs embryogenesis. The species *Amphimedon compressa*, *Monanchora arbuscula*, *Agelas dispar*, *Agelas clathrodes*, *Agelas* sp., *Aplysina* n.sp. 1, *Aiolochoiria crassa*, *Ircinia strobilina* and *Hyattella intestinalis* showed a more pronounced inhibitory effect of the cell division, as the treated cells were mostly undivided. The species *Geodia corticostylifera*, *Topsentia ophiraphidites*, *Aplysina* n.sp. 2, *Aplysina fistularis*, *Aplysina* ssp. *solageae*, *Callyspongia vaginalis* and *Verongula* sp. incited abnormal cell divisions, as the treated cells did not present regular sizes of their blastomeres or even a regular cell division pattern. The species *Aplysina fulva* had a mixed effect, as there were undivided and abnormal cells. *Niphates* sp., *Pseudosuberites* sp. and *Dragmacidon reticulatus* had no effect on the sea-urchin cell division.

Effects of feeding regimens on clearance and retention rates of the Mediterranean sponge species *Agelas oroides* (Demospongiae, Agelasida) and *Petrosia ficiformis* (Demospongiae, Haplosclerida)

Cristina Ferretti{ XE "Ferretti, Cristina" }^(1*); Simona Canepa{ XE "Canepa, Simona" }⁽¹⁾.
Antonio Sará{ XE "Sarà, Antonio" }^(1, 2, 4); Roberto Pronzato{ XE "Pronzato, Roberto" }⁽¹⁾.
Renata Manconi{ XE "Manconi, Renata" }⁽²⁾; Alan R. Duckworth{ XE "Duckworth, Alan R." }⁽³⁾

(1) DIPTERIS - Dipartimento per lo Studio del Territorio e delle sue Risorse, University of Genoa, Corso Europa, 26, I-16132 Genoa, Italy, cristina.ferretti@dipteris.unige.it; (2) DIPZAB - Dipartimento di Zoologia e Antropologia Biologica, University of Sassari, Via Muroni, 25, I-07100 Sassari, Italy; (3) AIMS - Australian Institute of Marine Science, PMB No 3, Townsville, QLD 4810, Australia

In vitro sponge culture is one of possible method to supply bioactive compounds with pharmaceutical potential. Considering that food may be one of the most important limiting factor in an *in vitro* sponge culture, we examined the effect of several diets on the growth of two Mediterranean sponge species, *Agelas oroides* and *Petrosia ficiformis*. Sponge explants (about 1 cm³ in size) fed a mixed diet composed by the microalgae *Chlorella sorokiniana* (2-3 µm in diameter), the Gram-negative bacterium *Escherichia coli* (2-3 µm in diameter), the yeast *Saccharomyces cerevisiae* (9-10 µm in diameter) at different concentrations and a commercial liquid food (CLF). To better represent the sponges' natural diet, two size classes of particles were considered on the base of the natural concentration (NC) from the sampling site: 1-4 µm (200000 cells/ml) and 4-10 µm (40000 cells/ml). The diets were 1NC, 3NC, 5NC and CLF. Explants were fed 3 times a week for 105 days. Explant growth, examining wet weight (g) (WW) and volume (ml) (V), was recorded at the start, end and every 10 days of experiment. We also examined the clearance and retention rates of the different diets by both species. Final survival rates of explants of both species were 97.50%, and all the explants of *A. oroides* and 45% of the explants of *P. ficiformis* regenerated their cut surfaces within 15-20 days. For both species, one-way ANOVA showed that final percentage of WW and V were similar between diets. Between all the treatments, *A. oroides* explants lost, on average, WW but increased their V to 115.11% of initial volume. Explants of *P. ficiformis* maintained their WW unchanged between all the treatments but their V increased, on average, to 118% of initial volume. Two-way ANOVA determined that clearance rate by both species was similar for the examined food type but differed for concentrations. The overall clearance rates of *A. oroides* and *P. ficiformis* were 9.9 l/h/gDW and 1.47 l/h/gDW, respectively. For both species, the mean retention rates of *C. sorokiniana* and *S. cerevisiae* differed among the examined diets.

Effects of crude extracts from the Mediterranean sponge species *Agelas oroides* (Demospongiae, Agelasida) and *Petrosia ficiformis* (Demospongiae, Haplosclerida) on human neuroblastoma cell lines

Cristina Ferretti { XE "Ferretti, Cristina" }^(1*); Cinzia Domenicotti { XE "Domenicotti, Cinzia" }⁽²⁾,
Barbara Marengo { XE "Marengo, Barbara" }⁽³⁾; Chiara De Ciucis { XE "Ciucis, Chiara De" }⁽²⁾,
Maria Adelaide Pronzato { XE "Pronzato, Maria Adelaide" }⁽²⁾; Roberto Pronzato { XE "Pronzato,
Roberto" }⁽¹⁾; Renata Manconi { XE "Manconi, Renata" }⁽⁴⁾

(1) DIPTERIS - Dipartimento per lo Studio del Territorio e delle sue Risorse -University of Genova, Corso Europa, 26, I-16132 Genova, Italy, cristina.ferretti@dipteris.unige.it; (2) DIMES - Dipartimento di Medicina Sperimentale -University of Genova, Via Leon Battista Alberti, 2, I-16132 Genova, Italy; (3) Istituto Giannina Gaslini, Largo G. Gaslini 5, 16148 Genova, Italy; (4) DIPZAB - Dipartimento di Zoologia e Antropologia Biologica - University of Sassari, Via Muroni, 25, I-17100 Sassari, Italy

On the entire marine fauna, sponges (Porifera) are a very rich source of bioactive molecules with biomedical potential and natural products, isolated from marine organisms including Porifera, may have a potential role of antioxidants. In this work, we analysed the effect of winter methanolic crude extracts from two Mediterranean sponge species, *A. oroides* and *P. ficiformis* on two human neuroblastoma cell lines. Our experimental model was represented by two human neuroblastoma cell lines: SK-N-BE(2)-C and LAN5, that were treated for 15 min and 30 min with 5, 10, 20 and 50 µg/ml of methanolic crude extracts of the two species. Treatments with *P. ficiformis* induced necrosis of both cell lines in all experimental conditions while exposures to *A. oroides* caused a different cytotoxic effect on the two cell lines. All concentrations of *A. oroides* extract tested at 30 min of exposure were highly cytotoxic inducing necrosis in both cell lines. A different behaviour was observed at 15 min of treatment; in particular, 5 µg/ml concentration did not show a cytotoxic effect while 10 and 20 µg/ml induced an increase by 35% and 43% (compared to control), respectively, of necrotic LAN5 cells. The same doses (10 and 20 µg/ml) caused respectively an increase (compared to control) by 85% and 66% of necrotic SK-N-BE(2)-C cells. Moreover, the maximal dose tested, 50 µg/ml of sponge extract, markedly (80%) increased the rate of necrosis in both cell lines. Analysing the appearance of apoptotic features, LAN5 cells treated with 5 or 10 µg/ml of sponge extract did not show a positivity to Annexin-V staining, and only 20 µg/ml concentration markedly enhanced (1.5 fold vs. control) apoptosis. The same doses were applied to SK-N-BE(2)-C cells and we found that exposure to 5 and 10 µg/ml reduced apoptosis by 42-48%, respectively vs. control; while 20 µg/ml did not cause any change. Interestingly, 5 µg/ml induced, in both cell lines, the appearance of a phenotype characterised by the presence of some membrane extensions that were associated neither with Annexin-V-positive staining nor propidium iodide labelling. To evaluate the ability of sponge extract to generate reactive oxygen species (ROS), we measured the number of DCF-DA positive cells. Both cell lines were characterised by basal ROS production and exposure to 10 µg/ml treatment enhanced ROS levels by 18% in SK-N-BE(2)-C while 20 µg/ml concentration increased ROS levels by 2.5 fold vs. control in LAN5 cells. These preliminary data suggest that *A. oroides* crude extract may have a biphasic effect dependent on the concentration. In fact, the minimal dose (5 µg/ml) keeps low basal ROS levels and seems to be ineffective on tumoral cells while the maximal dose 20 µg/ml has a pro-apoptotic effect more evident in LAN5 cells where it is probably triggered by the increased generation of oxidant species.

Sponge culture in a small harbour: The case study of the demosponges *Agelas oroides* (Agelasida) and *Petrosia ficiformis* (Haplosclerida) in the W-Mediterranean Sea

Cristina Ferretti{ XE "Ferretti, Cristina" }^(1*); Simone Vacca{ XE "Vacca, Simone" }⁽¹⁾; Roberto Pronzato{ XE "Pronzato, Roberto" }⁽¹⁾; Renata Manconi{ XE "Manconi, Renata" }⁽²⁾; Alan R. Duckworth{ XE "Duckworth, Alan R." }⁽³⁾

(1) DIPTERIS - Dipartimento per lo Studio del Territorio e delle sue Risorse, University of Genoa, Corso Europa, 26, I-16132 Genoa, Italy, cristina.ferretti@dipteris.unige.it; (2) DIPZAB - Dipartimento di Zoologia e Antropologia Biologica, University of Sassari, Via Muroni, 25, I-07100 Sassari, Italy; (3) AIMS - Australian Institute of Marine Science, PMB No 3, Townsville, QLD 4810, Australia

The potential of sponge culture for bioremediation was investigated in the harbour of S. Margherita (Ligurian Sea), an environment with a moderately low anthropic impact. New methods and technologies are used in this 6 months lasting experiment for the achievement of sponge biofilters to reduce the negative impact by organic pollution and to improve the environmental quality by production of natural products. The plant typology consisted of plastic box modules with a reticulated bottom (SAFEAQUAPLAN®) containing up to 24 sponge explants of about 27 cm³ in size. The structures were positioned in the small harbour at 1 and 2 m of depth under two jetties in different environmental conditions (outer area vs. innermost area). Three modules of 2 boxes were positioned under each jetty. Wild specimens of *Agelas oroides* and *Petrosia ficiformis* were collected at 10-20 m of depth from the Portofino Promontory in Autumn 2004. The collected sponges were cut using sharp and sterile scalpels into cube-shaped explants, approximately 27 cm³ in size. The volume of each explant was measured using the volume displacement method. All the fragments of *P. ficiformis* lost their typical violet colour showing a white pinacoderm due to the loss of autotrophic symbionts. This white colour is typical of individuals of the same species living in caves where the illumination is quite low or completely absent. Final survival rates of *A. oroides* and *P. ficiformis* were 37.50% and 55.56%, respectively. Overall, the growth of *A. oroides* explants slightly increased after 6 months, however two-way ANOVA showed that the growth significantly differed between jetties, depths and interaction, being greatest for explants cultured at the jetty at -1 m in the innermost area (170.66% of initial volume). Overall, *P. ficiformis* explants grew to 110.11% of initial volume and two-way ANOVA showed that the explants growth significantly differed between jetties, depths but no interaction. Overall, the greatest final percentage of volume was for explants cultured at the outer area jetty at -2 m (142.52% of initial volume).

***Aplysina aerophoba* - A model sponge for *ex situ* sponge biomass production?**

Rudolf Hausmann{ XE "Hausmann, Rudolf" }^(1*); Marco P. Vitello{ XE "Vitello, Marco P." }⁽²⁾;
Frank Leitermann{ XE "Leitermann, Frank" }⁽¹⁾; Sebastian Rühle{ XE "Rühle, Sebastian" }⁽¹⁾;
Matthias Voigt{ XE "Voigt, Matthias" }⁽¹⁾; Christoph Sylдатk{ XE "Sylдатk, Christoph" }⁽¹⁾

(1) Engler-Bunte-Institut, Lehrstuhl für Technische Biologie, Universität Karlsruhe (TH) Karlsruhe; (2)
University of Stuttgart, Institute of Biochemical Engineering, Stuttgart, Germany; now at the Max-Planck-
Institute for Biological Cybernetics, Tübingen, Germany

Sponges are a promising source of organic compounds of potential interest regarding industrial and medical applications. For detailed studies on such compounds, large amounts of sponge biomass are required. Obtaining that is at present extremely difficult because most sponges are relatively rare in nature and their mass cultivation in the laboratory has not yet been accomplished. It is notoriously difficult to keep these animals in a laboratory, and most sponges are prone to display a loss of biomass and ultimately die. In this study the possibility of culturing *Aplysina aerophoba* fragments in the laboratory was examined. *A. aerophoba* was chosen because of the relatively well studied microbial associations, the interesting bromo-compounds known, and because of the good availability of the species. The primary aim of this study was to set up a model sponge cultivation unit completely independent of natural sea water. This is a prerequisite for a further development of biotechnological sponge cultivation techniques. Therefore, a completely automated cultivation setup was constructed which allows the screening of cultivation conditions for sponge fragments under completely artificial, but controlled laboratory conditions. Sponge tissue was fragmented and the fragments were cultivated on three types of artificial substrates. The applicability of projected body area measurements for growth measurements proved to be unreliable, as the morphological variability of *A. aerophoba* is extremely high. Because of this plasticity, no assured growth rates could be described. Neither, any correlation between feed cells and morphological development could be found in *A. aerophoba* fragments. However, an essential result is that *A. aerophoba* fragments of a few mm size do have the potential to reorganize their body to a functional sponge, displaying an osculum. This is an important prerequisite for a technical reproduction of *A. aerophoba* in culture.

Screening of extracts of marine organisms from the South Adriatic coast for biological activity

Zoran Kljajic{ XE "Kljajic, Zoran" }^(*); Slavica Kascelan{ XE "Kascelan, Slavica" }; Vesna Macic{ XE "Macic, Vesna" }

Institute of Marine Biology, Dobrota bb, 85330 Kotor, Serbia and Montenegro, zkljajic@ibmk.org; skascelan@yahoo.com; marikultura@cg.yu

As a part of our search for compounds with biological activities from marine organisms, we have investigated antimicrobial, cytotoxic and hemagglutinating activities of lipid and water extracts from 94 marine organisms from the South Adriatic coast. We examined the biological activities of extract of 39 algal species (20 red algae, 14 brown algae and 5 green algae), 33 sponges, 6 coelenterates, 2 polychaete worms, 3 mollusks, 3 echinoderms and 8 tunicates. Antimicrobial activities (45 lipid and 25 water extracts) were tested against gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus*, a gram-negative bacterium *Escherichia coli*, and a yeast *Saccharomyces cerevisiae*. Most extracts of algae exhibited a weak antimicrobial activity. A moderate or a strong activity was detected with 25% Chlorophyta, 31% Rhodophyta, and 38% Phaeophyta. Among the invertebrates, 50% Porifera, 75% Anthozoa and 25% Tunicata showed the antimicrobial activity. The activity was moderate or strong with extracts of 45% species of Porifera and 50% of Anthozoa. Cytotoxic activity was determined against the L5178y mouse lymphoma cell *in vitro*. Most algal extracts again had some activity. A moderate or a strong activity was found with 14% Phaeophyta species, 45% Rhodophyta species and 67% Chlorophyta species. A moderate or a strong activity was exhibited by extracts of 44% sponges. Hemmagglutinating activity screening is a crucial initial phase for early selection of marine organisms for isolation of lectins. As lectins have different carbohydrate specificities, various types of red blood cells have been used (sheep red blood cells treated or untreated by neuraminidase, human red blood cells of types A, B and O). Among algae, 65% showed the activity (80% Chlorophyta, 50% Rhodophyta, and 79% Phaeophyta). Marine invertebrates had also a high incidence of activity: extracts of 78% Porifera, 83% Anthozoa, 50% Annelida, 67% Mollusca, 100% Echinodermata and 67% Tunicata agglutinated erythrocytes. From these preliminary investigations of various types of biological activities, species that merit further investigation have been selected. Statistically, our results are in general agreement with those obtained in other screening surveys.

Sponge farming in a shallow water pilot plant: Survival and life style

Renata Manconi { XE "Manconi, Renata" }^(*); Fabio Ledda { XE "Ledda, Fabio" }; Maria Francesca Ortu { XE "Ortu, Maria Francesca" }; Giacinta Stocchino { XE "Stocchino, Giacinta" }

Dipartimento di Zoologia e Antropologia Biologica dell'Università, Via Muroni 25, I-07100, Sassari, Italy,
r.manconi@uniss.it

Long-term sponge culture was carried out in an enclosed bay within the NW Sardinian Marine Protected Area “Capo Caccia - Isola Piana”. We aimed to create a small easily accessible *in situ* laboratory to test cultivability, sponge reactions to farming conditions and to investigate perspectives for a sustainable exploitation. The area selected for experiments has been located within a yachting marina in very shallow water (3 m depth). Two pilot plants, composed of two squared 1x1 m USAMA® modules and three basket case SAFEQUAPLAN® modules, were anchored to the submerged piers of the marina. Fragments of wild specimens belonging to 8 species (*Aplysina aerophoba*, *Sarcotragus spinosulus*, *Ircinia variabilis*, *Ircinia* sp., *Hippospongia communis*, *Petrosia ficiformis*, *Crambe crambe*, *Cacospongia scalaris*) were settled in the plants and monitored during the four years lasting experiments. Survival, growth rate and body remodelling after fragmentation, life styles in plant and adhesion to artificial substrates were evaluated. Sponge culture appears successful for most tested species that revealed a high ability to survive and to increase biomass in farming conditions producing over 300% of the starting biomass, like in the case of *S. spinosulus*. Cultured specimens showed different reproductive modes by asexual propagules (*A. aerophoba*) or sexually by larvae (*S. spinosulus*). Farming techniques will be furtherly set up and activities will be focused on biomass production, repopulation strategies and bioremediation processes in environments with different degrees of human impacts.

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Antimicrobial activity of Caribbean reef sponges (north-west Gulf of Urabá, Colombia)

Alejandro Martínez{ XE "Martínez, Alejandro" }^(1*); Elkin Galeano Jaramillo{ XE "Jaramillo, Elkin Galeano" }⁽²⁾; Diego F. Valderrama{ XE "Valderrama, Diego F." }⁽³⁾

(1) Universidad de Antioquia, Facultad de Química Farmacéutica. A.A 1226. Teléfono (574) 210 5473, Director del Grupo de Productos Naturales Marinos, amart@farmacia.udea.edu.co; (2) Universidad de Antioquia, Facultad de Química Farmacéutica. Grupo de Productos Naturales Marinos, elgaja@farmacia.udea.edu.co; (3) Universidade Federal do Rio de Janeiro, Instituto de Biologia - Departamento de Zoologia, Laboratório de Biologia de Porífera, Av. Pau Brasil, 211 - CCS - Bloco A - Sala A0-100, Ilha do Fundão - Rio de Janeiro - RJ - Brasil, diego_valderrama@lycos.com

Antimicrobial and antifungal activities of 72 extracts of different polarity (methanol, chloroform and n-hexane) from 24 species of Caribbean sponges were assayed against three pathogenic microorganisms: *Escherichia coli* (Gram-negative, ATCC 25922), *Staphylococcus aureus* (Gram-positive, ATCC 25923) and *Candida albicans* (fungus, ATCC 10231). Sponge samples were collected by scuba diving in shallow reefs (9-21 m depth) of Gulf of Urabá, in Colombia (southern Caribbean). Extracts were tested for antimicrobial activity at experimental concentrations of 500 µg/sensidisc by the agar diffusion assay method protocol described by Kirby-Bauer and modified by the World Health Organization (WHO) and the National Committee for Clinical Laboratory Standard (NCCLS). Bioassay results revealed that 19 species (79%) yielded crude extracts which displayed antimicrobial activity against at least one microorganism isolate. Seven sponge species (29%) crude extracts inhibited the three pathogenic microorganisms. *Leucetta* aff. *floridana* and *Cinachyrella kuekenthali* yielded the largest number (78%) of active extracts. *Aplysina archeri* and *Ircinia strobilina* "white" yielded extracts which exhibited the most potent antimicrobial activity, with values frequently exceeding those of a control antibiotic (Gentamicin-10 µg or Nistatin-100 units). Although the test panel of microorganisms tested here was very limited, the broad polarity range of extractions used was successful for detecting widespread antimicrobial activity of local reef sponges. It is strongly encouraged the search of sponge bioactive natural products in the poorly explored Gulf of Urabá, in Colombia.

Maintaining marine sponges in tanks: Effects of feeding and iron

Ronald Osinga{ XE "Osinga, Ronald" }^(1*); Michiel Kotterman{ XE "Kotterman, Michiel" }⁽²⁾

(1) Porifarma BV, Poelbos 3, 6718 HT Ede, The Netherlands, ronald.osinga@porifarma.com; (2)
Netherlands Institute for Fisheries Research, Haringkade 1, 1976 CP IJmuiden, The Netherlands,
michiel.kotterman@wur.nl

Three species of Mediterranean sponges, *Dysidea avara*, *Chondrosia reniformis* and *Acanthella acuta* were maintained for more than a year in indoor cultivation systems in The Netherlands. Different regimes for feeding were tested, which consisted of microalgae, bacteria and dissolved organic carbon (peptones). Recently, it was demonstrated that iron (Fe^{3+} ions) play an important role in sponge morphogenesis and physiology. Hence, we also added different concentrations ferric citrate to the seawater in the culture systems and compared pulsed administration of iron with continuous administration. None of the experiments resulted in a significant increase in biomass of the sponges. However, continuous addition of $3 \mu\text{M Fe}^{3+}$ in combination with deep-frozen *Phaeodactylum tricornutum* (a marine diatom) as a food source resulted in a positive physiological response of the sponges: all species increased their pumping activity under these conditions. The effect was most clear on *C. reniformis*. The presence of oscula had not been observed for more than 6 months on explants of this species. After the addition of ferric iron, the explants started to develop new oscula that showed a clear pumping activity. We conclude that continuous administration of Fe^{3+} is important to maintain pumping activity of sponges in tanks. Measurement on iron concentrations in natural seawaters support this view: we found high concentrations of iron in areas that have a rich sponge fauna. Within the framework of a joint European research effort (the SPONGES project), we will execute research to develop better feeds for sponges in culture and to further optimize the chemical water composition for sponge culture tanks

Antifouling activity of marine sponges

K. Padmakumar{ XE "Padmakumar, K." }^(*); M. Srikumar{ XE "Srikumar, M." }; S. Deepa{ XE "Deepa, S." }

Centre for Marine Biodiversity, Kerala University, Campus Kariavattom, Thiruvananthapuram, Kerala,
PIN-695581, India, k.padmakumar@vsnl.com

Many sedentary marine organisms have epibionts or biofoulers on their surface. But, a variety of sedentary plants and animals in the marine environment are also free from external fouling. These organisms have evolved strategies to defend themselves from the threat of fouling. Many marine sponges are among such organisms known to employ chemical defense to deter potential fouling organism. As a part of the search for marine derived non-toxic antifouling compounds, about 95 species of marine sponges collected from Indian coast was evaluated for their antifouling activity against potential foulers such as bacteria (*Vibrio alginolyticus*, *Vibrio vulnificus*, *Vibrio harveyi*, *Vibrio parahaemolyticus*), diatoms (*Navicula* spp., *Nitzschia palea*), barnacle (*Balanus amphitrite*) and mussel (*Perna indica*). Among this, 28% of the species showed antibacterial activity against fouling bacteria and 37% inhibited fouling diatoms, 18% showed antibarnacle cyprid settlement and 21% of sponges showed inhibition of mussel attachment. Through bioassay guided fractionation, antifouling compounds were isolated. The most promising organisms with potential antifouling activity are *Axinella tenuidigitata*, *Clathria frondifera*, *Dysidea fragilis*, *Dysidea herbacea*, *Haliclona cribricutis*, *Haliclona pigmentifera*, *Hyattella cribriformis*, *Iotrochota baculifera*, *Oceanapia fistulosa*, *Petrosia similis*, *Phycopsis* sp., and *Psammaphysilla purpurea*.

Effects of compounds isolated from the Brazilian sponge *Arenosclera brasiliensis* on the cytoskeleton integrity of cultured cells

Marisa Paula do Prado{ XE "Prado, Marisa Paula do" }⁽¹⁾; Marisa Rangel{ XE "Rangel, Marisa" }⁽²⁾; Katsuhiro Konno{ XE "Konno, Katsuhiro" }⁽²⁾; Gláucia Maria Machado-Santelli{ XE "Machado-Santelli, Gláucia Maria" }^(1*)

(1) Department of Cell and Developmental Biology, University of São Paulo, SP, Brazil; (2) Center of Applied Toxicology, Butantan Institute, São Paulo, SP, Brazil

Since 1951, when Bergman and Feeney first isolated an arabino-nucleoside from the marine sponge *Tectitethya crypta*, the attention on marine natural products has increased. Within marine animals, sponges (phylum Porifera) have the highest rate of cytotoxic compounds with 10% of the investigated species showing some cytotoxic activity. Many of these compounds are not general cytotoxic agents but rather are targeted towards specific cellular and biochemical events. Marine compounds that interfere with microtubules integrity can be used as antitumoral drugs since it is direct involved with mitosis. In our study, we isolated 2 compounds from the Brazilian marine sponge *Arenosclera brasiliensis* aiming to evaluate their cytotoxicity and their effects on microtubules organization of T47D cultured cells. The sponge was collected at Búzios, Rio de Janeiro and maintained in methanol. The organic extract of the sponge was subjected to solvent partitioning and the methanol/H₂O soluble fraction was then purified by SepPaK® C18 columns and HPLC. Two compounds showed prominent cytotoxic effects with an IC₅₀ = 5µg/mL, in an MTT assay. In a Confocal analysis, these two compounds also produced strong effects on the microtubule integrity of the treated cells. The decrease of normal filamentous structures that were observed, suggest that these compounds may participate in the disassembly of the microtubules networks. The discovery of novel compounds that act as microtubule destabilizing agents, mainly on the mitotic spindle, provides an opportunity to develop new antitumoral drugs improving the therapy or even develop specific tools in the study of cellular biology.

Anti-proliferative substances of the marine sponge *Geodia corticostylifera* from Brazilian coast

Marisa Rangel{ XE "Rangel, Marisa" }^(1*); Marisa Paula do Prado{ XE "Prado, Marisa Paula do" }⁽²⁾; Katsuhiko Konno{ XE "Konno, Katsuhiko" }⁽¹⁾; Hideo Naoki{ XE "Naoki, Hideo" }⁽³⁾; José Carlos de Freitas{ XE "Freitas, José Carlos de" }^(1,4); Glaucia Maria Machado-Santelli{ XE "Machado-Santelli, Glaucia Maria" }⁽²⁾

(1) Center for Applied Toxinology, Butantan Institute, Sao Paulo, 05503-900, Brazil, mrangel@butantan.gov.br; (2) Dept. of Cell Biology and Development, Biomedical Sciences Institute, University of Sao Paulo, Sao Paulo, 05508-900, Brazil; (3) Okinawa Health Biotechnology Research DeVelopment Center, 12-75 Suzaki, Gushikawa, Okinawa 904-2234, Japan; (4) Dept. of Physiology, Biosciences Institute, University of Sao Paulo, Sao Paulo, 05508-900, Brazil

Crude extracts of the marine sponge *Geodia corticostylifera* from Brazilian coast showed previously antibacterial, antifungal, cytotoxic, haemolytic and neurotoxic activities. At the present work we describe the isolation of the cyclic peptides geodiamolides A, B, H and I from the methanol extract of *G. corticostylifera* using RP-HPLC (Reversed Phase High Performance Liquid Chromatography). In a preliminary series of experiments these peptides inhibited the first cleavage of sea urchin eggs (*Lytechinus variegatus*), and EC₅₀ values varied from 100 to 600 nM. Duplication of nuclei without complete egg cell division indicated the mechanism of action should be related to microfilaments disruption. Further studies showed that the geodiamolides have antiproliferative activity against human breast cancer cells (T47D and MCF7), and EC₅₀ varied from 19 to 115 nM on T47D cells, and from 10 to 90 nM on MCF7. Using fluorescence techniques and confocal microscopy we found evidence that the geodiamolides A, B, I and H act disorganizing actin filaments of T47D and MCF7 cancer cells, in a similar way to other depsipeptides (such as jaspamide and dolastatins), without affecting microtubules. Normal cells lineages (human fibroblasts and rat liver cells) were much less affected by the treatment than tumor cells, thus indicating the biomedical potential of these compounds.

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Biotechnological cultivation of the marine sponge *Aplysina aerophoba*

Sebastian Rühle{ XE "Rühle, Sebastian" }^(*); Matthias Voigt{ XE "Voigt, Matthias" }; Rudolf Hausmann{ XE "Hausmann, Rudolf" }

Universität Karlsruhe (TH); Engler-Bunte-Institut, Lehrstuhl für Technische Biologie Engler-Bunte-Ring 1,
76131 - Karlsruhe, Germany, sebastian.ruehle@tebi.uni-karlsruhe.de; matthias.voigt@tebi.uni-karlsruhe.de;
rudolf.hausmann@tebi.uni-karlsruhe.de

For the here presented studies on the biotechnological cultivation of sponges the Mediterranean sponge species *Aplysina aerophoba* was chosen as a model organism. The ecological requirements of this species *e.g.* habitat and food requirements are relatively well studied. Furthermore the diversity and abundance of microorganisms associated with *A. aerophoba* are relatively well known. Sponges as suspension filter feeders are adapted to low but relatively constant bacterial concentration in seawater in dimension of 5×10^5 cells/ml. The Phylum Porifera is the only filter-feeding group able to retain particulate matter in the range from 0.2 to 1.0 μm size range. Also larger food particles in size 5-8 μm affect in high concentrations about 4×10^5 cells/ml the filtration rate of sponges. We have established a continuous feeding system, to ensure approximately the natural concentrations of unicellular microorganism in seawater in the cultivation set-up. In contrast to the easy feed of sponge biomass in milligram scale it becomes incredibly difficult in the hundreds of gram scale. Sponges are fed with two marine bacteria strains: *Janobacter limosus* and *Halomonas variabilis*, both with a diameter of approximately 1 μm . Cultivation of *A. aerophoba* is effective under completely artificial conditions for three months now. Experiments to estimate the filtration rates of the sponges and the measurement of oxygen demand have been carried out to enable the calculation of the required amount of supply organisms. For examination we apply sponge explants into small bioreactor units. First trials show a good adhesion on the artificial substrate. The arrangement of many parallel bioreactor units allows the screening of several experiments with different species at the same time. Long-term studies are supposed to show the influence of high cell concentrations to the sponge growth by monitoring cell counts, total organic carbon (TOC) and dissolved organic carbon (DOC). First measurements showed a decrease of decimal power from 8.6×10^5 to 8.6×10^4 cells/ml within 2 hours. The objective is to understand the quantitative and qualitative requirements of the model organism.

Biosilicification machinery in siliceous sponges: Key enzymes and proteins for silica nanobiotechnology

Heinz C. Schröder{ XE "Schröder, Heinz C." }; Anatoli Krasko{ XE "Krasko, Anatoli" };
Matthias Philipp Wiens{ XE "Wiens, Matthias Philipp" }; Werner E.G. Müller{ XE "Müller,
Werner E.G." }^(*)

Institut für Physiologische Chemie, Abteilung Angewandte Molekularbiologie, Universität, Duesbergweg 6,
D-55099 Mainz, Germany, hschroed@uni-mainz.de; krasko@uni-mainz.de; wiens@uni-mainz.de;
wmueller@uni-mainz.de

The major skeletal elements of the Demospongiae and Hexactinellida are spicules formed from amorphous silica (biosilica). Demosponges are unique in their ability to synthesize their silica skeleton enzymatically. In the past few years, we have cloned several isoforms of the silica-forming enzyme, silicatein, both from marine sponges (*Suberites domuncula*) and from freshwater sponges (*Lubomirskia baicalensis*). The silicateins are very similar to the cathepsins, a family of cysteine proteases which do not precipitate silica. In the silicatein sequence, the cysteine residue of the catalytic triad of these proteases is replaced by a serine residue which is essential for the catalytic mechanism of the enzyme. In addition, a hydroxy amino acid (serine) cluster is present in the molecules. Silicatein undergoes posttranslational modification by phosphorylation of the protein as revealed by 2D gel electrophoresis. Using primmorphs (a special form of 3D cell aggregates), we established that spicule formation begins intracellularly and is completed extracellularly. Immunoblotting and immunogold labeling experiments revealed that silicatein is not only present in the axial filament but also at the surface of the spicules. Applying the technique of differential display of transcripts, we identified a further enzyme, the silicase, in *S. domuncula*, which is able to depolymerize amorphous silica. The silicase shares highest similarity to the carbonic anhydrases, a family of zinc metal enzymes. The recombinant sponge enzymes, silicatein and silicase (patents granted), are key enzymes for a variety of potential applications in (nano)biotechnology and medicine, including (i) surface modification of biomaterials, (ii) encapsulation of biomolecules (drug delivery) and (iii) biofabrication of nanostructure materials for opto- and micro-electronics (lithography). The sponge enzymes allow the synthesis or modification of silica under ambient conditions that do not damage biomolecules. Silica is a component of bioactive glasses used as scaffolds in tissue engineering bone and cartilage. We showed that mineralization of bone-forming SaOS-2 cells is markedly increased when the cells are grown on silicatein (biosilica)-modified culture plates. The recombinant silicatein even retains its biocatalytic activity after immobilization of the protein onto gold surfaces and self-assembled polymer layers; formation of interconnected silica nanospheres with a diameter about 70-300 nm is observed. In addition, the immobilised silicatein is also capable of catalysing the synthesis of other metal oxides such as titania and zirconia, at neutral pH and room temperature. Moreover, we showed that silica spicules function as optical glass fibers with unique properties.

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**Does sponge chemical defense correlate with its pharmacological activities?
Examples from Western Pacific sponges**

Peter J. Schupp{ XE "Schupp, Peter J." }

University of Guam Marine Laboratory, UOG Station, Mangilao, 96923 Guam, pschupp@guam.uog.edu

The marine environment is a rich source of natural products. During the last three decades more than 14,000 natural products have been isolated from marine organisms with the focus being novel structures and new pharmacological activities. However, there are few studies that examine the correlation between the ecological and pharmacological activities of secondary metabolites from marine organisms. To assess possible correlations between ecological and pharmacological activities we examined extracts from Western Pacific sponges in terms of fish feeding deterrent properties as well as pharmacological activities. Sponges, being sessile and soft-bodied and thus almost unprotected, are known to employ bioactive secondary metabolites for protection against predators, bacterial pathogens or overgrowth by other organisms. The ecological bioactivity of sponge secondary metabolites often correlates with bioactivity in pharmaceutical assays. Our aim with this study was to 1) provide an overview of chemical defenses in sponges from Guam and surrounding Micronesian islands and 2) to examine possible correlations between ecological and pharmacological activities. To do so we analyzed existing published data on pharmacological activities including data from our collaborator's screenings efforts for anticancer (two leukemia and six solid tumors) and antimicrobial activities (*e.g. S. aureus*) for possible correlations with ecological functions of sponge secondary metabolites (*e.g. anti-feeding activities*). Feeding assays were conducted by incorporating sponge extracts into an artificial diet at natural concentrations and testing the deterrent effect of food containing sponge extracts with food containing solvents only. Sponge crude extracts incorporated in artificial diets were tested in field feeding experiments with omnivorous fishes and in the laboratory with the pufferfish, *Canthigaster solandri*. Preliminary results from 14 different sponges indicate that deterrent activity in fish feeding assays also correlates with activity in pharmacological assays.

Bycatch sponges of fishing nets: An eco-friendly collection method for preliminary bioactivity screening

Joseph Selvin{ XE "Selvin, Joseph" }^(1*); Aron Premnath Lipton{ XE "Lipton, Aron Premnath" }⁽²⁾

(1) Department of Microbiology Bharathidasan University, Tiruchirappalli 620 024, India, selvinj@rediffmail.com; (2) Marine Biotechnology Laboratory Central Marine Fisheries Research Institute, Vizhinjam 695 520, Trivandrum, India, liptova@yahoo.com

The successful development of drugs from the sponges completely relies on the continuous availability of the source organism and the cost effective collection methods. Moreover, it was reported that over-exploitation of the source organism may pose an imminent threat to the natural bed. Considering this lacuna, an eco-friendly collection of the sponges was attempted using the bycatches of fishing nets. Disposal of bycatch from fishing nets is a routine process of fishing operation. The selected station (southwest coast of India) was found to be an excellent area for the collection of diverse marine sponges as 'bycatch'. The landing status of sponges in this coast is more or less same, irrespective of the seasons. However, the trend of frequency and quantity of landings varied according to the fishing area and nets used. It was found that the bycatch landing was high in traditional fishing nets compare trawlers. Ample collection was obtained from the nets such as Echa vala (Greenish colour), which was operated during night hours at a depth of 10 'pakam' (1 'pakam' = 2 meters) and Velameen vala (in otherwise it is called othakundu kangoose vala), which was operated at a depth of 16-20 pakam. The major landing species were: *Callyspongia* sp. and *Sigmatocia* sp., followed by *Dendrilla nigra*, *Clathria gorgonoides*, *Axinella donnani* and other species. All the bycatch collections contained potent biologically active secondary metabolites. When compared to the netting and SCUBA methods, the bycatch collections were more reliable for drug discovery program due to economy, quantity, diversity and eco-friendly collection of the source organisms.

Development of closed-systems for high density culture of the Mediterranean sponge *Dysidea avara* (Demospongiae) for its pharmaceutically-important metabolite, Avarol

Marzia Sidri{ XE "Sidri, Marzia" }^(1*); Dominick Mendola{ XE "Mendola, Dominick" }⁽²⁾; René H. Wijffels{ XE "Wijffels, René H." }⁽²⁾; Ronald Osinga{ XE "Osinga, Ronald" }⁽¹⁾

(1) Porifarma B.V., Ede, The Netherlands; (2) Wageningen University, Food and Bioprocess Engineering Group (WUR)Wageningen, The Netherlands, www.marine.wur.nl

The Mediterranean sponge *Dysidea avara* is sought after for its sesquiterpene hydroquinone natural product avarol, a compound of pharmaceutical interest for its anti-psoriatic and anti-viral activities. Commercial-scale *in situ* cultivation of this species in Greek waters is presently supplying material to support the limited sale of anti-psoriatic skin creams in Germany. Culture of *D. avara* in closed-systems (which are inherently better suited for production of pharmaceutical products) is yet to be demonstrated at anything but the research scale. In this study maintenance and growth of explants made from wild-collected parent-stock (Blanes, Cataluña, Spain) is being accomplished using closed, controlled-environment culture systems currently under development jointly at WUR (Wageningen University, The Netherlands) and PORIFARMA BV (The Netherlands). Key culture parameters (temperature, salinity, dissolved oxygen, dissolved nitrate, and dissolved organic carbon) are monitored and at the out-set manually controlled. Special attention is being given to design of in-tank flow regimes for supporting optimal feeding, respiration, and other metabolic needs of the culture specimens. Special attention is also being paid to optimizing a varied, nutritious and economical diet formulation. We present preliminary growth data in relation to differing dietary components measured as a percentage of variation of underwater weight (uww) of explants. The goal is to improve the culture process and sponge biomass and avarol productivity by application of sophisticated process engineering principals and culture process control sub-systems developed at WUR and PORIFARMA BV. The system will then be expanded to a commercial prototype scale high density sponge culture tank. The system will be developed and perfected for culture of marine sponges in the frame of the SPONGES European Project, in which five innovative European (bio)tech companies and eight top class European research institutions are involved.

Antimicrobial activities in extracts of marine sponges from Rio de Janeiro coast, Brazil

Mara Flávia Lima da Silva{ XE "Silva, Mara Flávia Lima da" }⁽¹⁾; Juliana Andrade Biagio{ XE "Biagio, Juliana Andrade" }⁽¹⁾; Palloma Rodrigues Marinho{ XE "Marinho, Palloma Rodrigues" }⁽¹⁾; Ricardo Machado Kuster{ XE "Kuster, Ricardo Machado" }⁽²⁾; Marley Gomes da Silva{ XE "Silva, Marley Gomes da" }⁽¹⁾; Bruno Mendes Tavares{ XE "Tavares, Bruno Mendes" }⁽¹⁾; Leila de Souza Fonseca{ XE "Fonseca, Leila de Souza" }⁽¹⁾; Maria do Carmo de Freire Bastos{ XE "Bastos, Maria do Carmo de Freire" }⁽¹⁾; Kátia Regina Netto dos Santos{ XE "Santos, Kátia Regina Netto dos" }⁽¹⁾; Marcia Giambiagi-deMarval{ XE "Giambiagi-deMarval, Marcia" }⁽¹⁾; Guilherme Muricy{ XE "Muricy, Guilherme" }⁽³⁾; Marinella Silva Laport{ XE "Laport, Marinella Silva" }^(1*)

(1) Dept. Microbiologia Médica, Instituto de Microbiologia Prof. Paulo de Góes - UFRJ, 21941590, Rio de Janeiro, Brasil, mara_limasil@yahoo.com.br; juzinha_ab@yahoo.com.br; prm@predialnet.com.br; mcbastos@micro.ufrj.br; santoskrn@micro.ufrj.br; marciagm@micro.ufrj.br; marinella@micro.ufrj.br; (2) Núcleo de Produtos Naturais, CCS - UFRJ, 21941590, Rio de Janeiro, Brasil; (3) Dept. de Invertebrados - Museu Nacional - UFRJ, Rio de Janeiro, Brasil, muricy@acd.ufrj.br

The recent appearance of a growing number of bacteria resistant to conventional antibiotics has become a serious medical problem. To overcome this resistance, the development of antibiotics with novel mechanisms of action is a pressing issue. Marine sponges are rich sources of bioactive compounds, some of them can be useful for the development of new pharmacological tools and medicines. Sponges from varied localities have been screened for a variety of biological activities, *e.g.*, antimicrobial, antiinflammatory, antitumor, haemolytic and others. This study reports the *in vitro* screening of fourteen marine sponges collected in Rio de Janeiro, Brazil, in the search for novel antimicrobial compounds. Ethanol and aqueous extracts were tested for antibacterial activities. Ten of the fourteen species tested demonstrated activity in one or more of the bioassays. In antibacterial assays, aqueous extracts of *Cinachyrella* sp., *Mycale microsigmatosa*, *Petromica citrina* and *Polymastia janeirensis* showed a large action spectrum over Gram-positive cocci and *Mycobacterium tuberculosis* HRv37. Aqueous extracts of *Petromica citrina* demonstrated a MIC at 25 mg/mL and MBC \geq 100 mg/mL in an *in vitro* screening assay against *Staphylococcus aureus* ATCC 29213. These extracts are currently undergoing further analysis to identify the active constituents.

Large-scale production of pharmaceuticals by marine sponges: Sea, cell or synthesis?

Detmer Sipkema{ XE "Sipkema, Detmer" }^(1*,2); Harvey W. Blanch{ XE "Blanch, Harvey W." }⁽¹⁾; René H. Wijffels{ XE "Wijffels, René H." }⁽²⁾

(1) University of California Berkeley, department of Chemical Engineering, CA 94720, USA; (2) Wageningen University, Process Engineering group Bomenweg 2, 6700 EV Wageningen, The Netherlands

Marine sponges are known to produce an overwhelming array of secondary metabolites with pharmaceutical applications. The words ‘promise’ and ‘potential’ are frequently used in the conclusion of research papers about the discovery of new sponge-derived metabolites. We have assessed the shelf life of these qualifications by studying the technical and economical potential of using marine sponges for large-scale production of their secondary metabolites. We have considered two extreme cases (with respect to natural product concentration): The anticancer molecule halichondrin B from a *Lissodendoryx* sp; Avarol from *Dysidea avara* for its antipsoriasis activity. An economic and technical analysis was done for three potential production methods for halichondrin B and avarol: 1. Mariculture; 2. *ex situ* culture (in tanks), and; 3. cell culture. We concluded that avarol produced by mariculture or *ex situ* culture could become a viable alternative to currently used pharmaceuticals for the treatment of psoriasis. Production of halichondrin B from sponge biomass was found to not be a feasible process, mainly due to the extremely low concentration of the compound in the sponge. Technical feasibility was also analyzed for five alternatives: chemical synthesis, wild harvest, primmorph culture, genetic modification and semi-synthesis. It was concluded that the latter two approaches could prove to be valuable methods for the production of pharmaceuticals, based on chemical structures of secondary metabolites present in trace amounts in marine sponges.

Transfection of marine sponge cells to produce a cell line

Jane H. Thompson{ XE "Thompson, Jane H." }; Jay J. Bottesch{ XE "Bottesch, Jay J." }; Shirley A. Pomponi{ XE "Pomponi, Shirley A." }^(*)

Division of Biomedical Marine Research Harbor Branch Oceanographic Institution, Inc. 5600 U.S. 1 North
Fort Pierce, FL 34946

Previous attempts to produce a sponge cell line have been unsuccessful. We report the results of transfection of sponge cells using two separate transforming agents: Epstein-Barr virus (human herpes virus 4) and human telomerase reverse transcriptase plasmid (hTERT). Cells of *Axinella corrugata* and *Xestospongia muta* were exposed to media (RPMI 1640) infected with Epstein-Barr virions from a human lymphoblastoid cell line (1A2, ATCC). Cell cultures were treated in separate experiments with a liposome mediated transformation reagent (Lipofectamine) containing human telomerase reverse transcriptase plasmid (hTERT). For both, transfection was optimized by using a concentration gradient of transforming agent to a constant number of cells. Negative controls contained either media without Epstein-Barr virus or reagents without hTERT plasmid. Cells were incubated four to six weeks with a minimum of four complete media changes. After DNA extraction, PCR was performed for BALF2 (DNA binding protein of Epstein-Barr genome) and hTERT. Significant PCR products were produced in both Epstein-Barr and hTERT transformations, which indicates that cell cultures of *Axinella corrugata* and *Xestospongia muta* have been phenotypically modified by uptake of DNA from Epstein-Barr and hTERT plasmid. These results lay the groundwork for establishing both an immortalized cell line as well as a normal cell line with an extended life span.

Primmorphs production: Comparisons among species and importance of environmental factors

Laura Valisano{ XE "Valisano, Laura" }⁽¹⁾; Marco Giovine{ XE "Giovine, Marco" }⁽²⁾; Marina Pozzolini{ XE "Pozzolini, Marina" }⁽³⁾; Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽⁴⁾; Carlo Cerrano{ XE "Cerrano, Carlo" }^(1*)

(1) Dipartimento per lo studio del Territorio e delle sue Risorse, Corso Europa, 26, 16132, Genova, Italy, valisano@dipteris.unige.it; cerrano@dipteris.unige.it; (2) Dipartimento di Biologia, Viale Benedetto XV, 5, 16132, Genova, Italy, mgiovine@unige.it; (3) Advanced Biotechnological Center, Largo Rossana Benzi, 10, 16132, Genova, Italy, marina.pozzolini@unige.it; (4) Dipartimento di Scienze del Mare, Via Breccie Bianche, 60131, Ancona, Italy, bavestrello@univpm.it

In these last years, the discovery of a great number of new bioactive compounds produced by marine sponges has spurred the studies on sponge cell culture in order to obtain large amounts of biomass *in vitro*. In this research field, the most promising results are obtained with the development of a 3D growth system better known as "primmorphs" technique. Up today, these peculiar aggregates of cells have been obtained from 19 species, with great differences in shapes and cell growth rates. The species better investigated are *Dysidea avara*, *Suberites domuncula*, *Hymeniacidon perleve* and *Petrosia ficiformis*. From these species, primmorphs were used both for the production *in vitro* of bioactive compounds (e.g. avarol from *D. avara*) and as suitable models for sponge cell biology studies (e.g. biosilicification in primmorphs of *S. domuncula*). From a screening that we have conducted on 21 Mediterranean sponges, we obtained primmorphs from 13, evidencing a high variability among species concerning both the number and the dimensions of formed primmorphs. The dynamic of primmorphs formation varies among species, with specificity in the time required for the aggregation of cells, variability in the time of production of new small primmorphs, oscillation in growth and assessment of final dimensions. Moreover the formation process and trend of growth during one month of monitoring evidenced several patterns: slow and quick formation, short and long time survival and, in the case of long time survivor, the fission and the fusion models. Our work has the aim to evaluate the influence of sponge biological cycle in the primmorphs production using the common Mediterranean sponge *P. ficiformis* as cell source. A monthly monitoring, evidenced that both the number and the size of the primmorphs can deeply change depending on the period of the year the sponge is collected. April and July are the months that lead to the highest number, May and June are the months that leads to the biggest sizes of primmorphs, suggesting a possible relationships of these results with variation of temperature during the year and the reproductive period of *P. ficiformis*. Other relevant results of our researches demonstrate that the maintenance of sponges in aquaria before processing, the high temperatures or the addition of Fe³⁺ to the medium negatively affect primmorphs formation or growth.

Growth of gemmulae and tissue fragments of *Suberites domuncula* in shallow, dihedral spaces

Matthias Voigt{ XE "Voigt, Matthias" }^(*); Sebastian Rühle{ XE "Rühle, Sebastian" }; Rudolf Hausmann{ XE "Hausmann, Rudolf" }

Universität Karlsruhe (TH), Engler-Bunte-Institut, Lehrstuhl für technische Biologie, Engler-Bunte-Ring 1,
76131 Karlsruhe, Germany

The technical implementation of reproduction of sponges in *ex situ* cultivation is a crucial step towards biotechnological production of sponge biomass. In the presented study we report on the germination of gemmulae and tissue fragments of the marine sponge species *Suberites domuncula* in shallow, dihedral spaces. This technique has previously only been described for the fresh water sponge species *Spongilla lacustris* by Ankel & Eigenbrodt and by Brønstedt. Separated gemmulae were put on a microscope slide and overcast by a cover slip fixed with silicon glue on one side. The resulting gap width was approximately 0.5 mm. The microscope slides were put into 10 L basins with different food strategies: one basin with defined food concentration, one basin for checking long term effects and one basin without food for control purpose. The gemmulae in the first two mentioned basins were feed in a three day interval. The germination process was monitored and documented by weekly taken photographs. To our knowledge it is the first time that successful activation of germination of a marine sponge species gemmulae in artificial seawater is reported. The cells of the gemmulae have developed into a young sponge after a period of three weeks. Nearly 90% of the gemmulae have germinated in this time frame. These results open interesting perspectives for the biotechnical application of sponge cultivation to produce higher numbers of small sponges. In the second part of this study we have tested the growth behaviour of tissue fragments of *Suberites domuncula*. The fragments were put between two fixed microscope slides with a defined distance. The slides were inserted into aquaria with constant water flow for sufficient oxygen supply. The sponge fragments were cultivated in basins with (a) food supply and a water temperature of 25°C, (b) without food and 25°C water temperature and (c) with food supply in 20°C temperate water. As a preliminary result it was found that the tissue fragments show a laminar growth (on both sides of microscope slide). After one week the fragments have rounded the cutting edges and establish a thin cell layer around the centre fragment. The volume expansion is twice the width of the inserted tissue fragment. This growth behaviour provides perspectives for biotechnological production of sponges in cultivation.

Bioguided fractionation of an ethanol extract derived from *Amphimedon compressa* collected at the Pedra da Risca do Meio state park, Ceará, Brazil

Diego V. Wilke{ XE "Wilke, Diego V." }⁽¹⁾; Paula C. Jimenez{ XE "Jimenez, Paula C." }⁽¹⁾;
 Elthon G. Ferreira{ XE "Ferreira, Elthon G." }⁽¹⁾; Thiago A. Portela{ XE "Portela, Thiago A." }⁽²⁾;
 Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽³⁾; Claudia O. Pessoa{ XE "Pessoa, Claudia O." }⁽¹⁾;
 Manoel O. de Moraes{ XE "Moraes, Manoel O. de" }⁽¹⁾; Edilberto R. Silveira{ XE "Silveira,
 Edilberto R." }⁽²⁾; Letícia V. Costa-Lotufo{ XE "Costa-Lotufo, Letícia V." }^(1,4)

(1) Depto. de Fisiologia e Farmacologia - UFC; (2) Depto. de Química Orgânica e Inorgânica - UFC; (3) Museu Nacional - UFRJ; (4) Laboratório de Ciências do Mar - UFC

Marine sponges are a rich source of biologically active compounds. Among them, the genus *Amphimedon* is known mainly for their powerful hemolytic activity due to well known high weight molecules as halitoxin and amphitoxin. This study aimed on the bioguided fractionation of an extract derived from *Amphimedon compressa* collected off the coast of Ceará, Northeast Brazil. Material collection took place at the Pedra da Risca do Meio State Park, located at A, 3°33'800" S e 38°26'000" O; B, 3°36'000" S e 38°26'000" O; C, 3°36'000" S e 38°21'600" O; D, 3°33'800" S e 38°21'600" O, and depth raging from 17 to 30m. The samples were immersed in 100% ethanol and stored in low temperatures. Part of the storing EtOH was evaporated (1) and the rest was used in the homogenization of the material. The first extraction was done with EtOH/DCM 1:1. The solvent was removed and the residue (n water) was partitioned with DCM and EtOAc, originating, respectively, the DCM (2) and EtOAc (3) fractions and the water residue. The residue was washed with MeOH which originated the soluble (4) and non-soluble (5) fractions. The cytotoxicity off all fractions collected was evaluated by their hemolytic activity on mouse erythrocytes and their anti-proliferative activity on human tumor cell lines using the MTT assay. Fractions 4 and 5, both obtained from the water residue showed the highest hemolytic activity, while the organic fractions (2 and 3) did not induce hemolysis on the erythrocytes. The crude extract (1) had some hemolytic effect with an EC₅₀ = 255.1 µg/mL. The crude extract (1) showed also a strong anti-proliferative effect on all the assayed cell lines, with a mean IC₅₀ = 1.6 µg/mL. The DCM (2) and MeOH soluble (4) fractions also had a potent anti-proliferative activity over the cell lines with an IC₅₀ ranging from 1.7 to 12.8 µg/mL, for fraction 2, and from 3.8 to 14.6 µg/mL for fraction 4. The strong anti-proliferative effect observed for 4 might be due to its general hemolytic activity.

Growth and recovery in two wild donor sponge candidates for indigenous *in situ* dictyoceratid bath-sponge aquaculture in the Palm Island group, North Queensland, Australia

Carsten Wolff{ XE "Wolff, Carsten" }^(*); Alan R. Duckworth{ XE "Duckworth, Alan R." };
Elizabeth Evans-Illidge{ XE "Evans-Illidge, Elizabeth" }; Christopher Battershill{ XE
"Battershill, Christopher" }

Australian Institute of Marine Science (AIMS), Deriving benefits from marine biotechnology Group,
P.M.B. No. 3, Townsville 4810, Queensland, Australia, c.wolff@aims.gov.au; a.duckworth@aims.gov.au;
e.evansillidge@aims.gov.au; c.battershill@aims.gov.au

An indigenous bath-sponge aquaculture project has begun in the Palm Islands, a group of continental islands 70km to the north of Townsville, North Queensland, Australia. The Australian Institute of Marine Science (AIMS) supports the in-situ experimental scientific effort in this project. The two species which had been selected as the two most promising aquaculture candidates (see also Duckworth *et al.*) are *Rhopaloeides odorabile* and *Coscinoderma* n.sp.. Before an eventual up-scaling of the aquaculture venture could commence, a number of ecological as well as logistical concerns pertaining to population sizes and their commercial availability have to be addressed. Two wild populations of the dictyoceratid bath sponge candidate species, acting as donor sponges, were tested for their respective survivorship and re-growth in a small experiment. It commenced in September 2004 off the North East tip of Pelorus Island, the northernmost island in the Palm Island group, which has an abundance of both candidate species. Of the 30 sponges tagged, different levels of cut injury (30 and 70%), as well as no-injury control-sponges, were tested and individual biomass gains monitored in three dimensions on quarterly trips. A perhaps interesting result is the difference in recovery-type between the two species. Whereas thus far, no mortality has been recorded amongst either species - and *Rhopaloeides* has recovered biomass at a similar rate as *Coscinoderma* - the cut shape retention after injury is far more pronounced in *Rhopaloeides*. Biomass appeared to also be replaced at a faster rate in the injured specimens than in the control population. In addition and contrast to *Coscinoderma*, *Rhopaloeides* displays an initial re-growth lag of several months. Thus the two species appear to pursue different strategies when recovering from injury; which may be a reflection of their different growth habits per-se.

Potential of marine sponge *Hymeniacidon perleve* as a bioremediator of pathogenic bacteria in integrated aquaculture ecosystem

Wei Zhang{ XE "Zhang, Wei" }^(1*,3); Wantao Fu{ XE "Fu, Wantao" }^(1,2); Liming Sun{ XE "Sun, Liming" }^(1,2); Xichang Zhang{ XE "Zhang, Xichang" }^(1,2)

(1) Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; (2) Graduate School, Chinese Academy of Sciences, Beijing 100039, China; (3) Department of Medical Biotechnology, School of Medicine, Flinders University, Adelaide, SA 5042, Australia, WeiZhang@dicp.ac.cn

The recurrent incidences of aquaculture diseases world-wide have resulted in huge economic losses for aquaculture industry. One of the key triggering factors is the influx of bacterial pathogens from human and animal wastes through waterways. Sponges (Porifera), as sessile filter-feeding animals, have extremely high capability to filter the water column. The aim of this work is therefore to investigate the potential of using sponges as a bioremediator to remove pathogen bacteria in integrated aquaculture ecosystem. Using an intertidal marine sponge *Hymeniacidon perleve* as a model system, the ability of removing the most common pathogens *E. coli* and *Vibrio anguillarum* II in aquaculture waters were conducted in laboratory tests. In sterilized natural seawater (SNSW) supplemented with *E. coli* at $7.0\text{--}8.3 \times 10^6$ cells.ml⁻¹, *H. perleve* can remove an average 96% of *E. coli* within 10.5 h at a filter rate of ca. $7.53\text{--}8.03 \times 10^7$ cells h⁻¹.g⁻¹ of fresh sponge in two independent tests. Despite the removal efficiency and filter rate are similar, the clearance rates (the volume of water cleared of bacteria per unit time and sponge weight) vary significantly among individual sponge specimens and between two batches. For the tests of *V. anguillarum* II in SNSW, about 1.5g fresh sponges can keep the pathogen growth under control at a lower initial density 3.6×10^4 cells.ml⁻¹ of 200ml water volume. Further tests were done for 24 h using about 12 g fresh sponge in 2-L actual seawaters collected from two aquaculture sites that have ca. 8-fold difference in pathogen bacteria load. The concentrations of *E. coli*, *Vibrio* and total bacteria at 24 h in treatment groups were markedly lower, at about 0.9%, 6.2-34.5% and 13.7-22.5% respectively of those in the control. Using a fluoresce stain 1,1'-dioctadecyl-3,3',3'- tetramethylindocarbocyanine perchlorate, *E. coli* and *V. anguillarum* II cells were stained and fed to sponges. The confocal microscope observation confirmed that the sponges filtering-retained and digested these bacteria by phagocytosis. Potential may well exist for sponges as a bioremediator of bacterial pathogens in integrated aquaculture.

Advances in the development of *in vitro* cell cultures of sponge archaeocytes for the production of sponge-derived bioactive metabolites

Wei Zhang{ XE "Zhang, Wei" }^(1*,3); Liming Sun{ XE "Sun, Liming" }^(1, 2); Yuefan Song{ XE "Song, Yuefan" }^(1, 2); Yi Qu{ XE "Qu, Yi" }^(1, 2); Xupeng Cao{ XE "Cao, Xupeng" }^(1, 2); Yan Jin{ XE "Jin, Yan" }⁽¹⁾

(1) Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; (2) Graduate School, Chinese Academy of Sciences, Beijing 100039, China; (3) Department of Medical Biotechnology, School of Medicine, Flinders University, Adelaide, SA 5042, Australia, WeiZhang@dicp.ac.cn

Sponges (Porifera) are among the most successful life forms that are the phylogenetically oldest metazoan, with an estimated 15,000 species alive today. More than 10 percent of sponge species investigated have exhibited cytotoxic activity, which makes them inarguably the best source of novel bioactive compounds with therapeutic potential. However, only a few of these compounds have been tested in clinical trials, due to the limited availability of naturally-occurring sponge materials and the extremely low level of bioactive molecules. This is well known in the clinical development of marine-derived drugs as “pharmaco-apoptosis - programmed drug death”. To succeed in commercial development, sponge-based agents will have to be produced in relatively large amounts for detailed development, testing and clinical application. One of the most promising approaches that have been proposed is the application of *in vitro* culture of sponge primmorphs for the production of bioactive compounds in bioreactors developed by Prof. Müller’s group. It has been demonstrated that the primmorph culture of *Dysidea avara*, as a special form of sponge cell aggregates, can produce the anticancer agent, avarol. As a further improvement of the primmorph cultures, our laboratory has developed a new method to establish *in vitro* primmorph culture from archaeocyte-dominant cell population (ADCP) enriched by Ficoll gradient, rather than a mixed cell population (MCP). Our rationale is based upon the totipotency of archaeocyte cells and the different biological functions of various sponge cell types. A sponge *Hymeniacidon perleve* collected from China Yellow Sea was used as a model system for the investigation of cell proliferation. In a preliminary enrichment of up to 20% archaeocyte-population, distinct dynamics of primmorph formation were observed while significant increases in DNA synthesis, cell proliferation (up to 3-fold) and cell growth (up to 4-fold) were achieved. These results demonstrated the importance of archaeocyte and archaeocyte-like cells in successful establishment of *in vitro* sponge primmorph culture system. Our recent developments have been focusing on the development of advanced purification and fraction techniques to purify archaeocyte cells. An integrated 4-step process was developed which can purify archaeocyte cells up to 90% purity. The *in vitro* culture of sponge archaeocyte cells in various purities has clearly suggested that archaeocyte cells are possibly the proliferating cells among various sponge cell types. As a demonstration, the culture of archaeocyte cells of *H. perleve* has the ability to grow and biosynthesize the bioactive metabolites, however the differentiation is still a problem. To develop a continuous cell lines, factors controlling cell differentiation and promoting cell division will have to be determined.

Marine sponge *Hymeniacidon perleve* possesses high diversity of culturable actinobacteria: Isolation and phylogenetic diversity by 16S rDNA-RFLP analysis

Wei Zhang{ XE "Zhang, Wei" }^(1*,4); Haitao Zhang{ XE "Zhang, Haitao" }⁽²⁾; Yoo Kyung Lee{ XE "Lee, Yoo Kyung" }⁽³⁾; HongKum Lee{ XE "Lee, HongKum" }⁽⁴⁾

(1) Marine Bioproducts Engineering Group, Biotechnology Division, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China, WeiZhang@dicp.ac.cn; (2) Polar BioCenter, Korea Polar Research Institute (KOPRI), KORDI, Ansan P.O. Box 29, Seoul 425-600, Korea; (3) Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100039, China; (4) Department of Medical Biotechnology, School of Medicine, Flinders University, Bedford Park, Adelaide, SA 5042, Australia

The class Actinobacteria is a very important group of bacteria that has considerable value as a prolific producer of antibiotics and other therapeutic compounds. With the intense exploitation of terrestrial actinobacteria over many years, the discovery rate of novel bioactive compounds has fallen steadily, with an estimated 95% rediscovery rate of known compounds. To solve this problem, the isolation of novel actinobacteria from marine environments has been a fruitful area of research in the past decade. However, little is known about the diversity of actinobacteria from marine species compared to the diverse range of actinobacteria isolated from terrestrial environments. To better understand the actinobacteria diversity associated with marine sponges, this study attempted to isolate and cultivate actinobacteria from the intertidal marine sponge *Hymeniacidon perleve* for further physiological studies and to discover bioactive natural products. *H. perleve* is widely distributed along the coast of the Yellow Sea near Dalian, China. We recently isolated many antibacterial and antitumor compounds from this sponge. A culture-independent molecular approach has indicated high diversity of the associated bacteria, although no actinobacteria were identified. We wanted to discover whether the sponge harbors actinobacteria that were not detected because of a possible low abundance of Gram-positive actinobacteria. In this study, a total of 244 actinobacteria associated with the marine sponge *Hymeniacidon perleve* collected from the Yellow Sea, China were isolated using 13 different media. The number of species and genera of actinobacteria recovered from the different media varied significantly, underlining the importance of optimizing the isolation conditions. The phylogenetic diversity of the actinobacteria isolates was assessed using 16S rDNA amplification-restriction fragment length polymorphism (RFLP) analysis of 106 selected strains with different morphologies. The RFLP fingerprinting of selected strains by HhaI-digestion of 16S rDNA genes resulted in 11 different patterns. The HhaI-RFLP analysis gave good resolution for identifying the actinobacteria isolates at the genus level. A phylogenetic analysis using 16S rDNA sequences revealed a high diversity of seven genera of culturable actinobacteria including *Streptomyces*, *Nocardiosis*, *Pseudonocardia*, *Nocardia*, *Micromonospora*, *Rhodococcus*, and *Actinoballoteichus*. The dominant genus was *Streptomyces*, which represented 73.6% of the isolates. Three strains were candidates for new species.

Asconoid sponge motility as revealed by time-lapse microscopy

Calhoun Bond{ XE "Bond, Calhoun" }

Department of Natural Sciences. Greensboro College. 815 West Market St. Greensboro, NC 27401 USA,
bondc@gborocollege.edu

Time-lapse studies have revealed various motile phenomena (locomotion, contractile “waves”, etc.) in leuconoid sponges, but these earlier studies neglected sponges with simpler canal designs. Recent time-lapse videos of intact asconoid sponges (*Leucosolenia* sp.) show motile behaviors, such as locomotion and contractile waves, that are similar (in appearance and speed) to those seen in leuconoid sponges (e.g.: *Aplysilla longispina*). In asconoids, contractile waves propagated at speeds of 30-50 $\mu\text{m}/\text{min}$, and they involved systemic constriction then dilation of the slender canal-body tubes. Apparent locomotion of the asconoid tubes is an outward movement (200-400 $\mu\text{m}/\text{hr}$) away from the central conglomeration of tubes; such movements were always accompanied by spicule rearrangements. These spicule manipulations provide an apparent mechanism for locomotion (and perhaps anchorage) in these asconoid sponges, rather than (or in addition to) the amoeboid cell crawling and other contractile events that propel leuconoid sponges. This evidence suggests that, as in leuconoid sponges, asconoid sponges use such motile behaviors to generate adaptive responses to environmental change; these responses include adjustment of flow patterns and anchorages, repulsion of intruding organisms, and competition for living space.

Variations and limits of thermotolerance in the common Mediterranean sponges
Chondrilla nucula* and *Axinella polypoides

Franz Brümmer{ XE "Brümmer, Franz" }^(*); Martin Pfannkuchen{ XE "Pfannkuchen, Martin" };
Ralph O. Schill{ XE "Schill, Ralph O." }

(1) Biological Institute, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany,
franz.bruegger@bio.uni-stuttgart.de; martin.pfannkuchen@bio.uni-stuttgart.de; ralph.schill@bio.uni-stuttgart.de

Species of Porifera comprise a major portion of the marine benthic communities. Zonation patterns of sublittoral communities are less well described due to problems of accessibility and lack of wide-scale applicability. Many biological and physical factors are involved in the distribution of sponges including sedimentation, substrate, temperature, light and oxygen availability, water flow and nutrient levels. These factors and consequently the habitats, change with increasing depths. In this study the focus is on the vertical distribution patterns of the common Mediterranean sponges *Chondrilla nucula* and *Axinella polypoides* in the Limski channel, Rovinj, Croatia. We investigated the heat-shock (Hsp70) responses of the sponge species at temperatures of 18°C, 25°C, 28°C, 30°C and 34°C. A rise in temperature from 25°C to 28°C induced a stress-reaction in *C. nucula* and elevated the Hsp70 level two-fold. A further temperature shift from 30°C to 34°C led to a further increase in the level of Hsp70 with the maximal induction at 34°C. However, the survival rate during a recovery period at 25°C was 100% at all temperature regimes except 34°C. At the same temperatures mentioned above *A. polypoides* showed a different marked effect of stress-response. At 30°C the sponges showed a decreased Hsp70 level of about 20% relating to the temperature at 28°C. In the sponges the highest temperature result in a decreased Hsp70 level by half, compared to the control at 25°C. Up to 28°C there was no mortality at all during the recovery period. *A. polypoides* seems to reached the upper thermal limit between 28°C and 30°C. Up to 28°C there was no mortality at all during the recovery period. The comparisons of heat-shock protein responses of *C. nucula* and *A. polypoides* suggest that differences exist between species in the strength of the heat shock reaction as well as in the upper thermal limits of protein synthesis in general. In the light of the stress protein response, thermal abilities has implications for the vertical distribution ranges of these species

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How and why do sponges incorporate foreign material? Strategies among Porifera

Carlo Cerrano{ XE "Cerrano, Carlo" }^(1*); Barbara Calcinai{ XE "Calcinai, Barbara" }⁽²⁾; Laura Valisano{ XE "Valisano, Laura" }⁽¹⁾; Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽²⁾

(1) Dipartimento per lo studio del Territorio e delle sue Risorse, Corso Europa, 26, 16132, Genova, Italy, cerrano@dipteris.unige.it; (2) Dipartimento di Scienze del Mare, Via Breccie Bianche, 60131, Ancona, Italy

The selection and incorporation of foreign materials in sponges is a complex phenomenon: it involves both a system of recognition of pinacocytes towards sand grains mineralogy and a system of coordination among cells, which transport and engulf particles in definite areas of the sponge. Regarding mineralogy, it seems that quartz particles, when incorporated, could play an important role in collagen production. Among incorporating species, two different modalities can be outlined, depending on the life style of species: i) hard bottom species select particles on the ectosome by pinacocytes, in relation to the particles size and mineralogy, and then incorporate them; this mechanism is generally related to skeletal arrangement (*e.g.* genus *Chondrosia* and *Ircinia*) ii) soft bottom species engulf particles mainly from the base and select mainly the size of particles, with little attention to mineralogy of sand; this mechanism is generally related to the need of a stable anchoring (*e.g.* genus *Oceanapia*, *Tectitethya*, *Cliona*). In psammobiontic sponges, a sediment coat can be present but until now its relation with sponge physiology is only hypothesized.

Ion channels in *Haliclona melana* (Haplosclerida, Chalinidae)

Cláudio Corrêa-Porto{ XE "Corrêa-Porto, Cláudio" }^(1*); Márcio Reis Custódio{ XE "Custódio, Márcio Reis" }⁽¹⁾; Luis Carlos Salomão{ XE "Salomão, Luis Carlos" }^(1,2); Antônio Carlos Cassola{ XE "Cassola, Antônio Carlos" }⁽³⁾

(1) Departamento de Fisiologia, Instituto de Biociências. Universidade de São Paulo. Rua do Matão - Travessa 14 - n. 321. Cidade Universitária. CEP 05508 900. São Paulo (SP). Brazil, claudio@intron.com.br; mcust@usp.br; (2) Faculdade de Ciências Biológicas, Exatas e Experimentais. Universidade Presbiteriana Mackenzie. Rua da Consolação, 930 - CEP 01302-907. São Paulo (SP). Brazil; (3) Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas - Universidade de São Paulo. Av. Prof. Lineu Prestes, 1.524. Cidade Universitária. CEP 05508-900. São Paulo (SP). Brazil, cassola@icb.usp.br

Although sponge physiology is based on specialized cells, without developed organs or tissues like other metazoans, these animals are capable of several highly coordinated processes. Little, however, is known about the mechanisms involved in the integration of the different cells in these organisms. Among the existing structures for this purpose, ionic channels can be involved in a great variety of processes that control interactions between cells. Such proteins have already been described in practically all phyla, but at present in Porifera only one article describes an ion channel using patch clamp technique. These experiments were performed with the objective to identify possible ionic channels in cells of the sponge *Haliclona melana* Muricy and Ribeiro, 1999. Cells were obtained with gentle agitation of a small sponge fragment, and were homogenized and submitted to successive centrifugation and ultra-centrifugation steps in sucrose gradients. Native vesicles of plasma membrane, containing ionic channels were kept in liquid nitrogen. Lipid bilayers were obtained by painting the orifice (0.33mm) of a cup of Delrin with azolecitin, which, inserted in one of the two excavated contiguous compartments in a PVC block, delimited two compartments (CIS and TRANS), both containing symmetrical solutions of KCl (150mM) plus CaCl₂ (100μM). To the CIS side, 20μl of native vesicles was added into the solution, and in approximately 40% of the attempts, incorporation of the native vesicles into the lipid bilayer was observed. The changes of the conductance of this bilayer, indicating the incorporation of ionic channels, have been monitored using a 200A Axopatch amplifier, connected to a digital oscilloscope (Gould). The analog signals were converted into digital signals (PCM2b) and transferred to a microcomputer using an interface card (Digidata 1200A). The analyses were made using the pCLAMP-6 program. The analysis of the data indicated the incorporation of two different types of ionic channels with distinct kinetics: One with conductance of about 50 pS and other with about 300 pS. This is the first registry in Porifera of the activity of isolated ionic channels using the technique of native vesicle incorporation on artificial lipid bilayers. The technique used in this work will allow the biophysical characterization of the ionic channels in this group of animals in the same way it did on other organisms.

Ultrastructure analysis on the anchoring spicule of deep sea sponge *Monorhaphis chuni* (Schulze, 1904) - Implications for spiculogenesis in Hexactinellids

Carsten Eckert{ XE "Eckert, Carsten" }^(1*); Richard Wirth{ XE "Wirth, Richard" }⁽²⁾; Michael Wiedenbeck{ XE "Wiedenbeck, Michael" }⁽³⁾; Klaus Kropf{ XE "Kropf, Klaus" }⁽⁴⁾

- (1) Museum für Naturkunde, Zentralinstitut der Humboldt-Universität zu Berlin, Institut für Systematische Zoologie, Invalidenstr. 43, D-10115 Berlin, Germany, carsten.eckert@museum.hu-berlin.de; (2) GeoForschungsZentrum, Department 4.1., Chemistry of the Earth, Telegrafenberg, D-14473 Potsdam, Germany; (3) GeoForschungsZentrum, Department 4.2., Inorganic and Isotope Geochemistry, Telegrafenberg, D-14473 Potsdam, Germany; (4) Institut für Physiologische Chemie, Abteilung Angewandte Molekularchemie, Universität Mainz, Duesbergweg 6, D-55099 Mainz, Germany

Since the discovery of the glass sponge *Monorhaphis chuni* (Schulze, 1904) on the 1st German Deep Sea Expedition (RV "Valdivia") very little has been learned about the life habit of this animal. Worldwide this sponge has been documented only in few sampling sites, suggesting a wide distribution in the deep sea (Indian Ocean to West Pacific). The anchoring spicule of the sponge is exceptional in that it can reach lengths of up to 3 m with a maximum diameter of 8.5 mm. *Monorhaphis chuni* thus produces the largest biosilica structure known on Earth. The *Monorhaphis chuni* sample which was the focus of our research was recovered by dredging during the Valdivia-Expedition off the coast of East Africa at a depth of 1600 meters. The spicule fragment has a length of 30 cm with a maximum diameter of 6 mm. Further samples from a Soviet deep sea cruise (RV "Vityaz II") off Madagascar's East coast and from a French cruise (RV "Coriolis") off New Caledonia were also examined. Polished cross sections confirm the existence of up to 500 highly regular rings which are centred around a 150 µm diameter, structureless core. Visible at the middle of this core is the square axial channel with an approx. 2 µm thick protein filament which passes through the entire spicule from end to end. The thickness of rings is nearly constant in any sample, but this thickness varies between sample locations (e.g., Somalia basin - 8-10 µm thick silica strata; Madagascar basin - 3-5 µm). The content of the thin boundary layers between rings was analyzed by TEM and EDAX. These results provide important new details about the mechanism of spiculogenesis. The role of the protein axial filament and of the syncytial layer, which covers the surface of spicules along their whole length, will be shown. The anchoring spicule attaches the sponge organism to the seafloor and has the important physical properties of being both flexible and stable. Additional to this spicule, the sponge body of *Monorhaphis chuni* contains approx. 30 other spicule types with lengths ranging between a few micrometers and 5 cm. However, the functions of these morphologies are poorly understood. The ring structure provides the possibility of determining the absolute age of the central and outer strata using the radiogenic isotope ³²Si and to estimate the growth rate of the organism (pers. comm., U. Morgenstern, GNS New Zealand). Under the assumption of an age model we tested the use of *Monorhaphis* for resolving younger paleoenvironmental processes. Secondary Ion Mass Spectrometry analysis revealed variations in the minor element concentrations in biosilica. Point profiles using a step distance of 50 µm found significant variations in trace element concentrations as a function of radial distance from the axial channel. This observation has led to the hypothesis that the concentric bands in *Monorhaphis chuni* may provide a recording of bottom water chemistry.

***In vitro* sponge culture: Morphological changes in the aquiferous system of the Mediterranean sponge species *Agelas oroides* (Demospongiae, Agelasida)**

Cristina Ferretti{ XE "Ferretti, Cristina" }^(1*); Michela Pizzorno{ XE "Pizzorno, Michela" }⁽¹⁾;
Roberto Pronzato{ XE "Pronzato, Roberto" }⁽¹⁾; Renata Manconi{ XE "Manconi, Renata" }⁽²⁾;
Alan R. Duckworth{ XE "Duckworth, Alan R." }⁽³⁾

(1) DIPTERIS - Dipartimento per lo Studio del Territorio e delle sue Risorse, University of Genoa, Corso Europa, 26, I-16132 Genoa, Italy, cristina.ferretti@dipteris.unige.it; (2) DIPZAB - Dipartimento di Zoologia e Antropologia Biologica, University of Sassari, Via Muroni, 25, I-07100 Sassari, Italy; (3) AIMS - Australian Institute of Marine Science, PMB No 3, Townsville, QLD 4810, Australia

The Atlanto-Mediterranean demosponge *Agelas oroides*, common at the bathymetric range of 2-40 m, is the experimental model of our investigations. This orange sponge is characterized by a massive, variably lobate-digitate growth form, up to 25 cm in height with a firm but compressible consistency related to a well developed spongin skeleton. This species contains several secondary metabolites with pharmaceutical potential. One of these compounds is the alkaloid Taurodispacamide A with an antihistaminic activity. The key role of the sponge biomass production in this applied field of research, suggested us to perform a preliminary (4 months) *in vitro* culture of *A. oroides* to evaluate its survival rate and suitability to be cultured for the production of bioactive molecules. The survival of the experimental model was tested by evaluation, using light microscopy, of the morpho-functional characters of the aquiferous system during culture, in that all the biological activities (feeding, respiration, excretion and reproduction) dependent on water flow efficiency of the aquiferous system. Considered traits were the size of choanocyte chambers and canals, and the density of choanocyte chambers and spherulous cells (number/area 1 μm^2). We compared the aquiferous system of explants of *A. oroides* to that of wild specimens. Explants were randomly collected from the culture system after 1, 12, 60 and 120 days. Other explants were maintained in seawater without flow to study the degenerative process of this species. The diameter of choanocyte chambers and canals showed significant changes during the culture experiment being greatest at 12 days compared to wild specimens and explants collected at 60 and 120 days. This could be due to reorganization processes of the aquiferous system of explants suggesting that this sponge adapts itself to the new body size and environmental conditions. The density of choanocyte chambers and spherulous cells differed significantly during the culture experiment, being greatest at 60 days and 120 days, respectively. The increase of the number of spherulous cells mainly along the cut surface of explants could be related to the production of biomass ex novo and showed a good regenerative ability of this species.

Sponges feeding mainly on dissolved organic matter (DOM)? Encrusting sponges play a key role in DOM removal in coral cavities

Jasper M. de Goeij{ XE "Goeij, Jasper M. de" }^(1*,2), Fleur C. van Duyl{ XE "Duyl, Fleur C. van" }⁽¹⁾

(1) Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB, Den Burg, Texel, The Netherlands; (2) CARMABI Foundation, P.O. Box 2090, Piscaderabaai z/n, Willemstad, Curaçao, Netherlands Antilles, jdegoeij@nioz.nl

Coral cavities are among the largest but least explored habitats of coral reefs. We studied dissolved organic matter (DOM) removal by cavity sponges in the fore reef slope of coral reefs along Curaçao, Netherlands Antilles. In an earlier study, we found significant dissolved organic carbon (DOC) depletion in cavity water compared to ambient reef water. Influxes of DOC in a coral cavity of 100 l in volume averaged 75.8 mmol C.m⁻².day⁻¹. Suspension feeders, of which the greater part are sponges, inhabit more than 50% of the cavity walls, and therefore considered an important factor of carbon depletion. Sampling was done in 1 l and 1.7 l chambers with stirring devices at *in situ* depth and light conditions. We used 2 species of encrusting cavity sponges, *Halisarca caerulea* and *Mycale microsigmatosa*. Water samples were taken in 30 to 90 min time series. Significant reductions in DOC concentrations in *H. caerulea* and *M. microsigmatosa* (8 to 27% and 9 to 16% respectively) were found. Influxes of DOC averaged 0.49 mol C.m⁻².day⁻¹ and 0.38 mol C.m⁻².day⁻¹ for *H. caerulea* and *M. microsigmatosa* respectively. Largest concentration drops were observed in the first 10 min, comparable with DOC depletion patterns in cavities. Blanks of bare substrate show no significant flux of dissolved carbon. We conclude that cryptic habitats are sinks of DOM, cavity sponges take up DOM and play a significant role in DOM removal in cryptic habitats. In this study DOC was the largest source of carbon intake for the examined species.

Feeding selectivity within the bacterioplankton in seven sponge species

Anne Kathryn Kauffman{ XE "Kauffman, Anne Kathryn" }^(*); Mark J. Butler{ XE "Butler, Mark J." }; Andrew S. Gordon{ XE "Gordon, Andrew S." }

Department of Biological Sciences. Old Dominion University Norfolk, VA 23529 USA,
amkauffm@odu.edu

Sponges are conspicuous members of many marine communities, both in their abundance and their diversity. As filter feeders with high throughput, and uptake mechanisms that can accommodate particles ranging over two orders of magnitude in diameter, sponges have a direct effect on water column microbial communities. While much work has been dedicated to measuring the quantitative aspects of sponge feeding ethology, with respect to selective removal of specific size fractions, there have been fewer experiments designed to address qualitative selection within size fractions. Results of the few studies demonstrating selection from within the bacterioplankton suggest that the particle removal process may be more than a mechanical process. We addressed qualitative feeding selectivity within the bacterioplankton in seven species of sponge as part of a larger study that also assessed quantitative aspects of sponge feeding in the laboratory and *in situ* during both spring and summer. Our experiment was conducted at Keys Marine Laboratory using water collected from Florida Bay near Long Key, Florida, during both spring and summer. Sponges were held in static 13-liter tanks for the duration of the experiment and water samples were collected from these tanks at 40 minutes for molecular fingerprint based comparisons among species and to the source water. The seven species selected for study include: large sponges that are major structural features of hard-bottom habitat (*Ircinia campana*, *Spherospongia vesparium*), widespread and highly abundant sponges (*Cinachyra* sp., *Ircinia* sp.), and commercial sponges (*Spongia barbara*, *Hippospongia lachne*, *Spongia chereis*). Molecular fingerprints of tank filtrates will be made using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA. Comparisons of fingerprints will be made by analysis of shifts in relative intensities of DGGE bands across species when using equal amounts of DNA extract and variation across species in the relative intensities of fingerprint bands will be indicative of selection. Many demosponges are known to be larval provisioners with vertically transmitted microbial communities. Broader investigation of selectivity in uptake and rejection from with the bacterioplankton will provide insight into the mechanisms of interaction between sponges and their resident and transient microbial communities.

How (fast) sponges metabolize particulate organic carbon

Marieke Koopmans{ XE "Koopmans, Marieke" }^(*); René H. Wijffels{ XE "Wijffels, René H." }

Food & Bioprocess Engineering Group, Department of Agrotechnology and Food Sciences, Wageningen University Research Centre, P.O. Box 8129, NL-6700 EV, Wageningen, marieke.koopmans@wur.nl

We culture sponges in controlled bioreactors for production of secondary metabolites. Sponges are very slow growing suspension feeders, which makes it difficult to culture them economically. Despite the interest in sponges, still little is known about their nutritional demands. Filtration and clearance rate experiments show that particles are filtered from the seawater efficiently. Although a lot of 'food' is taken up, growth is very limited. In our study we want to understand the particle uptake and metabolization of these particles. We applied different feeding strategies and followed substrate uptake and metabolism within the sponge. Two different sponges, *Haliclona oculata* and *Haliclona xena* were used. *H. oculata* and *H. xena* were fed with different algae that have different cell sizes. Particle uptake was measured by following cell concentration during different feeding experiments. Besides particle uptake, respiration rates and metabolic rates were measured to determine the amount of particles and carbon taken up and metabolized by the sponge and the effect of this to oxygen consumption rate. Metabolic rates were measured using NMR. NMR can be used to measure the carbon isotope ^{13}C in different molecules and quantify the amount of these carbon-containing molecules in liquid or solid state. Of all carbon present in nature only 1.11% is ^{13}C , the rest is ^{12}C . This means that only a small fraction of the carbon present in the sponge is measured using NMR. When sponges were fed with ^{13}C labeled algae all metabolized algae gave an increase in ^{13}C inside the sponge. By measuring the ^{13}C profile at different time intervals we measured which compounds were accumulated and how fast this was done. This method gave us the opportunity to find out how and how fast particulate organic carbon was metabolized by the sponge. The complete picture of carbon uptake, respiration and metabolism enables us to design a good and complete diet to use for cultivating sponges.

Silicon use by marine sponges: A re-examination of ecological and histological aspects

Manuel Maldonado{ XE "Maldonado, Manuel" }^(*); Maria Carmen Carmona{ XE "Carmona, Maria Carmen" }; Ana Riesgo{ XE "Riesgo, Ana" }

Department of Aquatic Ecology, Centro de Estudios Avanzados de Blanes (CSIC), Acceso Cala St. Francesc 14, Blanes 17300, Girona, Spain, maldonado@ceab.csic.es

Silicon (Si), in the form of silicic acid, is a fundamental nutrient for diatoms, silicoflagellates, radiolaria, and many sponges. The biological use of Si has relevant ecological effects, including direct control of marine primary productivity and indirect control of CO₂ exchange between the ocean and the atmosphere. Our current understanding of the Si cycle in the ocean assumes that diatoms are the only Si users responsible for shaping the main parameters of the cycle, with negligible contributions by sponges, radiolarians and silicoflagellates. However, after investigating Si use by several sponge communities, we have found evidence that retention of Si by sponges in some sublittoral and bathyal communities is substantial. Sponge populations function as Si sinks that may affect Si cycling dynamics and Si availability for diatoms, particularly in Si-depleted environments. Yearly production rates of siliceous skeletons (biogenic silica) by sublittoral sponges in different ocean environments were estimated to be similar to values known for diatom assemblages. These results again suggest that Si consumption by sponges may be affecting the capability of some local marine environments to generate primary production. We have also re-investigated some cytological aspects of the Si consumption and production of siliceous skeletons by sponges, a process traditionally known to occur intracellularly within specialized, amoeboid, cells (*i.e.*, sclerocytes) of the sponge mesohyl. Because there are recent claims that axial filaments exocytosed by the sclerocytes appear to direct intercellular secretion of spicules in the mesohyl of some sponges, we have re-examined the histology of such a process. We have found evidence suggesting that axial filaments are exocytosed by reasons unrelated to intercellular spicule secretion and that, if intercellular secretion is to occur, it must take place by other mechanisms. In addition, we have found a new pathway for spicule secretion, which does not involve the participation of the sclerocytes.

Discovery of the Gaba-ergic system in freshwater and marine sponges

Renata Manconi{ XE "Manconi, Renata" }^(1*); Cristina Ferretti{ XE "Ferretti, Cristina" }⁽²⁾;
Lorenzo Gallus{ XE "Gallus, Lorenzo" }⁽³⁾; Grazia Tagliafierro{ XE "Tagliafierro, Grazia" }⁽³⁾;
Roberto Pronzato{ XE "Pronzato, Roberto" }⁽²⁾; Paola Ramoino{ XE "Ramoino, Paola" }⁽²⁾

(1) Dipartimento di Zoologia e Antropologia Biologica dell'Università, via Muroni 25, I-07100, Sassari, Italy, r.manconi@uniss.it; (2) DIP.TE.RIS. dell'Università, Genova; Corso Europa, 26, I-16132 Genova, Italy; (3) DI.BI.SA.A. dell'Università, Viale Benedetto XV, 5 I-16132 Genova, Italy

The existence of a coordination system in Porifera is till now hypothetical although sponges display prompt reactions to both environmental and experimental stimuli and are capable of directional movements both at the larval and adult stages of their life cycle. Electrical recording was however evidenced and various types of neurotransmitters, namely epinephrine-like, norepinephrin-like and serotonin-like, were detected by histochemical methods. Here we report on the first approach to detect the presence of GABAB-like system in freshwater and marine sponges. Samples were fixed by standard methods, labelled with specific antibodies and examined under a confocal laser scanning microscope Olympus-FluoView300. Immunofluorescence localization and distribution of the γ -amino butyric acid (GABA), the enzyme L-glutamic acid decarboxylase (GAD), the vesicular GABA transporter (vGAT) and the GABAB R1 and R2 subunit receptors were detected in free-swimming larvae of the freshwater sponge *Ephydatia fluviatilis* and in adults of *Agelas oroides* and *Chondrilla nucula*. These findings indicate the existence of a complex intercellular communication system in sponges, and strongly suggest that their mobile and very sensitive larvae are a suitable experimental model to investigate the first steps of the evolutive history of nerve cells and their integrative system.

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A sponge with a “skin”: Structural and chemical antimicrobial activity in the Mediterranean *Chondrilla nucula*

Wilfred Meyer{ XE "Meyer, Wilfred" }^(1*); Marzia Sidri{ XE "Sidri, Marzia" }⁽²⁾; Franz Brümmer{ XE "Brümmer, Franz" }⁽³⁾

(1) Institute of Anatomy, University of Veterinary Medicine, Bischofsholer Damm 15, 30173 Hannover, Germany, wilfried.meyer@tiho-hannover.de; (2) Porifarma BV, Poelbos 3, 6718 HT Ede, The Netherlands;

(3) Institute of Biology, Department of Zoology, University of Stuttgart, Pfaffenwaldring 57, 70550 Stuttgart, Germany

Studies on aquatic vertebrates, including mammals, demonstrated that general antimicrobial functions of the skin surface can be due to free sugars derived from glycoconjugates. The present study considers for the first time free sugars as basic defense against microbial proliferation in a sponge, using sensitive lectin histochemistry supported by LM and TEM methods. Additionally, the presence of complex carbohydrates was generally demonstrated by the AB-PAS method. Both methodical approaches evidenced the occurrence of acidic glycoconjugates in the exopinacoderm layer and of neutral complex carbohydrates in the cells and the amorphous extracellular matrix of the mesohyl. The glycoconjugates in the exopinacoderm may be responsible for the production and compact elasticity of the outer mucous cover, typical of this species. Among the free sugars identified by lectin staining, some show an antimicrobial activity by inhibiting the adherence and growth of bacteria and fungi to the surface of *Chondrilla nucula*. This defense strategy is in many aspects comparable to that one already described in some aquatic vertebrates.

Ecology and physiology of mesohyl plasticisation in *Chondrosia reniformis*

Lorenzo Parma{ XE "Parma, Lorenzo" }^(1*); Dario Fassini{ XE "Fassini, Dario" }⁽¹⁾; Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽²⁾; Iain C. Wilkie{ XE "Wilkie, Iain C." }⁽³⁾; Francesco Bonasoro{ XE "Bonasoro, Francesco" }⁽¹⁾; Maria Daniela Candia Carnevali{ XE "Carnevali, Maria Daniela Candia" }⁽¹⁾

(1) Dipartimento di Biologia "Luigi Gorini", Università degli Studi di Milano, via Celoria 26, 20133 Milano Italia, lorenzo.parma@unimi.it; (2) Istituto di Scienze del Mare, Università Politecnica delle Marche, via Breccia Bianche, 60131, Ancona Italia; (3) Department of Biological and Biomedical Sciences, Glasgow Caledonian University, 70 Cowcaddens road, Glasgow G4 0BA, Scotland

Chondrosia reniformis is a marine demosponge consisting mainly of a massive collagenous mesohyl, which is an extremely adaptable material: for example, the mesohyl reacts to mechanical stimulation by stiffening. In addition, it has often been observed in nature that parts of sponges can undergo slow elongation and attenuation resulting in the formation of propagules. This represents an obvious advantage, in terms of asexual reproduction and dispersion. This phenomenon of mesohyl creep appears to be initiated by the fragmentation of the substrate to which the sponge is attached, and can be interpreted as a possible response to gravity. Echinoderm mutable collagenous tissue is the only known example of collagenous tissue that is able to undergo rapid and obvious changes in its mechanical properties. It could be hypothesized that connective tissue mutability is not a unique feature of echinoderms but is an adaptive strategy present also in primitive animals such as sponges. This hypothesis is supported by morphological and physiological evidence provided by our preliminary studies in *C. reniformis*, which suggest that the mechanical properties of the mesohyl are under cellular control. The aim of the present work is both to provide more information on the creeping phenomenon of *C. reniformis* specimens in nature and to explore the biological mechanisms responsible for changes in mechanical properties of the mesohyl. In the present work these aspects are being addressed by an integrated approach, which consists of: 1) a field survey; 2) an experimental field study; 3) and an experimental laboratory study. Field survey: the phenomenon was explored in parallel in three different regions of the Italian coasts. The specimens from the different areas showed a clear correlation between *C. reniformis* behaviour and the specific substratum, the water temperature and the trophic conditions. Sponges growing on a substratum mostly composed of mussel shells in eutrophic environments showed the highest creeping in natural conditions; sponges living on a more solid substratum in oligotrophic water showed infrequent phenomena of natural creeping. Experimental field study: the creeping phenomenon was experimentally induced in the field by attaching suitable weights (from 2 to 40 g) to the sponges. The consequent elongation, which was similar to the natural phenomenon, demonstrated that gravity is a responsible factor. Experimental laboratory study: the response of mesohyl tensility to changing parameters (temperature, salinity) was tested in physiological experiments. The results confirmed a close relationship between water temperature and mesohyl stiffness. In the light of these results we intend to search for evidence of mesohyl adaptability in other sponges, to explore the hypothesis that this occurs in other phyla and thereby to broaden traditional evolutionary views on the adaptive implications of collagenous tissues in metazoans.

Sponge activity *in situ* - Biotechnological and ecological implications

Martin Pfannkuchen{ XE "Pfannkuchen, Martin" }^(1*); Gisela Fritz{ XE "Fritz, Gisela" }⁽¹⁾;
 Sabine Schlesinger{ XE "Schlesinger, Sabine" }⁽¹⁾; Ralph O. Schill{ XE "Schill, Ralph O." }⁽¹⁾;
 Kristina Bayer{ XE "Bayer, Kristina" }⁽²⁾; Ute Hentschel{ XE "Hentschel, Ute" }⁽²⁾; Friederike
 Hoffmann{ XE "Hoffmann, Friederike" }⁽³⁾; Franz Brümmer{ XE "Brümmer, Franz" }⁽¹⁾

(1) Biological Institute, Department of Zoology, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany, martin.pfannkuchen@bio.uni-stuttgart.de; gisela.fritz@bio.uni-stuttgart.de; sabine.schlesinger@bio.uni-stuttgart.de; ralph.schill@bio.uni-stuttgart.de; franz.bruegger@bio.uni-stuttgart.de; (2) Research Center for Infectious Diseases, Röntgenring 11, 97070 Würzburg, Germany, kristina.bayer@mail.uni-wuerzburg.de; ute.hentschel@mail.uni-wuerzburg.de; (3) Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany, fhoffman@mpi-bremen.de

Sponges as so called inner filter feeders are actively producing a current through their bodies thus aspiring food and oxygenated water. Descriptions of anaerobic microbes inhabiting the sponge mesohyl have questioned the general assumption of a permanently well-oxygenated sponge tissue. Assumptions of anaerobic processes and anoxic conditions in sponges, however, not only result in implications on biotechnological issues like *in vitro* culture approaches and nutrient supply. They also suppose a possible regulatory mechanism for sponges to control associated microorganisms by providing an ecologically challenging habitat by changing from aerobic to anaerobic states only allowing adapted organisms to persist within sponges. As various activity patterns are already published the pumping activity would allow sponges to actively control not only their inhabitants but also their inhabitants' metabolism by switching from an aerobic to an anaerobic state. Microelectrode measurements on *Aplysina aerophoba* in the lab showed that this species is well oxygenated when pumping, but when it stops pumping, the major part of the tissue turns anoxic within minutes. We therefore investigated the pumping behaviour of *Aplysina aerophoba in situ* and present the resulting implications on possible metabolic interactions as well as the biotechnological implications. To address this question in a non-invasive manner we employed SCUBA diving techniques and snorkelling. A tracer was applied *in situ* to visualize the water current through 2,144 oscules on 259 individual *Aplysina* at different seasons and different times of day. Negative effects of the tracer used on the sponges' activity could be excluded by control experiments. All oscules investigated were active and showed a clear tracer discharge. Therefore we deduce a permanent aerobic condition for *Aplysina aerophoba* tissue *in situ*. This fits with the detection of betaproteobacterial 16S rRNA genes of the *Nitrosospora* group and of genes encoding for ammonia monooxygenase (*amoA*). In contrast, no 16S rRNA genes of anaerobic ammonium oxidizers (anammox) or genes encoding for nitrite reductase (*nirK*) of denitrifying bacteria could be found in *Aplysina aerophoba* and associated bacteria.

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How do dictyonal Hexactinellida build their skeletons?

Henry M. Reiswig{ XE "Reiswig, Henry M." }

Department of Biology, University of Victoria, and Natural History Section, Royal British Columbia Museum, P.O. Box 3020 Stn CSC, Victoria, B.C. V8W 3N5, Canada, hmreiswig@shaw.ca

How dictyonal skeletons of hexactinellids are built remains largely unexplored. Zittel considered this information necessary for unraveling the relationships between dictyonal groups, but he was unable to advance the subject. Later specialists, Schulze and Ijima, turned to other characteristics of dictyonal sponges to characterize the various families and their relationships. At present, dictyonal hexactinellids are arranged in a series of families on the basis of framework type (Reid's farreoid, eurentoid, aulocalycoid) and the form of channelization (epirhyses, aporhyses, diplorhyses, diarhyses, schizorhyses, amarrhyses, or none). While we employ these criteria, in reality we understand almost nothing about how they arise and find it difficult to erect defensible definitions of them. The case in point here derives from recent recognition and definition of the aulocalycoid framework type as the extension of individual continuous axial filaments in formation of longitudinal dictyonal strands. In the typical aulocalycoid framework, each strand, once begun, grows by extension of the axial filament and silicification of that filament as a potentially infinitely long dictyonal ray, the "ray-without-end" model. New dictyonalia are never, ever, added onto the strands. This contrasts to the well established and widely figured model of farreoid and eurentoid frameworks where, in growth, new dictyonalia are added like bricks to the growing margin of every dictyonal strand, the "add-to-tip" model. In attempting verification of quasi-aulocalycoid skeletons, it was necessary to re-examine claims of framework organization made originally by Zittel. The add-to-tip model has proven to be imaginary. The framework of *Farrea occa*, arguably the simplest dictyonal skeleton, has a single layer of parallel dictyonal strands which are extended to accomplish growth. In the add-to-tip model, every section through a dictyonal strand should carry two axial filaments (occasionally only one or three), one from each of the adjacent basal and distal dictyonalia. In actuality, longitudinal dictyonal strands in *F. occa* skeletons carry a bundle of five to ten axial filaments which pass by the axial cross of individual dictyonalia. The ultimate source of these filaments is difficult to determine but they clearly originate from dictyonalia located many meshes more basal in the skeleton, perhaps even to the origin of the strands. At growing tips, new dictyonalia are added to the sides, not the tips of the strands which are already silicified and extend beyond the added dictyonallium. The farreoid pattern is clearly nothing like the classical add-to-tip model. The pattern of growth in lychnisc frameworks (*Neaulocystis grayi*) is reminiscent of Zittel's original model in that here all beams, longitudinal and transverse, have only one or two axial filaments.

First ultrastructural study of living tissues of an Amphidiscophoran - *Sericolophus hawaiiicus* Tabachnick and Lévi, 2000

Henry M. Reiswig{ XE "Reiswig, Henry M." }^(1*); Chaman L. Singla{ XE "Singla, Chaman L." }⁽²⁾; Craig M. Young{ XE "Young, Craig M." }⁽³⁾

(1) Department of Biology, University of Victoria, and Natural History Section, Royal British Columbia Museum, P.O. Box 3020 Stn CSC, Victoria, B.C. V8W 3N5, Canada, hmreiswig@shaw.ca; (2) Department of Biology, University of Victoria, P.O. Box 3020 Stn CSC, Victoria, B.C. V8W 3N5, Canada, csingla@hotmail.com; (3) Oregon Institute of Marine Biology, University of Oregon, P.O. Box 5389, Charleston, OR 97420, USA, cmyoung@darkwing.uoregon.edu

Fine structure of hexactinellid tissues has until now been known from several species, all of which are members of the subclass Hexasterophora. Without knowledge of tissue structure in subclass Amphidiscophora, the authority with which the general structural pattern can be applied to the entire class Hexactinellida remains limited. We report here our transmission and scanning electron microscope (TEM, SEM) study of the living tissues of *Sericolophus hawaiiicus* and compare the results to those of hexasterophorans. As in all hexasterophorans examined to date, tissues of *S. hawaiiicus* consist mainly of a trabecular syncytium, supported by a very thin, fibrous, presumably collagenous, mesolamella. Flagellated chambers have the typical structure of an outer wall (R1) of mesolamella-supported trabecular tissue encompassing a stolon-connected network of widely-spaced enucleate collar bodies. The hourglass-shaped collars are connected at their midpoints to the well-known inner reticulum (R2) of the trabecular syncytium, unsupported by mesolamella. Archeocytes or choanoblasts occur singly or in small groups in the thin mesohyle space within the trabecular syncytium as in most hexasterophorans. Typical plugged junctions occur between trabecular tissues, archeocytes, stolons of the collar body network and spermatocytes. Large congeries of archeocyte are not present but they are represented by derivative spermatocysts and oocytes in early feeding stages. Accessory cells appear to be very sparse and have not been encountered in TEM. Symbiotic bacteria are common in the basal cup of collars and in the thin mesohyle space. We have noted some features that may be distinct from those reported in Hexasterophora. The collar villi are more numerous in *S. hawaiiicus* consisting of a ring of 57 instead of the typical 30-50. Open gaps occur in R2 which are clearly not associated with collars; these form short-circuits between the marginal and central regions of the flagellated chambers. The overall pattern of tissue organization is nonetheless very similar to that known for hexasterophorans; it can now be confidently extended to encompass the entire class Hexactinellida.

Correlating pumping behavior and oxygen gradients in the tissue of *Dysidea avara* and *Haliclona xena* (Porifera: Demospongiae)

Marie-Lise Schläppy{ XE "Schläppy, Marie-Lise" }^(1*); Friederike Hoffmann{ XE "Hoffmann, Friederike" }⁽¹⁾; Hans Røy{ XE "Røy, Hans" }⁽¹⁾; René H. Wijffels{ XE "Wijffels, René H." }⁽²⁾; Dominick Mendola{ XE "Mendola, Dominick" }⁽²⁾; Marzia Sidri{ XE "Sidri, Marzia" }⁽³⁾; Dirk de Beer{ XE "Beer, Dirk de" }⁽¹⁾

(1) Max Planck Institute for Marine Microbiology, Microsensors Group, Celsiusstr. 1, 28359 Bremen, Germany, mlschlae@mpi-bremen.de; (2) Wageningen University, Food and Bioprocess Engineering Group, P.O. Box 8129, 6700 EV Wageningen, The Netherlands, www.marine.wur.nl; (3) Porifarma, Poelbos 3, 6718 HT Ede, The Netherlands

A Clark-type microelectrode was used to measure oxygen gradients near and inside the tissue of live *Dysidea avara* and *Haliclona xena* sponges held in a flow chamber. Oxygen measurements were carried out concurrently with measurements of flow rate exiting an osculum by applying the particle tracking velocimetry (PTV) technique. This is a novel manner of combining two methods for correlating pumping behavior and its effect on oxygen levels within a live sponge. The results showed that if the sponge was pumping, the oxygen concentration in the tissue was only marginally lower than that of the ambient water. However, if the sponge stopped pumping, the oxygen profiles were typically of the diffusive type, with a clear boundary layer. The combination of these two techniques allows us to calculate fine-scale oxygen fluxes in-and-out of active sponges, even at low pumping rates. These combined methods may have applications to ascertain the physiological state of sponges held in *ex situ* aquaculture tanks.

Glass sponges arrest pumping in response to increased sediment loads

Gabrielle J. Tompkins{ XE "Tompkins, Gabrielle J." }^(*); Sally P. Leys{ XE "Leys, Sally P." }

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9,
gjt1@ualberta.ca; sleys@ualberta.ca

Glass sponges are unique in that the bulk of their tissue is a giant syncytium. This tissue continuity allows them, in the absence of nerves, to respond to mechanical stimuli by propagating electrical signals that shut down the feeding current. Flow records show that sediment added to flow-through seawater chambers caused the reef-forming cloud sponge (*Aphrocallistes vastus*) and the non reef-forming boot sponge (*Rhabdocalyptus dawsoni*) to arrest feeding. Sediment applied topically as a suspension (10-500 mL of 0.5-1 g.L⁻¹) to sponges in 0.5-2.5 L chambers caused immediate arrests of the feeding current; sponges typically resumed pumping within 2 minutes but often underwent sequential arrests. *A. vastus* arrested fully in response to the first small topical sediment doses (10 mL) but subsequent applications of 10-300 mL elicited incomplete arrests. Larger doses caused repeated arrest-recovery cycles sometimes persisting for several hours. In contrast, *R. dawsoni* arrested fully in response to each topical dose of sediment. The two sponges differed in their response to fine sediment (<25 µm) added gradually at 3 mL.min⁻¹ to the incurrent water. The arrest phase in *R. dawsoni* was 25-60s, whereas in *A. vastus* the feeding current slowed gradually over 1.5-4.5 min, sometimes with a stepped pattern. Prolonged addition of filtered sediment to aquaria, for 1-3 hours following the initial arrest response, reduced pumping rates in both sponges. Pumping rates remained low as long as sediment was added and recovery to full pumping rates took up to 12 hours. Sequential arrest-recovery cycles were triggered in both sponges and could be a mechanism for flushing sediment from the sponge. In *R. dawsoni* only a few arrests were triggered before pumping declined to negligible levels, however *A. vastus* resumed pumping immediately after each arrest. Robust pumping of *A. vastus*, relative to *R. dawsoni*, was confirmed *in situ* by testing pumping with fluorescein dye. Data indicate that while both glass sponges are physiologically sensitive to increased sediment loads, *A. vastus*' pumping is more robust during short-term sediment exposure. *A. vastus*' ability to continue pumping in the presence of sediment could allow it to survive in sediment-rich environments like the Fraser Reef, British Columbia, Canada.

Poriferan apoptotic cell death: Of sponges and man

Matthias Philipp Wiens{ XE "Wiens, Matthias Philipp" }

Institut für Physiologische Chemie/Abteilung für Angewandte Molekularbiologie, Duesbergweg 6,
Universität, 55099 Mainz, Germany, wiens@uni-mainz.de

Programmed cell death (PCD) and apoptosis - its morphological manifestation - are a prerequisite for the development of multicellular animal life. PCD is also responsible for the elimination of cells following exposure to countless stimuli, physiological and non-physiological. Sponges (phylum Porifera) represent the phylogenetically oldest metazoan taxon. Thus, the discovery of core components of the poriferan apoptotic cell death machinery contributes to advancing our understanding of both molecular evolution and basic control of apoptotic mechanisms. Those apoptotic molecules that have been preserved from Porifera to man include members of the Bcl-2 and IAP families (apoptotic regulators), death domain proteins (adapters), caspases (effectors), and various proteins, whose part in PCD has not been completely elucidated yet. Through the combination of both primmorph culture and poriferan graft fusion/rejection system, *in vitro* investigations on apoptotic pathways in sponges became possible. The results thus obtained demonstrated that, in many respects, the functional mechanisms of poriferan apoptotic cell death are well conserved and resemble those found in higher Metazoa. However, in several cases they reveal sponge-specific features, *e.g.* concerning the arrangement of domains. Nevertheless, when expressed in mammalian cell lines, BHP2 - a poriferan Bcl-2 homologue - displayed its anti-apoptotic function even in this heterologous system. Furthermore, since filter feeders are abundantly exposed to marine microorganisms representing a potential threat, sponges are provided with efficient defense mechanisms. We demonstrated the existence of a poriferan recognition system for Gram-negative bacteria, that involves a receptor on the cell surface recognizing the bacterial endotoxin LPS (lipopolysaccharide), the adapter MyD88 (bearing a death domain and a TIR domain) and the executing molecule MPEG (a perforin-like protein). Combined with an effective apoptotic death machinery those features might lay out the molecular basis for the remarkable poriferan regeneration capacity and longevity.

**Growth rate variations of the calcareous skeleton of the Caribbean sclerosponge
Ceratoporella nicholsoni: *In situ* measurement versus calculated methods**

Philippe Willenz{ XE "Willenz, Philippe" }

Royal Belgian Institute of Natural Sciences Department Invertebrates 29 Rue Vautier B-1000 Bruxelles
Belgium, philippe.willenz@naturalsciences.be

The growth rate of the aragonitic skeleton of the Caribbean "sclerosponge" *Ceratoporella nicholsoni* has been studied by *in situ* staining of specimens with calcein in a reef tunnel at 28m below sea level, near Discovery Bay, Jamaica over a period of 21 years. Experiments were performed up to eight times from 1984 to 2005. After each staining, skeletal chips were removed from the periphery of sponges for analysis, and specimens were left in place for further studies on growth and regeneration. Ground sections perpendicular to the surface were photographed under epifluorescence microscopy. Annual skeletal growth rates were calculated from measurements of the linear extension between calcein stained lines along growth axes. From the initial population of 10 specimens ranging from 10-20 cm maximum diameter, only 4 specimens allowed measurements after 21 years; the 6 others were either lost or had been excavated by boring organisms. Data confirmed previous observations indicating that although average annual growth rates remained in the same range for different periods, significant differences occurred from one individual to another within the same period. The annual growth rate of a given individual also varied significantly in time. This is the longest survey on living individuals of *Ceratoporella*. The variability in growth rates previously demonstrated with this method is compared to age models based on U/Th dating and Sr/Ca cycles. It is an essential step in using sclerosponges as proxies of sea water temperature.

***In situ* measurement of feeding and metabolism in fjord sponges**
(invited lecture)

Gitai Yahel{ XE "Yahel, Gitai" }⁽¹⁾; Sally P. Leys{ XE "Leys, Sally P." }^(2*)

(1) Department of Biology, University of Victoria, PO Box 3020 Stn CSC, Victoria, BC, V8W 3N5, Canada, yahel@uvic.ca; (2) Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada, sleys@ualberta.ca

Glass sponges are conspicuous inhabitants of the deep benthic community of North East Pacific fjords. Using the Canadian remotely operated submersible ROPOS and a new *in situ* device for clean sampling of the seawater inhaled and exhaled by the sponges, we measured directly the removal of total organic carbon (TOC) and living cells, as well as the excretion of nitrogenous waste, by two species of glass sponge at 165 m. At the deep fjord habitat, ambient water resembled true deep sea water with low oxygen concentrations ($<2 \text{ cm}^3 \cdot \text{L}^{-1}$), high dissolved silica ($>55 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$), and low organic content ($\text{TOC} < 53 \pm 6 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$). The turbid water ($<35\%$ m^{-1} transmissivity) contained high suspended sediment load ($7.1 \pm 0.9 \text{ mg L}^{-1}$) and the available organic carbon was surprisingly low ($>2\%$). Heterotrophic bacteria ($<1 \text{ } \mu\text{m}$) were the most nutritionally valuable and predictable food source at the sponge habitat ($8.4 \pm 2.2 \times 10^5 \text{ cells cm}^{-3}$). Both *Aphrocallistes vastus* and *Rhabdocalypus dawsoni* were effective bacteriovores, removing up to 95% of the bacteria (*A. vastus* $73 \pm 16\%$, *R. dawsoni* $66 \pm 23\%$, $n=26$; 5 paired water samples, respectively). Nano heterotrophic protists ($4\text{--}10 \text{ } \mu\text{m}$) were also removed at similar efficiencies. While the distribution of this plankton was extremely patchy (Lloyd's Patchiness index 3.5), it contributed a significant proportion ($\sim 30\%$) of carbon to the sponges' nutrition due to its size. Plankton removal ($2.2 \pm 1.3 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) accounted for the entire carbonic uptake and ammonia excretion ($0.20 \pm 0.13 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) by the sponge with no evidence for dissolved organic uptake. Curiously, despite the massive siliceous skeleton of both sponges, silica uptake was below detection levels ($0.28 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$). This low silica uptake supports previous suggestion of low growth rates in Hexactinellida. Our results suggest that glass sponges are efficient filter feeders, selectively removing the scarce microbial cells from a 'soup' of suspended clay and detritus particles. Reported abundances of up to 250 individuals 10 m^{-3} suggest that the filtering activity of these sponges could significantly impact benthic-pelagic mass exchange at NE Pacific fjords.

**Embryo development and transfer of symbiotic bacteria from adults to larvae in
Corticium candelabrum (Porifera: Demospongiae)**

Sònia de Caralt{ XE "Caralt, Sònia de" }^(1*,2); Maria-J. Uriz{ XE "Uriz, Maria-J." }⁽²⁾; René H. Wijffels{ XE "Wijffels, René H." }⁽³⁾

(1) Food and Bioprocess Engineering Group, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands; (2) Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Accés a la cala St. Francesc, 14. 17300 Blanes, Girona, Spain; (3) Food and Bioprocess Engineering Group, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Oocytes, spermatogonia and embryo development and the origin of the symbiotic bacteria of *Corticium candelabrum* (Homosclerophorida) a sublittoral Mediterranean sponge are described through SEM and TEM. *C. candelabrum* proved to be a simultaneous hermaphrodite species. Gametogenesis starts in January-February. Oocytes and spermatogonia were clearly differentiated in April. Embryos developed from June to July when larvae released spontaneously. Bacterial transmission from the parental sponge takes place in early oocytes, when symbiotic bacteria are concentrated in spherical clusters. At this stage, firmly interlaced follicular cells from the parental tissue surround the embryo. A thin collagen layer is below the follicular layer. After cleavage, bacteria occupy free spaces between blastomers, and mainly accumulate at the embryo periphery. The blastocoele is formed (blastulation) by cell migration to the periphery, and at this stage several morphotypes of symbiotic bacteria occupy the central cavity (blastocoele). The collagen, which was spread through the whole cavity in immature larvae, is arranged in mature larvae in a dense layer that separates cells from the central cavity (basement membrane). Many examples of engulfed bacteria in larval cells were observed, which is an indication of their use as a complementary energy source.

The contribution of Urmetazoa *Tlx/Hox11* homeobox gene for origin and early evolution of gastrulation process
(invited lecture)

Cristiano C. Coutinho{ XE "Coutinho, Cristiano C." }^(*); Rodrigo N. Fonseca{ XE "Fonseca, Rodrigo N." }; Guilherme A. Maia{ XE "Maia, Guilherme A." }

Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Instituto de Ciências Biomédicas,
Dept. Histologia & Embriologia, Bloco B lab. 25, Av. Pau Brasil s/n. CEP 21.940-590, Rio de Janeiro,
Brasil, ccoutinho@histo.ufrj.br

The concept of gastrulation is more than germ layer establishment and separation of primordial adult structures. Gastrulation is a deep conserved developmental process among Metazoans because it is also associated with gene interactions for axis patterning, when cells execute a program of differentiation according to the body axes. The highly evolutionarily conserved homeobox genes are such an example. They have a pivotal role in genetic programs involved in patterning the embryonic axes and are regulated according to the gastrulation process. Comparison of sponge homeobox sequences shows they do not share homology with genes dictating anterior-posterior patterning, except perhaps to some extent with the NKL homeobox gene complex of mouse and *Drosophila*. In *Drosophila* NKL complex (93DE) is involved in a network of gene interactions that governs progressive cell fate decisions during mesoderm patterning. Only the *Tlx-Lbx* and *NK3-NK4* linkages have been retained in chordates and are believed to have evolved from an ancient gene cluster common in ancestral bilaterians. The sponge homeobox gene of the *Tlx* family was further analyzed by a heterologous model, providing evolutionary evidence associating *Hox11/Tlx/EmH3* expression with proliferation and delay or abrogation of terminal differentiation of immature sponge and human precursor cells. The cell differentiation/proliferation program of amoeboid phagocytic cells, represented by archeocytes in sponges and vertebrate macrophages of the extraembryonic mesoderm, are the primary self-recognition system of all animals and *Tlx/Hox11* is now speculated to be homologically involved in this process. The data suggest that mesodermal structures may have originated earlier than is commonly thought, as also supported by morphological, stem cell system and genetic data from Cnidaria and Porifera. Moreover, a clump of substrate-adherent amoeboid cells is necessary and sufficient to generate new sponges, without the maintenance in adult form of patterned body axes and layers; which is an attribute of the gastrulation process. The assumption of germ layers in sponges is also problematic, since the contribution of the hypothetical endoderm and ectoderm to adult sponge tissues is quite different from what is known in other metazoans. As a general conclusion a speculative scenario is proposed where primitive spongeomorph mesohyl (mesenchyma) was the precursor for all metazoan germ layers, and the *Tlx* gene family (NKL) would be a primary molecular control for mesenchymal lineage, controlling proliferation and differentiation.

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Comparative study on spermatogenesis in the Homoscleromorpha

Sofia M. Efremova{ XE "Efremova, Sofia M." }^(1*); Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }⁽²⁾; Yulia I. Mukhina{ XE "Mukhina, Yulia I." }⁽¹⁾; Philippe Willenz{ XE "Willenz, Philippe" }⁽³⁾; Nicole Boury-Esnault{ XE "Boury-Esnault, Nicole" }⁽⁴⁾

(1) Biological Institute of St. Petersburg State University, Oranienbaumskoye sch. 2, Stary Peterhoff, 198504, St. Petersburg, Russia, smefremova@mail.ru; juliamuchina@mail.ru; (2) Department of Embryology, Faculty of Biology, University of St-Petersburg, Universitetskaya nab, 7/9, Saint Petersburg, Russia, aereskovsky@mail.ru; (3) Department of Invertebrates, Royal Belgian Institute of Natural Sciences, Rue Vautier, 29, B-1000 Bruxelles, Belgium; (4) UMR CNRS 6540 DIMAR, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France, esnault@com.univ-mrs.fr

The Homoscleromorpha have a very simple organization and have been considered as more primitive among the Demospongiae but recently some advanced characters in their morphology and development were found which pointed to a higher evolutionary level. These are the presence of two true epithelial tissues and collagen IV and of the acrosome in spermatozoon. We made a comparative TEM and SEM investigation of spermatogenesis of the species of genera *Oscarella* (*O. tuberculata*, *O. lobularis*, *O. imperialis*, *O. microlobata*, *O. viridis*, *Oscarella* sp.) and *Pseudocortidium* (*P. jarrei*) to evaluate the variability of the spermatogenesis characters within homoscleromorph species and between Homoscleromorpha and the other clades of Porifera. The sponges were collected by Scuba diving from June to August 1999-2003, in the western Mediterranean Sea, at depth of 5-25 m and *Oscarella* sp. was collected in the Martinique Island (Caribbean Sea). Of all the species studied only *O. imperialis* contained simultaneously the eggs and spermatocysts, the others were gonochoristic. Spermatogenesis proceeds in the cysts, which are the former choanocyte-chambers or, more exactly, choanocyte-canals. The events of spermatogenesis develop along the cyst's perimeter. Different generations of the germ cells are seen in transverse or longitudinal sections of the canals. Spermatogonia have the same dimensions as the choanocytes and retain the nucleolus and the flagellum. Their basal side rests upon the basal membrane. At least one spermatogonial division takes place at transverse plane at that one daughter cell is connected with the substrate. Different combinations of the male cells are observed along the cyst's perimeter and from the border to cavity: spermatogonia-primary spermatocytes, primary spermatocytes-secondary spermatocytes-early or late spermatids, early spermatids-late spermatids-spermatozoa, only spermatozoa. Taking into account the presence of such compartments we suppose the existence of wave-like pattern of male germ cells maturation in these sponges conditioned by canal form of the spermatocysts. In all species except *O. microlobata* acrosome of the mature spermatozoa is electron-dense, flattened and slightly bow-like, as it was described early in *O. lobularis* (= *tuberculata*). The acrosome of *O. microlobata* represents a round granule above the nucleus. Our preliminary data on the oogenesis of *O. imperialis* and *O. viridis* allow us to connect the presence of acrosome with the creation of follicular envelope around the oocytes which might make difficulty the fecundation by the spermatozoon without this organelle.

Sponge development: The past and the future
(invited lecture)

Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }

Department of Embryology, Biological Faculty, Saint-Petersburg State University, Universitetskaya nab.
7/9, 199034 Saint-Petersburg, Russia, aereskovsky@mail.ru

Sponge development investigation has a 140 year history, beginning from the work of Haeckel. The role of embryological studies in establishing the relationships between animals has been paramount since the 1866 and what became known as the “biogenetic law.” Embryological investigations made important contributions to spongiology: the creation of the subkingdom Enantiozoa, the separation of Calcinea and Calcaronea in the Calcarea, and the separation of Tetractinomorpha and Ceractinomorpha within Demospongiae. Our ultrastructural investigations of Homoscleromorph sponge development have cast of doubt on the position of this group in Demospongiae. But in spite of a long history of sponge embryology, now we have information about embryonic development for about 100 sponge species. There are some demosponge orders (Verongida, Agelasida, Lithistida, Astrophorida) for which there is no description of embryology and development has only been investigated in two species of Hexactinellida. The difficulties for the phylogenetical interpretation of comparative embryology of Porifera are connected with the high degree of polymorphism of their development. The same cleavage pattern and blastula type may be characteristic of several different larval types. On the other hand, the same larval types may develop from different cleavage patterns and morphogenesis paths. Nevertheless, this material is sufficient for sponge development typisation. Each of the developmental types represents an established sequence of invariant stages characteristic of the development of animals within high taxonomic groups (above the Order level). The basal phylogenetic position of Porifera among the Metazoa and its suggested paraphyly make new investigations on embryology and larvae especially actual. Relatively few homologues of developmental genes are known in the Porifera. Comparison of transcription factors that regulate genes expression during development will provide an evolutionary perspective to relationships among basal metazoan phyla.

Asexual reproduction in *Oscarella* (Porifera; Homoscleromorpha)

Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }^(1*); Daria B. Tokina{ XE "Tokina, Daria B." }⁽²⁾

(1) Department of Embryology, Biological Faculty, Saint-Petersburg State University, Universitetskaja nab. 7/9, 199034, St-Petersburg, Russia, aereskovsky@mail.ru; (2) Zoological Institute of Russian Academy of Science, Universitetskaja nab. 1, 199034, St-Petersburg, Russia, polinaist@mail.ru

Asexual reproduction by external budding in representatives of Homoscleromorpha is reported for the first time. Two Mediterranean sponge species were studied, *Oscarella lobularis* and *O. tuberculata*. Buds are formed in the marginal basal part of the sponge. Budding takes from one to four days. We have investigated this process under light and scanning electron microscopy and defined three budding stages. First, small irregular protuberances, consisting of external parental tissue, are formed. Second, they elongate and acquire more regular, nipple-like shape. These protuberances are tube-like, with their internal cavity derived from parental exhalant canal. Their wall consists of three layers: external layer is flagellated exopinacoderm, internal one is flagellated endopinacoderm, and intermediate one is a thin layer of mesohyl. Third, a spherical bud with a large central cavity is formed. During budding, we did not observe cell proliferation or transdifferentiation either in the zone of budding or in any special mitotically active region. The bud attached to the substrate is similar to the rhagon that develops after metamorphosis of the cinctoblastula larva, *i.e.* it has a syconoid-like organization. Morphogenetically, budding in *Oscarella* has profound differences from that in other sponges. Occurring by epithelial morphogenesis, it is similar to morphallaxis during regeneration, because the budding region transforms completely into a new individual, which has proportions characteristic of the adult sponge. The presence in Homoscleromorpha of epithelial morphogenesis is unique among sponges. This feature is shared by Homoscleromorpha and Eumetazoa.

Reproduction strategies and reproductive effort of *Oscarella tuberculata* (Demospongiae, Homoscleromorpha) from the Northwest Mediterranean

Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }^(1*); Daria B. Tokina{ XE "Tokina, Daria B." }⁽²⁾; Chantal Bézac{ XE "Bézac, Chantal" }⁽³⁾; Thierry Pérez{ XE "Pérez, Thierry" }⁽³⁾

(1) Department of Embryology, Faculty of Biology, University of St-Petersburg, Universitetskaya nab, 7/9, Saint Petersburg, Russia, aereskovsky@mail.ru; (2) Zoological Institute Russian Academy of Sciences, Universitetskaya nab, 1, Saint Petersburg, Russia; (3) UMR CNRS 6540 DIMAR, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France

Life history and reproduction cycles of sponge species are needed for a better understanding of their evolution and role in marine ecosystems. This work presents the reproductive cycle of *Oscarella tuberculata* (Demospongiae, Homoscleromorpha) and its variation in relation to both biotic and abiotic factors. We particularly compared life cycles of two distinct color morphs (blue and yellow-green) of *O. tuberculata*, and correlated the reproductive effort of the yellow-green morph with natural temperature variations. Samples of the two color morphs were collected within several communities in eight distinct localities of the NW Mediterranean (France & Spain). For assessing the reproductive effort, we sampled about ten yellow-green individuals of a given population every month between October 2001 and August 2003 in a site equipped with a permanent temperature recorder (Maire Island, Marseille, France). Then, samples were preserved and cut for histological and cytological works. *Oscarella tuberculata* is quite common in shallow waters (2-24m) of the NW Mediterranean basin. This species is viviparous and rarely hermaphrodite. Male and female gametes derive from choanocytes. Eggs, embryos and larvae are generally concentrated into the central part of sponge and patched around large exhalant canals. Spermatocysts are also localized in the central part of the choanosome, but their distribution is less patchy than oocytes. Spermatogenesis occurs asynchronously in spermatocysts. Eggs are isolecithal and rich in yolk inclusions. Cleavage is holoblastic, equal and asynchronous and leads to a morula. A coeloblastula pre-larva develops from the morula by multipolar egression. The result of the cell division and differentiation processes is a ciliated cinctoblastula larva. Symbiotic bacteria are present extracellularly during all development. Reproduction (gametogenesis and embryogenesis) leads to localized destruction of parental tissue. Spermatogenesis occurs in the middle of July. Oocyte vitellogenesis occurs from June to the end of July. Embryo development starts in mid-July and release of larvae is generally observed from the end of July to the beginning of September. During the embryogenesis, the reproductive effort of *O. tuberculata* may be up to 32% of the maternal tissue volume. There is a significant difference between reproduction of different color morphs of *O. tuberculata*. In the blue morph, the development of embryos occurs throughout a period between June and September (the hydrological summer), whereas the green morph shows embryos only in June.

The larval biology of the chemically rich sponge *Luffariella* sp

Piers Ettinger-Epstein{ XE "Ettinger-Epstein, Piers" }^(*); Dianne M. Tapiolas{ XE "Tapiolas, Dianne M." }⁽²⁾; Rocky de Nys{ XE "Nys, Rocky de" }⁽¹⁾

(1) School of Marine Biology & Aquaculture, James Cook University, Townsville, QLD, Australia; (2) Australian Institute of Marine Science, Townsville, QLD, Australia

A serious obstacle to the development of marine natural products (from sponges and other taxa) that are in commercial/clinical trials is supply. This is because the concentrations of many highly active compounds from marine organisms are often low. Aquaculture is often viewed as an alternative for producing compounds of interest. A key step in developing aquaculture of chemically rich species is the closure of lifecycles by settling and raising larvae, however the basic biology of most sponge species is unknown. This study examined the larval biology and settlement preferences of *Luffariella* sp. (Demospongiae; Dictyoceratida; Irciniidae) as a model for the production of pharmacologically active compounds. *Luffariella* produces manoalide and analogues which are potent inhibitors of the enzyme Phospholipase A2 (PLA2). *Luffariella* is a hermaphrodite and releases larvae from mid-November to late December in the Palm Islands, Queensland, Australia. Up to 600 larvae were released sporadically during the day by individual sponges over at least one month. Larvae were approximately 400 µm long and had a band of cilia at one pole, which appeared to maintain directional control. Settlement occurred within 30 hours and larvae demonstrated a strong negative phototaxis. Furthermore, larvae settled more rapidly under dark conditions; at higher larval densities and in the presence of settled conspecifics. Cues often associated with invertebrate larval settlement, such as crustose coralline algae, had no effect on the settlement of *Luffariella*; however biofilmed surfaces induced settlement.

Reproductive strategies of three halichondriid species in the White Sea

Elena Igorevna Gerasimova{ XE "Gerasimova, Elena Igorevna" }⁽¹⁾; Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }^(2*)

(1) Department of Invertebrate Zoology, Faculty of Biology and Soil Sciences, St. Petersburg State University, 7/9 Universitetskaya Embankment, 199034, St. Petersburg, Russia, eigerasimova@mail.ru; (2) Department of Embryology, Faculty of Biology and Soil Sciences, St. Petersburg State University, 7/9 Universitetskaya Embankment, 199034, St. Petersburg, Russia, aereskovsky@hotmail.com; aereskovsky@pisem.net

Life history investigations of Arctic sponges provide observations on the reproductive adaptations of these primitive metazoans in the unstable environment. Sponges of the family Halichondriidae are quite common in high latitudes and can be an excellent subject for life history studies. The present study has been aimed at the initial stages of life cycles of three halichondriid species inhabiting the White Sea - *Halichondria panicea* (Pallas, 1766), *Halichondria sitiens* (Schmidt, 1870) and *Vosmaeria crustacea* Fristedt, 1885. Sponges were sampled in June-September 2004 and 2005 at about two week intervals in the Kandalaksha Bay and examined for reproductive elements using standard histological techniques. All three species studied appeared to be gonochoric. Two species of *Halichondria* were characterized by asynchronous gametogenesis both within their populations and in individual sponges. The early stages of oogenesis and spermatogenesis in *H. panicea* were observed in the beginning of June right after the ice thawing. In late June many gametes were mature and ready for spawning. Embryogenesis was observed from early July and intensive larval release occurred at the end of that month when the water temperature reached its annual maximum. Oogenesis and spermatogenesis in *H. sitiens* began in the middle of June. Mature oocytes were found at the beginning of August. The presence of embryos was recorded during the following six weeks and larvae started to be released at the end of September. The differences in terms of reproduction between *H. panicea* and *H. sitiens* can be explained by their geographical distribution and environmental preferences. The White Sea is one of the extreme sites for the first species distribution while the last species is known up to the Greenland and Kara Sea in the North. Therefore gametogenesis of *H. panicea* in the White Sea was relatively rapidly and larval release was correlated with the peak temperature. To the contrary gametogenesis of *H. sitiens* was relatively long and the larvae released under lower temperature. In contrast to both *Halichondria* species all reproductive events observed in the specimens of *V. crustacea* were synchronized. Oogenesis started in early July while spermatogenesis began at the end of this month. In early September mature oocytes were found near the exhalant canals of the sponges. Their size was smaller than the mature oocytes of *Halichondria* (about 50 µm in *V. crustacea* versus 170 µm in *H. panicea* and *H. sitiens*). No reproductive elements were found in *V. crustacea* sampled during the second half of September. The results suggest *Vosmaeria* may be oviparous. Thus, three halichondriid species demonstrate different reproductive strategies in the Arctic.

Differentiation of the ciliated cells during the embryogenesis of *Halisarca dujardini* (Demospongiae, Halisarcida)

Elisaveta L. Gonobobleva{ XE "Gonobobleva, Elisaveta L." }

Department of embryology, Faculty of Biology and Soil Sciences, St. Petersburg State University,
Universitetskaya nab. 7/9, St. Petersburg 199034, Russia, gonobol@pochtamt.ru

Embryogenesis is a complex of morphogenetic events that leads to the formation of the adult and/or larval structure. Cellular differentiation is one of them. Little is known about cellular differentiation during embryonic development in sponges. In this work we have investigated ultrastructural details of the differentiation of larval ciliated cells in *Halisarca dujardini* Johnston (Demospongiae, Halisarcida). The external layer of the embryo is separated during cleavage. Once formed, the external cells undergo mitotic divisions and differentiation. The cytoplasm of the blastomeres in the 2-16 cell embryo does not have polar localization of the organelles. At this stage, such main ultrastructural details characterize the blastomeres: the nucleus is situated in the central region of cytoplasm and possesses fibrillar nucleoli. Chromatin is dispersed. A centriole is present in the perinuclear cytoplasm. Golgi complexes do not interconnect with the centriole. Blastomeres are connected each other with filopodia and membrane apposition. During the 32-64 cell stage, the external cells polarity is gradually established. The steps of formation the polarized (ciliated) cells are well known: duplication of centriole; migration of centriole to the apical cell surface to become basal body; elongation of cilia containing the axoneme; formation of accessory structures of basal body. At the ~128-256 cell stage such main ultrastructural details characterize external cells layer: the nucleus possesses nucleoli and locates in the apical part of the cells. Nucleoli have fibrillar and granular components and possess several large holes. Chromatin is denser, and granular material appears. The nascent basal body attaches to the central part of the apical cell membrane and nuclear membrane. Golgi complexes are located in the upper part of the perinuclear cytoplasm. Cilia pockets begin to form. Surface cells join together by intercellular junctions in the apical lateral parts of the cells. At the ~500-1000 cell stage the axonemes appear. Oblique striated rootlets arise from the proximal part of the basal body. After this stage, differentiation involves increasing the nucleus-plasma ratio, changing the form of nuclei and cilia pockets, developing the endoplasmic granular reticulum and utilizing the yolk. Two main conclusions can be made. 1. At the 128-cell stage transcriptional activity in the nuclei is significantly changed. The external cells form an integrative epithelium (layer) with the appearance of cell contacts. These two events are characteristic of the mid-blastula transition point in embryonic development of some metazoans. 2. The process by which ciliated cells form is similar to that which has been described for ciliated epithelia of other animals. Unusual is appearance the oblique striated rootlets in the period of embryogenesis. The ciliated cells of *H. dujardini* larvae have the fibrillar nonstriated oblique rootlets.

**New data about morphology, nutrition and metamorphosis of sponge larvae
Halichondria panicea (Pallas, 1766) (Demospongiae, Halichondrida) from Barenz
sea**

Ludmila V. Ivanova { XE "Ivanova, Ludmila V." }

Department of Zoology, Biological Faculty, Russian State Pedagogical University, River Moika Emb., 48,
191186 St. Petersburg, Russia. tel. (812)3517204

We observed the release of larvae from *Halichondria panicea*, their morphology, and metamorphosis in Nature and in marine aquaria with the running water. It was found that the release of larvae in this species, which inhabits the littoral area of Barenz Sea, begins at the end of August and lasts three weeks. Larvae, originating from the same sponge, differ in size, shape and morphology. Size of the larvae varies from 500 x 300 μm to 1.6 x 0.5 mm. Smaller larvae have an oval shape, while the larger ones are oblong. The period of free swimming is relatively short for large larvae (several hours), while for small larvae it may last up to 3 days. Free-swimming is followed by the long period of gliding and crawling, when larvae settle and move over the substrate up to 9 days. During the free-swimming period they exhibit what was called "corkscrew" swimming, moving forward in a spiral path, constantly rotating in a clockwise direction through the water. During the gliding period larvae elongate, and their form becomes changeable. Many of them form multiple surface folds at the posterior end of their bodies. Larvae prefer dispersed light and tend to choose vertical surfaces for settling. Right after settlement and attachment larvae undergo metamorphosis, which lasts up to 14 days and includes a stage of the «pupa». During this period the aquiferous system of the juvenile sponge is formed. The analysis of semi-thin and ultra-thin sections showed that parenchymella-larvae of *Halichondria panicea* from Barenz Sea are capable of phagocytosis. We have found successive stages of phagocytosis of organic particles, bacteria and small flagellates of 2-4 μm in size (evidently zooflagellata) by the ciliated surface cells of larvae. These cells form one or more distinctive globate protrusions (lobopodia) at the distal cell end, which participate in active phagocytosis (it was previously assumed that all sponge larvae are "non-feeding" (lecitotrophic)). According to our research, ciliated cells also take part in fibrous collagen production and in formation of the young sponge's body. Thus, the larval ciliated cells of *H. panicea* appear multifunctional and multipotent.

A mathematical model for the genetic regulation of growth and canal formation in a sponge with radiate accretive growth

Jaap A. Kaandorp{ XE "Kaandorp, Jaap A." }^(1*); Joke G. Blom{ XE "Blom, Joke G." }⁽²⁾;
Werner E.G. Müller{ XE "Müller, Werner E.G." }⁽³⁾

(1) Section Computational Science Faculty of Science University of Amsterdam Kruislaan 403 1098 SJ Amsterdam The Netherlands, jaapk@science.uva.nl; <http://www.science.uva.nl/~jaapk/>; (2) Center for Mathematics and Computer Science (CWI) Kruislaan 413, NL-1098 SJ Amsterdam The Netherlands, Joke.Blom@cwi.nl; (3) Institut fuer Physiologische Chemie Abt. Angewandte Molekularbiologie Johannes Gutenberg-Universitaet Mainz Duesbergweg 6 D-55099 Mainz Germany, wmueller@mail.uni-mainz.de

We present a mathematical model of the genetic regulation controlling skeletogenesis and the influence of the physical environment on an adult branching sponge with accretive growth (for example *Haliclona oculata* or *Lubomirskia baikalensis*). This model consists of a biomechanical component (a model of the deposition of the skeleton), a regulatory component (a model of the regulatory network controlling skeletogenesis and the formation of the aquiferous system) and an environmental component (model of flow and distributions of nutrients in the environment of the sponge). From previous work it is known that high concentrations of silicate induce spicule formation and upregulates the silicatein, collagen and myotrophin genes. The upregulation of the genes activates locally the production of spicules in the sponge and the deposition of the skeleton. Furthermore it is known that the expression of the gene *Iroquois* is induced by increased water current and activates the formation of oscula and the aquiferous system. In previous work it was demonstrated that *Frizzled* works as polarity factor in the sponge *Suberites domuncula*, *Frizzled* is expressed at the surface (the pinacoderm) of the sponge. It is hypothesized here that *Frizzled* works as positional information system, indicating the “up”-direction (the direction towards the external environment in a branching sponge). Based on this previous work we propose a regulatory network controlling the separation in time and space of the skeletogenesis and the formation of the aquiferous system. The regulatory network is closely linked with environmental influences. For building a skeleton, silicate is absorbed from the environment. In our model silicate is transported by advection and diffusion through the environment and absorbed at the surface of the sponge resulting in silicate gradients emerging in the neighborhood of the sponge. The developmental regulatory network and the spatial and temporal gene expression patterns in this model are described using a system of partial differential equations. The unknown parameters in the model (the quantitative relations between genes, diffusion coefficients and decay parameters) can be approximated using an optimization procedure, by comparing the difference between modeled and actual spatial and temporal gene expression patterns. The distribution of the silicate in the environment is modeled using an advection-diffusion model. We have coupled the developmental regulatory network model and the model of the environmental distribution of silicate with a three-dimensional model of accretive growth of the sponge. Growth of the sponge is modeled by a surface-normal deposition process in which the thickness of newly added layers is determined by the combined effect of environmental silicate and genetic regulation. During the presentation we will show a few animations of the (simulated) development of the sponge.

Gastrulation in sponges: Epithelialization of the larva and juvenile
(invited lecture)

Sally P. Leys{ XE "Leys, Sally P." }

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada,
sleys@ualberta.ca

Gastrulation is the process by which germ layers are formed in multicellular animals, one of the principal features that distinguishes them from their colonial protozoan ancestors. Molecular evidence now firmly places Porifera within the Metazoa, yet embryogenesis is highly varied, gastrulation processes are not well understood, and even the presence of germ layers is much debated. Calcarean sponge embryos and larvae - the focus of early studies and source of Haeckel's term 'gastrula' - have several unusual epithelial-like movements that resemble invagination processes in other metazoans. Early development involves the formation of a cup-shaped embryo, similar to that known from the Volvocales. The cup turns inside out placing the ciliated epithelium at the anterior pole of the fully differentiated larva. At metamorphosis this pole invaginates to internalize the ciliated cells, which dedifferentiate and go on to form choanocytes of the juvenile. As in other sponges, gastrulation in the Calcarea (Calcispongia) is a two step process involving epithelialization of the differentiated larva and invagination at metamorphosis to reorganize these layers to produce the feeding epithelium. Unlike other sponges, however, calcarean embryos use epithelial rather than mesenchymal movements to reposition cells. Epithelial processes, such as the invagination that occurs at metamorphosis in Calcarea, may have been co-opted for formation of a sac-like gut in later animals. Thus gastrulation as we know it in other metazoans may be recognized as distinct cell migrations and epithelial transitions that give rise to cell layers in different Porifera.

Glass sponges become syncytial by fusion of blastomeres

Sally P. Leys{ XE "Leys, Sally P." }⁽¹⁾; Nicole Boury-Esnault{ XE "Boury-Esnault, Nicole" }⁽²⁾

(1) Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada, sleys@ualberta.ca; (2) UMR CNRS 6540 DIMAR, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France

The tissue of adult glass sponges (Hexactinellida) consists largely (~75%) of a giant multinucleated syncytium. Cellular components also exist, but these are joined to the multinucleated tissue by cytoplasmic bridges so that the entire animal is cytoplasmically continuous. It is this construction that allows glass sponges to propagate electrical signals that cause the arrest of the feeding current, and which has prompted the proposal that they be separated from other sponges in the subphylum Symplasma. How does the syncytial tissue arise? We have studied early embryogenesis in the lyssaccine glass sponge *Oopsacas minuta* using serial section light microscopy combined with transmission and high resolution scanning electron microscopy. Early cell division is equal and holoblastic; the embryo is cellular until the 32 cell stage. Thereafter unequal cleavage yields a collection of variously sized small cells (micromeres) at the surface of the embryo and large yolk and lipid-filled cells (macromeres) that line a small blastocoel. The peripheral micromeres become joined to one-another by cytoplasmic bridges. The central macromeres extend large pseudopodia to gradually fill the blastocoel and at the same time their membranes fuse to form a giant syncytial tissue. This tissue - the future multinucleated trabecular reticulum of larva and adult sponge - then reaches around to envelop the embryo, covering and at the same time forming cytoplasmic bridges with the remaining uninucleate cells. This event results epithelialization of the embryo, an event that could be interpreted as gastrulation. The resulting larva consists of a continuous multinucleate (syncytial) tissue that permeates both anterior and posterior poles and forms a smooth epithelium that covers the entire larva. Cellular components connected to this tissue and to one another by way of cytoplasmic bridges include: multiciliated cells that form a girdle around the middle of the larva; multinucleated sclerocytes that span the length of the larva; spherulous cells; and branched choanocytes that form young flagellated chambers in the posterior pole. Thus glass sponge syncytia arise from cellular embryos by fusion of the blastomeres at a very early stage in embryogenesis. The data support the hypothesis that siliceous sponges arose from a common cellular silica-secreting ancestor. Following this hypothesis Demospongiae + Hexactinellida would constitute the clade Silicispongia.

Heterologous analyses of sponge *Tlx/Hox11* homeobox gene regulation suggesting deep homology

Guilherme A. Maia{ XE "Maia, Guilherme A." }^(*); Rodrigo N. Fonseca{ XE "Fonseca, Rodrigo N." }; Cristiano C. Coutinho{ XE "Coutinho, Cristiano C." }

Departamento de Hsitolgia e Embriologia, UFRJ, Av Pau Brasil s/n, Predio do CCS, CEP 21940-590, Rio de Janeiro, RJ, Brazil, gamaia@histo.ufrj.br

The sponge *EmH-3* gene has been recently attributed as a member of the *Tlx/Hox11* homeobox family, a gene family highly conserved throughout evolution. *Tlx/Hox11* homeobox family genes are known to act in the control of proliferation and differentiation codifying regulatory factors. In this study, we investigated the functional homology of the *EmH-3* promoter with the human *Hox11* promoter, also a member of the *Tlx* family. Different constructions of the *EmH-3* promoter fused with a reporter gene (luciferase) were transiently transfected into a primitive hematopoiesis human cell lineage (K562), which expresses the *Hox11* gene and can be induced to differentiate. Expression of the endogenous *Hox11* gene and the reporter gene were investigated under normal conditions and after induction of differentiation. Results have shown that the reporter gene and *Hox11* are expressed in a similar pattern; both are expressed in proliferating cells and downregulated in differentiating cells. Previous studies have shown, through Ficoll gradient and RT-PCR analyses, that *EmH-3* is expressed in sponge totipotent cells, the archeocytes, in proliferating state but not when undergoing differentiation. Structural analysis of the *EmH-3* promoter region 1 kb upstream from the translation-initiation site was done by alignment with the corresponding region of *Tlx* genes from mouse human and *Drosophila* genomes. Putative binding sites for transcription factors of the TCF-1, CAAT binding protein, LMO2, USF-1 and Ikaros families were found in similar positions in all *Tlx* promoters analyzed. All these factors are known to control cell proliferation.

Deuterostome-like sperm cells in Demospongiae: Evolutionary implicationsManuel Maldonado{ XE "Maldonado, Manuel" }^(*); Ana Riesgo{ XE "Riesgo, Ana" }Department of Aquatic Ecology, Centro de Estudios Avanzados de Blanes (CSIC), Acceso Cala St.
Francesc 14, Blanes 17300, Girona, Spain

Sponges are widely regarded to be the ancestors of the extant metazoans. Nevertheless, it is puzzling that sponges are characterized by not only primitive features, but also by a few highly specialized traits that are hard to be reconciled with the ancestral status of the group. Here we report on a feature that apparently stresses such a mismatch. By studying the spermatogenesis of several demosponges, we have found a notable diversity in both the process of sperm formation and the ultrastructure of the resulting spermatozoon. In one of these demosponges, all the cells in the spermatid cysts develop synchronously, experiencing dramatic transformation at the spermatid stage. Spermatids, far from differentiating into a conventional primitive sperm cell as it would correspond to an archetypical ancestral metazoan, undergo a remarkable lengthening, exocytosing most of the cytoplasm to produce a needle-like cell. There is also a lengthening of the nucleus, which, besides condensing its chromatin, reshapes itself into a thin spiral. During the reshaping process, the cell body also bends about 180° at the point of insertion of the cilium and a large cytoplasmic pocket is formed to harbor the basal portion of the cilium. As a result, the sperm becomes V-shaped, with the cell body being posterior -in the direction of movement- to where the cilium inserts. A cone-shaped acrosome is also formed close to the base of the cilium, which, unlike conventional sperm cells, has become the anterior pole of the sperm cell. The cilium, which shows a typical 9+2 structure with a conventional basal body and anchoring points, develops 9 long rootlets that run parallel to the nuclear membrane and extend over the whole length of the sperm body. More interestingly, these giant rootlets are not smooth but striated, a feature common in higher metazoans but uncommon in Demospongiae. After a process of mitochondrial fusion, a large single mitochondrion is produced to lay in close association with the ciliary rootlets, at the posterior end of the cell. Spermatozoa with similar structure are only known from some basal deuterostomes. The evolutionary and ecological meaning of such sperm specialization is intriguing and several hypotheses are discussed.

Skeletal fibers of *Sarcotragus spinosulus* and *Aplysina aerophoba* (Porifera, Demospongiae): A histochemical study during regeneration

Renata Manconi{ XE "Manconi, Renata" }^(*); Maria Francesca Ortu{ XE "Ortu, Maria Francesca" }; Fabio Ledda{ XE "Ledda, Fabio" }; Giacinta Stocchino{ XE "Stocchino, Giacinta" }; Gavina Corso{ XE "Corso, Gavina" }

Dipartimento di Zoologia e Antropologia Biologica dell'Università, Via Muroni 25, I-07100, Sassari, Italy

The structural analysis of fibers during morphogenetic processes can help to understand the morpho-functional performances of the skeletal network of horny sponges. *Sarcotragus spinosulus* Schmidt, 1862 and *Aplysina aerophoba* Schmidt, 1862 from the W-Mediterranean Sea were studied by a histochemical approach to investigate developmental phases and rapid growth to produce ex novo the skeletal network during regenerative processes after the experimental fragmentation following the USAMA® technique. Buds of *A. aerophoba* and regenerated portions of *S. spinosulus* collected after 3 months from transplant were processed for histochemical analyses and compared with wild specimens. Different staining methods (Alcian blue, Dane Herman, Pasini, PAS) were applied on about 50 sections for each stain. Young fibers of *A. aerophoba* bear, a wide axial core of glycosaminoglycans (GAGs), surrounded by two thick layers of glycoprotein and collagen respectively. The surface of mature fibers is lined by an outer layer of laminar material of scleroproteins secreted by a peculiar concentric structure of spongioblasts located at the growing apices. In *S. spinosulus* growing fibers are made of scleroproteins showing scattered areas rich of GAGs in the central portion. Glycosaminoglycans appear to be involved in regenerative processes of skeletal elements in *A. aerophoba* and *S. spinosulus*. These findings match well the previous biochemical analyses on the distribution of GAGs in sponge body and their key role in structural and morphogenetic processes.

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The events of metamorphosis in the demosponge *Halisarca dujardini* Johnston, 1842, studied with immunocytochemical method

Yulia I. Mukhina { XE "Mukhina, Yulia I." }⁽¹⁾; Vadim V. Kumeiko { XE "Kumeiko, Vadim V." }⁽²⁾; Olga I. Podgornaya { XE "Podgornaya, Olga I." }⁽³⁾; Sofia M. Efremova { XE "Efremova, Sofia M." }^(1*)

(1) Biological Institute of St. Petersburg State University, Oranienbaumskoye sch. 2, Stary Peterhoff, 198504, St. Petersburg, Russia, juliamuchina@mail.ru; smeefremova@mail.ru; (2) Institute of Marine Biology FEB RAS, Palchevski street, 17, 690041, Vladivostok, Russia, vkumeiko@yandex.ru; (3) Institute of Cytology RAS, Tikhorezkiy pr. 4, 194064, St. Petersburg, Russia, podg@IM1632.spb.edu

Sponge larval flagellated cells (FC) are known to form an external layer of the larva but their subsequent fate and morphogenetic role is still unclear unless a certain marker is found. We used Percoll density gradient fractionation to separate different larval cell types of *Halisarca dujardini* (Demospongiae, Halisarcida). A total of 5 fractions were obtained. Fraction 1 contained about 100% of FC and its polypeptide composition differed greatly from the rest fractions. The polyclonal antibodies (AB S) against a specific protein of 68 kDa (S) expressed in larval flagellated cells were raised. By means of double immunofluorescence using both the AB S and commercial anti-tubulin antibodies (AB T), we studied the distribution of protein S in larval FC and its fate at successive stages of metamorphosis under LSM5 PASCAL confocal laser scanning microscope and LEICA fluorescent microscope. The S protein was found all over the cytoplasm of the FC and was absent in the flagellum. The inner cells of the swimming larva were not stained by AB S suggesting that it is sufficiently specific for FC. AB T stained brightly the flagellum and basal body and only weakly the plasma membrane. No staining of cytoplasm or AB S-labeled structures was observed. Thus, AB T and S, when used together, are suitable to trace FC fate during metamorphosis. Before settlement, posterior FC lost flagella. However, anterior - lateral FC retained them at the end of free swimming period. FC were directly involved in metamorphosis. Just after settlement the narrow monolayer of lateral FC attached to the substrate was formed around the flattened larva. Their flagella were internalized and surrounded by FC cytoplasm. Gradually AB S label spread inward the metamorphosing larva; both AB S and AB T staining were confined to internal cell mass. In 3 days post-settlement most of the former FC composed the choanocyte-chambers. AB S labels granules in the cell body, as it did in premetamorphic FC. Diffusive AB S and AB T staining is visible also in T-shaped dermal pinacocytes on sponge surface. Still, these cells do contain both antigen characteristics of FC. A newly-formed adult sponge develops a definitive network of the choanocyte chambers within one month after settlement. At that time AB S label is seen in the choanocytes, but it disappears in the pinacoderm cells (the T-shaped pinacocytes). Protein S (p68 kDa) is concentrated now at cells' apical part, exactly in the collars. Amoebocytes - the other cell type visualized in the sections - display no AB S staining. Thus in the development of *H. dujardini* the larval FC participate directly in construction of definitive organs such as the choanocyte-chambers and the upper pinacoderm.

Life-cycle of *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004 (Calcarea: Porifera)

Emilio Lanna{ XE " Lanna, Emilio" }⁽¹⁾; Leandro C. Monteiro{ XE "Monteiro, Leandro C." }⁽²⁾;
Michelle Klautau{ XE "Klautau, Michelle" }^(1*)

(1) Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Pau Brasil 211, CCS, 21941-590, Rio de Janeiro, RJ, Brazil, mklautau@biologia.ufrj.br; (2) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, 20940-040, Rio de Janeiro, RJ, Brazil

Paraleucilla magna is one of the most abundant calcareous sponges in Rio de Janeiro state. Nonetheless, curiously it had never been noticed before the 1990s. This species lives attached to hard substrates in sciaphylous or photophylous habitats, frequently associated with calcareous algae and bryozoans. The objective of the present work is to describe the life cycle of *P. magna*. This is the first time that the life cycle of a calcareous sponge has been studied in the Southern Hemisphere. Five to twelve specimens of *P. magna* were collected monthly from January 2004 to October 2005 in Vermelha Beach (Rio de Janeiro, Brazil). Sampled specimens were fixed with 100% ethanol and preserved in 70% ethanol. Each specimen had its external morphology, colour, size and associated organisms observed. Thick sections (150 µm) were made and stained with alcohol acidic fucine to search for reproductive structures and to analyse the organization of the skeleton. When reproductive structures were found, the specimens were decalcified for three days with 10% EDTA, washed with distilled water, dehydrated with ethanol and xylene, and embedded in paraffin wax. Thin sections (15 µm) were made and stained with Hematoxylline/Eosine to observe the details of the reproductive process. Reproductive structures were found in January, February and March. In 2005 these structures could be observed until April. Early stages of oocytes were observed in January, larger oocytes and early larvae were present in February, and mature larvae appeared in March. The observed oocytes are large cells apparently derived from choanocytes. They have a large nucleus and granular cytoplasm. Most of them are found surrounded by nurse-cells. Larvae are amphiblastula, composed of macromers and micromers, and measuring about 45 µm. *P. magna* has just one reproductive period, starting in mid-summer and finishing in late summer or beginning of fall. This reproductive period matches the seasonality of calcareous sponges that inhabit the Northern Hemisphere.

**Life cycle and reproductive effort of the Mediterranean commercial sponge
*Spongia officinalis***

Thierry Pérez{ XE "Pérez, Thierry" }^(1*); Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }⁽²⁾; S. Correia{ XE "Correia, S." }⁽¹⁾; Chantal Bézac{ XE "Bézac, Chantal" }⁽³⁾; Nicole Boury-Esnault{ XE "Boury-Esnault, Nicole" }⁽¹⁾; Jean Vacelet{ XE "Vacelet, Jean" }⁽¹⁾

(1) UMR CNRS 6540 DIMAR, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France, perez@com.univ-mrs.fr; (2) Department of Embryology, Faculty of Biology, University of St-Petersburg, Universitetskaya nab, 7/9, Saint Petersburg, Russia; (3) UMS 2196, Service des Moyens Communs, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France

There is an urgent need for baseline studies on reproductive cycles of marine invertebrates in order to assess the possible effect of the environmental changes at the population level. This is all the more crucial for species with an important economic value frequently affected by disease outbreaks largely influenced by thermal anomalies as it is the case for commercial sponges. In this context, we revisited the successive reproductive phases of the Mediterranean bath sponge *Spongia officinalis* and studied for the first time its reproductive effort in relation to natural temperature fluctuations. Samples of a given population, in a site equipped with a permanent temperature recorder, were collected monthly and preserved for histology and cytology. We characterized two entire reproductive cycles by analyzing a total of 215 individuals. Among them, less than 50% were reproductive. This species is gonochoric and among the samples 37% were female whereas only 6% were male. Spermatozooids come from choanocytes. Although the spermatogenesis is synchronous, the differentiation of cysts is asynchronous. Cysts are about 17 to 80 µm and ovoid. The origin of oocytes is unclear. We found (i) young oocytes, spherical, from 19 to 44 µm in diameter, with a big nucleus and few yolk inclusions within the cytoplasm; (ii) larger oocytes, spherical, from 47 to 82 µm, with a large nucleus and abundant yolk granules in the cytoplasm. The size of mature oocytes is 95 - 186 µm. We did not observe fertilization. Oocytes and embryos can be found in the same individual. Cleaving embryos were frequently observed. Development leads first to a morula measuring from 156 to 564 µm, and then to a parenchymella larva that will be expelled into the water. The parenchymella larvae are ovoid and ciliated, 530 x 342 µm, with dark pigmentation and cells with long cilia at the posterior region. Spermatogenesis and oogenesis occur at very distinct periods. Spermatogenesis was always observed during the cold season. The production of spermatocysts by a sponge measuring 500 cm³ is from a minimum of 3300 to a maximum of 19000. Production of oocytes is highly variable over the year, from a minimum of 50 to a maximum of 460 per sponge measuring 500 cm³. Embryos are also observed all over the year, but their production is also highly variable, from about 200 at the end of summer to about 2000 embryos per sponge in December. There is no significant correlation between temperature fluctuations and oogenesis, but we nevertheless observed a maximum in winter (mean temperature 13.5°C) when the spermatogenesis occurs. This is also the period of a maximal production of embryos, whereas larvae were only observed in the summertime. After this work, an enigma remains to be solved. How can *S. officinalis* produce embryos throughout the year while spermatogenesis only occurs during a short period in winter? We discuss two different hypotheses that will need further research.

Dynamics of gametogenesis and embryogenesis in a temperate demosponge

Ana Riesgo{ XE "Riesgo, Ana" }^(*); Manuel Maldonado{ XE "Maldonado, Manuel" }

Department of Aquatic Ecology, Centro de Estudios Avanzados de Blanes (CSIC), Acceso Cala St.
Francesc 14, 17300-Blanes, Girona, Spain

We have documented gametogenic and embryogenic dynamics in a Mediterranean population of the homosclerophorid demosponge *Corticium candelabrum* using both light and electron microscopy. In this hermaphroditic sponge, spermatogenic cysts were detected in the mesohyl immediately underneath the endopinacoderm for several weeks in late spring and early summer. In contrast, production of oocytes was a continuous process at the population level, being active even during the time of larval release, which occurred for just a couple of weeks in mid-summer. Most of the members of the population were found to be engaged in sexual reproduction. Nearly 100% of the studied individuals contained oocytes at any time of the year and about 100% of them contained sperm at the peak of spermatogenesis, suggesting that sexual maturity in these sponges is reached soon after settlement. The timing of both male and female gametogenesis was found not to be directly related to seawater temperature. Our histological monitoring allowed us to estimate, for the first time, the success of internal fertilization in a demosponge, which reached extremely high values (99.27%). We also checked for embryo mortality during development, and found it to be negligible. Because effective reproduction (*i.e.*, fertilization and larval release) occurs only within a narrow time window during the warm season, the reasons why oogenesis extends all over the year, contradicting the seasonal pattern known for most other temperate demosponges, remain unclear. Several ideas are put forward, including the fact that oocytes appear to synthesize most of the yolk by themselves, with scarce involvement of nurse cells from the mother sponges.

The reproductive cycle of *Geodia barretti* (Bowerbank, 1858) (Porifera, Astrophorida) in two Scandinavian fjords

Frank Spetland{ XE "Spetland, Frank" }⁽¹⁾; Hans Tore Rapp{ XE "Rapp, Hans Tore" }^(1*);
Friederike Hoffmann{ XE "Hoffmann, Friederike" }⁽²⁾; Ole Secher Tendal{ XE "Tendal, Ole Secher" }⁽³⁾

(1) Department of Biology, University of Bergen, Bergen High-Technology Center, PO Box 7800, N-5020 Bergen, Norway, frank.spetland@bio.uib.no; hans.rapp@bio.uib.no; (2) Max Planck Institute for Marine Microbiology, Celsiusstr. 1, D - 28359 Bremen, Germany, fhoffman@mpi-bremen.de; (3) Zoological Museum, University of Copenhagen, Universitetsparken 15, DK - 2100 Copenhagen, Denmark, ostendal@snm.ku.dk

Sponges belonging to the Astrophorida form mass occurrences on the outer shelf and upper slope over large areas of the NE Atlantic. *Geodia barretti* is the dominating sponge species on such grounds along the Norwegian coast, around the Faroes, in the Barents Sea, and between Iceland and Greenland. Reproduction and recruitment of sponges on the grounds are largely unknown. Here we describe the gametogenesis in *Geodia barretti* in two Scandinavian fjords through a year cycle. The samples were collected in Korsfjorden, western Norway, and in Kosterfjorden on the Swedish west-coast. The reproductive cycle is annual for both populations, with one or two periods of gamete release per year. The onset of the reproduction coincides with the phytoplankton blooms in both fjords. The gametes in the Korsfjorden population are released in May/June, just after the phytoplankton spring bloom is over, and the sedimentation of dissolved and particulate organic matter is at its highest. In Kosterfjorden release of gametes occurs at the same time after the spring bloom; however, in this fjord an additional release of gametes was observed in October, just after the autumn phytoplankton bloom came to an end. Individuals within the same local population reproduce simultaneously within a restricted period of time. *Geodia barretti* is a dioecious and oviparous sponge, with oocytes (up to 100 micrometers in diameter) and spermatocysts (up to 125 micrometer in diameter) organised in clusters within the mesohyl. The sponge has asynchronous spermatogenesis, and synchronous oogenesis. Asexual reproduction has not been observed.

Spermatogenesis in the genus *Iophon* (Acanthidae, Microcionina, Poecilosclerida, Demospongiae) from Chilean Patagonia and the White Sea: A comparative study

Philippe Willenz{ XE "Willenz, Philippe" }^(1*); Alexander Ereskovsky{ XE "Ereskovsky, Alexander" }⁽²⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽³⁾

(1) Department of Invertebrates, Royal Belgian Institute of Natural Sciences, Rue Vautier, 29, B-1000 Bruxelles, Belgium, philippe.willenz@naturalsciences.be; (2) Department of Embryology, Biological and Soil Sciences Faculty, St. Petersburg State University, Universitetskaya nab. 7/9, Saint-Petersburg 199034, Russia, aereskovsky@mail.ru; (3) Museu Nacional, UFRJ, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil, hajdu@acd.ufrj.br

Late stages of spermatogenesis have been compared in a specimen of *Iophon proximum* from Fjord Comau, Chile and a specimen of *Iophon piceus* from Kandalaksha Bay, White Sea. As in all Demospongiae, spermatogenesis occurs within spermatocysts in both species. The origin of the spermatogonia could not be determined on these incidental observations. Spermatocysts appear at different stages of development, from spermatocyte I stage to mature spermatozoa and are located at random in the choanosome. Within each cyst, the development is homogeneous and synchronous. Their shape is spherical to ovoid with a mean size ranging from 34.4 µm to 47.7 µm, depending on the number of male gametes. Both species show elongated spermatozooids (about 4.0 x 0.9 µm in *I. piceus* and 4.5 x 0.8 µm in *I. proximum*) with a nucleus of cylindrical shape bearing a trapezoidal anterior side. The surface of the nucleus has a wavy aspect. Other shared features are cytoplasmic invaginations occurring around the basal part of the flagellum and an elongated basal body (1.7 µm in *I. piceus* and 1.1 µm in *I. proximum*). Acrosome is absent in both species.

Population dynamics of two West Australian *Haliclona* species (Porifera: Demospongiae: Haplosclerida: Chalinidae)

David Abdo{ XE "Abdo, David" }

School of Plant Biology (M090), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, 6009, Western Australia, Australia, abdod01@student.uwa.edu.au

The Western Australian species *Haliclona* sp. is a subtidal temperate marine sponge with two morphologies, possibly belonging to the same species. *Haliclona* sp. has been identified as a potentially economically important species due to the potent and unique bioactive compound (Salicylhalamide) it produces. However, the only information on this species relates to its chemistry with crucial information on the species biology and ecology lacking. The main aim of the study was to understand how the reproductive strategy and larval characteristics of *Haliclona* sp. structure and maintain the species populations. This involved firstly determining their taxonomic placement and relationship to each other using both traditional, chemical and molecular approaches. The study also examines the sexual (*e.g.* mode, sex phenotype and periodicity) and asexual reproduction characteristics of the two morphs. In addition, the larval characteristics (*e.g.* settling preferences, lifespan and morphology) of both morphs were also examined. To gain a better understanding of the dynamics of *Haliclona* populations, the natural growth of the species was monitored to assess any relationship between growth and processes (*e.g.* abiotic factors and competition/predation) which may be acting on the sponge populations. The study has gained valuable data on two *Haliclona* sponges in Western Australia which possibly has the lowest amount of published data on sponges in Australia, if not the world.

Morphometric measurements of complex sessile epibenthic organisms using a novel photogrammetric technique

David Abdo{ XE "Abdo, David" }^(*); James Seager{ XE "Seager, James" }; Euan Harvey{ XE "Harvey, Euan" }

School of Plant Biology (M090), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, 6009, Western Australia, Australia, abdod01@student.uwa.edu.au

The biomass and size of sponges have been measured in various ways from simple morphometric measurements, to measurements of weight (wet or ash-free), as well as traditional measurements of surface area (foil wrap) and volume (displacement). Many of these methods are destructive in nature (*e.g.* wet/dry weight), while others have reduced accuracy (*e.g.* length, height and weight measurements). This paper details and evaluates a photogrammetric technique developed to provide an alternative method for obtaining accurate, non-contact, non-destructive measurements of sponge's in-situ. The aim of the research was to evaluate the technique and determine the applicability of the technique as a research tool. The study involved capturing stereo images of six plasticine models using a specifically developed stereo digital still camera system. The models had three levels of surface complexity (complex, moderate and simple) and two sizes (approximately 2.5 L and 0.5 L). Using purpose fully developed software, three-dimensional reconstructions of the models were made to determine their volumes. To evaluate the technique as a method for obtaining morphometric measurements of sponges four parameters were examined: observer bias, accuracy, precision and efficiency. No significant ($P > 0.05$) difference was seen between the observer's volume estimates for any of the six models. The mean accuracy of volume estimates using this technique was $\pm 3\%$, with the lowest mean accuracy being $\pm 7\%$. This is significantly better than any previously reported non-destructive technique for determining the volume of sponges. The precision of the technique were also high, with the greatest imprecision being 2% of the volume estimate. The technique also proved to be time efficient with a mean measurement time of 60 minutes. When the technique was tested in a field situation on different sponge morphologies (flabellate, tubular and massive/mounding sponges), the accuracy, precision and efficiency were within the results seen during the laboratory trials. The photogrammetric technique trialed allows for non-destructive, un-observer biased, accurate, precise and efficient volume measurements of morphometrically complex sessile epibenthic invertebrates. Underwater photogrammetry and associated three-dimensional reconstruction has many advantages over traditional sampling techniques, and it is clear that this technique will be beneficial in the research of benthic organisms.

Reading the sponge community structure code in coral reefs (invited lecture)

Pedro Alcolado{ XE "Alcolado, Pedro" }

Institute of Oceanology, Ave. 1a, No.18406, Playa, Havana, Cuba, alcolado@ama.cu

The non-cryptic coral reef sponge community structure, due to its high connectivity with physical environment (sensu Bradbury), can be considered as a readable coded message reflecting the physical deterministic environment. Sponges have been used as environmental bio-indicators by Alcolado, Alcolado and Herrera-Moreno, Muricy, Zea, Carballo *et al.*, Carballo and Naranjo, and Vilanova *et al.*. Attempts carried out in Cuba to explore the potential use of sponge communities as bio-indicators are described. Species composition and relative abundance were obtained from 1 m² quadrats. Shannon's heterogeneity index (H') and Pielou's equitability index (J') were calculated cumulatively till stabilization (natural logarithms). Meta-analyses were done by pooling data for delimiting ranges of variation H' and J', and for obtaining trends of community variables, for environmental inference purposes. The results discussed ahead come from a number of coral reefs sites sampled around Cuba since 1976. *Clathria venosa* and *Iotrochota birotulata f. musciformis* dominated in much polluted reefs (Alcolado and Herrera-Moreno). *Scopalina ruetzleri* was abundant or dominated in moderately polluted reefs (Alcolado and Herrera-Moreno, Zea). *Mycale microsigmatosa*, *S. ruetzleri* and *Amphimedon viridis* have been found dominating under sewage stress (Muricy). In close agreement with Wulff, *Aplysina cauliformis* was tolerant to strong waves. A condition of stress is inferred exclusively when the dominance of some of those species is coupled to low values of species richness or species heterogeneity. Both *Cliona vesparia* (alfa stage) and *Tectitethya crypta* indicate sedimentation and wave joint stress. Species can also be used as indicators by summing up the numerical percentages of species with similar tolerance features. Cluster analysis has successfully segregated polluted sites (Alcolado and Herrera-Moreno, Carballo and Naranjo, Vilanova *et al.*). Preston and Preston's scheme for inferring six different environmental predictability and favorability situations by using gorgonian communities, (based on H' and J'), was applied and modified by Alcolado for sponges. This modification consisted in a scatter graph diagram of pairs of values of H' and J' from 104 sites. The resulting scatter area was divided into 11 "environmental inference ranges" reflecting different situations of predictability and favorability. Additionally, scatter graphs of variability with regard to depth of diversity indices, population density and sponge cover were obtained from many reef sites. They are reference patterns to comparatively infer the community condition within stress gradients. 1. The analysis of community's diversity indices can be used as a complementary tool to infer the predictability and degree of benignity of physical environment. 2. The dominance of certain species helps to infer the environmental factors determining the structure and composition of sponge communities (waves, sedimentation, low salinities, illumination, etc.). 3. Greater amounts of direct measurements of environmental physical factors supposedly related to sponge community features (rather than simply deductive inferences) are needed to improve the bio-indication potential of sponges.

Community structure of sponges in coral patches at upwelling zone, Northeast Venezuela

Adriana Alvizu{ XE "Alvizu, Adriana" }⁽¹⁾; Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }⁽²⁾;
José Luis Palazón{ XE "Palazón, José Luis" }⁽³⁾

(1) Universidad de Oriente N.E.- Escuela de Ciencias Aplicadas del Mar, Venezuela, adri_alvizu@yahoo.com; (2) Museo Marino de Margarita, Venezuela, crisdiaz@ix.netcom.com; (3) Universidad de Oriente N.E. - Instituto de Investigaciones Científicas, Venezuela - Instituto de Ciencias Marinas de Andalucía Puerto Real, Cádiz, España, jluis.palazon@icman.csic.es

Sponges are organisms that show high diversity and a wide geographic distribution in tropical marine ecosystems. Their abundance is partly determined by physical factors, such as water movement, light, substrate availability, and among of sedimentation. The present study aimed to understand major community structure parameters (H' , J' , species richness, and abundance) of sponge populations at two coral communities. The study area was Cubagua Island, located in a region affected by upwelling, where the coral communities grow in the form of patches with low coral diversity. The two study sites have distinct topographic composition and sedimentation rates. The first station is Las Cabeceras where the substrate is dominated by the hydrocoral *Millepora* sp., zoanthids, and some colonies of *Diploria strigosa* and *Porites astreoides*, and the second station is El Mercado, which is dominated by large colonies of *Montastraea annularis*, *Colpophylla natans* and *D. strigosa*. In both sites, two sub-areas were selected, and at each sub-area five transects were randomly located (parallel to coast) between 2 and 4 m deep. A 1 m² quadrant was placed continuously along each transect, and the occurrence and area covered by each sponge was recorded. Sediment traps (cylinders 24 cm high and 8 cm in diameter) were set at each station bi-monthly (from March to September 2005) and harvested every 14 days. The sedimentation rate was different between both localities, with the highest values at El Mercado (221.54 ± 111.60 gr/m²/day). Fifteen species were recorded within the transects in both stations, however the total number observed was 27 species in Las Cabeceras, and 19 in El Mercado. Two of the species found at Las Cabeceras (*Desmacella meliorata* and *Clathria calla*) are first reports for Venezuela. The sponge cover was very similar between the sub-areas, but different between stations. Las Cabeceras presented the highest sponge cover ($6.18 \pm 2.03\%$) represented mostly by three species: *Amphimedon erina* ($2.54 \pm 1.11\%$), *Tedania ignis* ($1.397 \pm 0.773\%$) and *Chondrilla nucula* ($1.17 \pm 0.47\%$). In El Mercado the sponge cover was low ($0.93 \pm 0.79\%$) and the most abundant and dominant species were *T. ignis* ($0.28 \pm 0.4\%$), *Niphates erecta* ($0.23 \pm 0.17\%$) and *A. erina* ($0.09 \pm 0.12\%$), which are very common at nearby mangrove and corals reef ecosystems. The diversity and equitability index were similar for both stations: Las Cabeceras, $H' = 2.17$ bits/ind and $J' = 0.55$, and El Mercado, $H' = 2.21$ bits/ind, and $J' = 0.56$. This means that at these two sites sponges have low diversity but some species prevailed over others. Analyzing relative density shows Las Cabeceras presenting the highest density (470 ± 109 ind/m²), compared to El Mercado (39.3 ± 32.83 ind/m²). Our results indicated that the large sedimentation rate at El Mercado is probably a major factor causing the low cover and density of sponges, and the exclusion of several sponges species when compared to Las Cabeceras.

Spongivory by angelfish (Family Pomacanthidae) in Salvador, Bahia state, Brazil

Bárbara Rustum Andréa{ XE "Andréa, Bárbara Rustum" }⁽¹⁾; Daniela Batista{ XE "Batista, Daniela" }^(1, 2); Cláudio L.S. Sampaio{ XE "Sampaio, Cláudio L.S." }⁽³⁾; Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾

(1) Depto. de Invertebrados - Museu Nacional - Universidade Federal do Rio de Janeiro. Quinta da Boa Vista, s/no., São Cristóvão. 20940-040 Rio de Janeiro, RJ, Brasil; (2) Departamento de Biologia Marinha, Universidade Federal Fluminense. Outeiro São João Batista s/n Niterói, Rio de Janeiro, Brasil; (3) Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba

The sponges are widely distributed in many marine ecosystems, but only a few animals consume them. There are few studies on spongivory by reef fishes, mostly in the Caribbean. The genera *Pomacanthus* and *Holacanthus* (Family Pomacanthidae) are considered the most important spongivorous of the Caribbean, with sponges comprising more than 70% of their gut contents. The goals of this study were to identify the sponge species which are part of the diet of the angelfish *Pomacanthus paru*, *Holacanthus ciliaris* and *Holacanthus tricolor* in Salvador, Bahia state, Brazil; to estimate the frequency of occurrence of each sponge species in the stomachs of fishes; and to calculate the accumulated richness of the sponge species in each species of angelfish. The studied specimens were obtained through ornamental fisheries. A total of 16 specimens were studied: five of *P. paru*, five *H. ciliaris* and six *H. tricolor*. The stomachs were removed and their contents were separated according to the aspect of the fragments. Pieces of the sponge fragments were immersed in paraffin to prepare transversal sections for identification. In Salvador, 34 species of sponges were found in 16 specimens of angelfish. Fourteen species of sponges were found in the diet of *Pomacanthus paru*, 23 in *Holacanthus tricolor*, and 13 in *H. ciliaris*. These findings indicate that all three species of angelfish are generalists in the consumption of sponges in Brazil, as they were already shown to be in the Caribbean. Besides sponges, the angelfish ate other organisms such as seaweed, zoanthids, bryozoans, and corals. The most frequent sponges in angelfish gut contents were *Tedania ignis*, *Mycale* sp., and *Spirastrella* sp., all with 37.5% of frequency in the 16 stomachs analyzed. *Tedania ignis* was not consumed by *H. ciliaris* in Salvador, but it was consumed by the other fish species, being the most frequent in *H. tricolor* e *P. paru*. The accumulated richness of sponge species in the diet of angelfish showed a tendency towards stability, but more specimens are required to estimate the total richness of sponge species in diet the diet of each reef fish in Salvador. There are differences between the sponge species consumed by angelfish in Brazil and those reported from other studies in the Caribbean. These differences are probably related to the variation in the abundance and toxicity of the sponges in the two regions.

Environmental factors affecting the distribution and abundance of the tropical sponge *Rhopaloeides odorabile* on the Great Barrier Reef (GBR)

Raymond Bannister{ XE "Bannister, Raymond" }^(1*); Christopher Battershill{ XE "Battershill, Christopher" }⁽²⁾; Rocky de Nys{ XE "Nys, Rocky de" }⁽¹⁾

(1) School of Marine Biology and Aquaculture, James Cook University, University drive, 4810, Townsville, Australia; (2) Australian Institute of Marine Science, QLD 4810 Townsville, Australia

Rhopaloeides odorabile is an abundant Dictyoceratid sponge common to the outer- and mid-shelf coral reefs of the Great Barrier Reef (Australia). However, it is rare on inshore reefs and where present has a smaller mean size. We investigated the factors driving this change in abundance and population structure focusing on the feeding biology and the impacts of sediment. One hypothesis to support the differential distribution is the increased ability to benefit from mixo-trophy in clear offshore waters. Using respirometry chambers we determined that under light conditions up to 1000 mmol photons.m⁻².s⁻¹ *Rhopaloeides odorabile* did not utilise photosynthesis as an energy source demonstrating no net energy contribution from symbionts. An alternative explanation for the distribution of *Rhopaloeides odorabile* is the differential impact of sediment across the continental shelf. The rate of sedimentation decreases towards the continental shelf. The quality and type of sediment also varies across the shelf with inshore sediments dominated by fine (< 63 mm) particle sizes, which are absent offshore. The effect of these sediment fractions on feeding biology is being experimentally determined using controlled feeding studies. While these studies contribute to an understanding of the factors influencing the distribution and abundance of *Rhopaloeides odorabile*, they are also a component of a broader program to determine optimum sites for the aquaculture of this species as a commercial bath sponge on the Great Barrier Reef.

**Effects of shading, current and predation on the distribution of the sponge,
Suberites sp. in temperate Australian coastal lakes**

Peter Barnes{ XE "Barnes, Peter" }

School of Biological Sciences, University of Wollongong, Northfields Ave, NSW 2522, Australia,
pbb47@uow.edu.au

The sponge, *Suberites* sp. is found in many temperate saline coastal lakes in NSW, Australia. It is an upright sponge, contains cyanobacteria, may grow attached or unattached to the substratum and individuals can be moved around within lakes by wind-driven currents. Previous work found that *Suberites* sp. is patchily distributed within lakes, but when they do occur, are most common in areas with a mosaic of bare sediment and patchy seagrasses or macroalgae. *Suberites* sp. is rarely found in areas with a dense canopy of seagrasses (particularly *Zostera*). This study used manipulative experiments to examine possible processes which may explain the absence of *Suberites* sp. from areas with dense seagrasses; 1. shading by seagrass canopies, 2. reduced water flow amongst seagrass, 3. other mechanisms associated with areas of dense seagrasses. Individual sponges were tagged *in situ* in Smiths Lake. Sponges were then shaded, had currents reduced or were transplanted to areas with dense *Zostera*. In addition, appropriate untouched and procedural controls were included. There were no effects of shading nor reduced current on individual sponges. In contrast, sponges transplanted to seagrasses were absent or had dramatically reduced in size by the end of the study. Examination of the surviving sponges in seagrasses and a repeated series of transplants revealed numerous bite marks, typical of small fish. These results suggest that predation may be an important mechanism in determining the small scale distribution of *Suberites* sp. within a lake. Although fish were not directly observed feeding on sponges in seagrasses, from the nature of the marks and the species of fish known to be in Smiths Lake at the time, small leatherjackets (Family: Monacanthidae) are most likely to be responsible.

Spongivory by *Pomacanthus paru* (Perciformes: Pomacanthidae) in Brazil

Daniela Batista{ XE "Batista, Daniela" }^(1*, 2); Bárbara Rustum Andréa{ XE "Andréa, Bárbara Rustum" }⁽¹⁾; Roberto Villaza{ XE "Villaza, Roberto" }⁽²⁾; Carlos Eduardo Leite Ferreira{ XE "Ferreira, Carlos Eduardo Leite" }^(2, 3); Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾

(1) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ), Quinta da Boa Vista s/n São Cristóvão, Rio de Janeiro, RJ, Brasil, danibiomar@uol.com.br; (2) Departamento de Biologia Marinha, Universidade Federal Fluminense (UFF), Outeiro São João Batista s/n Niterói, Rio de Janeiro, Brasil; (3) Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM), Rua Kyoto s/n, Arraial do Cabo, Rio de Janeiro, Brasil

Few animals feed on marine sponges. In the Caribbean, reef fish species of genera *Holacanthus* and *Pomacanthus* (Family Pomacanthidae) are the main spongivorous vertebrates, and sponges may comprise more than 70% of their gut contents. So far, all studies about spongivory by reef fishes were done in the Central Atlantic and Pacific Oceans, and there is no information about the subject in Brazil and the South Atlantic. The goals of this study were: (1) to identify which and how many sponge species are predated by the angelfish *Pomacanthus paru* in Brazil; (2) to quantify the proportion of higher taxonomic groups consumed by *P. paru*; and (3) to quantify the feeding rates (number of bites/min) in each site. Three sites were studied: Ilha Grande (23°01'S), Abrolhos (17°58'S) and Atol das Rocas (3°52'S). We used underwater observations complemented with gut content analysis. We observed 3391 bites by angelfish through visual census, in which the algae was the main taxonomic group consumed (77%), followed by undetermined material (21%), corals (1%), and sponges (1%). Only four sponges species were observed being predated by *P. paru*: *Spirastrella hartmani* (48%), *Chondrilla nucula* complex (32%), cf. *Mycale microsigmatosa* (11%), and *Scopalina ruetzleri* (9%). Feeding rates of *P. paru* varied significantly between the sites ($p < 0.001$), but not between the hours of the day, from 9 a.m. to 2 p.m. ($p > 0.05$). Total bite rates were significantly lower in Ilha Grande than in Abrolhos ($p < 0.03$) and Atol das Rocas ($p < 0.01$). This variation in predation intensity along the Brazilian coast may be related to the latitudinal reduction of water temperature. The major taxonomic groups in gut contents of *P. paru* were algae, sponges, tunicates and cnidarians. A total of 36 species were identified. The species more frequent in gut contents were *Chondrilla nucula* (60%), *Spirastrella hartmani* (47%), *Acervochalina* sp. 1 (33%), cf. *Amphimedon compressa* (33%), Chalinidae not identified 1 (30%) and *Dragmacidon* sp. (30%). The sponge and algae consumption varied significantly between sites. Sponge consumption was significantly higher at Ilha Grande than in Abrolhos and Atol das Rocas ($p < 0.03$). Algal consumption was lower at Ilha Grande than in Atol das Rocas ($p < 0.03$), but not between Abrolhos ($p > 0.05$). The differences between the sites in the proportion of algae and sponges in the diet of *P. paru* can be related to the availability of the two groups of organisms in the ecosystem. The large difference in sponge richness in the diet of *P. paru* as determined by the two methods used is probably related to the cryptic habitat of most sponge species in Brazil, which hampers the observation of the predation in visual census. *P. paru* is a generalist reef fish, which diet range is a function of the prey availability in the environment.

Substrate related differences in the excavating patterns of boring sponges

Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽¹⁾; Francesca Azzini{ XE "Azzini, Francesca" }⁽¹⁾; Carlo Cerrano{ XE "Cerrano, Carlo" }⁽²⁾; Barbara Calcinai{ XE "Calcinai, Barbara" }⁽¹⁾

(1) Dipartimento di Scienze del Mare, Università di Ancona, Via Brece Bianche I-60131 Ancona; (2) Dip.Te.Ris., Università di Genova, Corso Europa 26 I-16100 Genova

Eroding sponges produce a series of connected holes and galleries into calcareous substrata where they live; the excavations are realised by chemical substances secreted by special cells that remove the substratum as characteristic, small fragments (chips) that are expelled through the oscula. The boring activity results in typical scars (pits) on the wall chambers. It is well known that only calcium carbonate is etched by sponge activity but no comparative data are available about different forms of carbonate. In this work we present data regarding the erosion rate of the fast growing tropical boring sponge *Cliona albimarginata* on different kinds of carbonates. In particular we have tested portions of the shell of the large bivalve *Hippopus* sp. and of the branches of the stony coral *Acropora* sp. together with different kinds of carbonatic stones as Carrara marble, Majolica of the Conero Promontory, stone of Finale, stone of Prun and stone of Vicenza. The experiment was performed from June 2004 to January 2005 in the area of the Marine Park of Bunaken (North Sulawesi, Indonesia). In this period five cubic pieces (about 8 cm³) for each material were weighted and fixed on the surface of a unique large specimens of *Cliona albimarginata*, some square metres wide. After six months the pieces were collected, cleaned by hydrogen peroxide and weighed again to calculate the percent of mineral eroded by the sponge action. In the same period a panel of the same substrata was tested in the same place but not on the sponge surface to verify the action of other dissolving processes unrelated with the sponge activity. These controls revealed that the dissolution due to other boring organisms or to the action of the sea waters is negligible. On the other hand all the pieces placed on the sponge were covered by the sponge tissue and more or less dissolved. The dissolution rates of different kinds of carbonate are highly variable. Between the shell and the stony coral the first shows a rate of dissolution significantly higher than the other (48 vs. 40%) thus confirming the idea that the more dense material are easily etched. Among stone the highest dissolution rate was shown by the stone of Finale (34%), the marble of Carrara and the Majolica of the Conero and the stone of Vicenza have a dissolution rate ranging from 25 to 18% while the stone of Prun shows a very low erosion rate (8%). Also the shape of the excavating pattern is different in relation with the kind of carbonate. The differences in dissolution rate and erosion pattern may be explained in terms of crystal shape and aggregation, degree of metamorphism and presence of other minerals.

Inter- and intra-specific sponge competition on a coral reef

James J. Bell{ XE "Bell, James J." }

Institute of Biological Sciences, University of Wales, Aberystwyth, Edward Llwyd Building, Aberystwyth,
SY233DA Wales, UK

Sponges are an important component of coral reef benthic fauna in the Indo-Pacific and as such are frequently involved in inter- and intra-specific spatial competition. The results of these interactions are likely to influence the distribution and abundance of both sponges and competitors. In this study the static outcomes of spatial competition between sponges and other dominant reef organisms (including corals, encrusting algae, colonial ascidians, soft corals) are reported on two reefs experiencing different sedimentation regimes. Sponges frequently encountered corals, with many species demonstrating an ability to overgrow coral polyps, while most species were not overgrown by corals. Since many sponges are superior competitors to corals on Indo-Pacific reefs, we might expect sponges to be the dominant group on reefs rather than corals. The factors responsible for preventing the domination of sponges are discussed including predation, growth and the differential effects of physical disturbance on sponges and corals. The transitivity of this tropical sponge assemblage was calculated using the index of Tanaka and Nandakumar and compared with that of a temperate sponge assemblage. The Indo-Pacific sponge assemblage had a higher index of transitivity compared with the temperate sponge assemblage indicating the tropical assemblage to be more hierarchically organised than the temperate assemblage. These results are discussed in the context of increased inter- and intra-specific competition on coral reefs compared to temperate reefs.

Morphological monitoring of subtidal sponge assemblages

James J. Bell{ XE "Bell, James J." }^(*); Mark Burton{ XE "Burton, Mark" }; Blaise Bullimore{ XE "Bullimore, Blaise" }; Philip B. Newman{ XE "Newman, Philip B." }; Kate Lock{ XE "Lock, Kate" }

Institute of Biological Sciences, University of Wales, Aberystwyth, Edward Llwyd Building, Aberystwyth, SY233DA Skomer Marine Nature Reserve, Countryside Council for Wales, Fishermans Cottage, Martins Haven, Haverfordwest, Pembrokeshire SA62 3BJ Wales, UK

The management of the marine environment requires effective temporal monitoring of communities and assemblages to detect any change above the level of natural variability. Even though sponges are usually abundant in subtidal hard substratum environments and have the ability to significantly influence other community members they have often been excluded from monitoring programs because they are taxonomically difficult and often hard to quantify compared to other marine groups. We describe the use of a morphological method to examine photoquadrat data collected at three hard substratum sites over a 10-year period at Skomer Marine Nature Reserve, south west Wales. Differences in the morphological assemblages and abundance were apparent between years, but the sponge assemblages showed rapid recovery (within 1 year) to their original assemblage composition and abundance following declines. The changes were attributed to natural biological variation or a short-term impact, rather than any response to prolonged environmental change and there was no correlation between any of the changes in assemblages observed and the environmental variables measured. A comparison of morphological and species data enabled the same sponge assemblages to be identified at different sites, validating the method. This morphological approach to monitoring represents a cost-effective and realistic way of monitoring sponge assemblages.

The ecology of sponges subject to fishing and ecosystem change

Mark J. Butler{ XE "Butler, Mark J." }; Anne Kathryn Kauffman{ XE "Kauffman, Anne Kathryn" }^(*)

Department of Biological Sciences Old Dominion University Norfolk, VA 23529 USA,
amkauffm@odu.edu

Shallow, hard-bottom habitat is a ubiquitous feature of the shallow waters within the Florida Keys marine ecosystem, and sponges are a prominent component of these communities. Yet, remarkably little is known about the structure, ecological function, or resilience of hard-bottom communities or about the population ecology of the large sponges that dwell there. Our poor understanding of these communities has been highlighted in recent years by questions about the possible impacts of ecosystem change and resource exploitation on hard-bottom habitat. For example, hard-bottom communities in Florida Bay have been subject to algal blooms and massive sponge die-offs. Furthermore, there are concerns about the possible ecological impacts of Everglades restoration (*e.g.*, salinity change) and commercial sponge fishing on hard-bottom community structure and function. In the case of sponges, resolution of these issues has been hampered because there is no stock assessment, the most basic population dynamics for the pertinent sponge species are largely unknown, their tolerance to changes in water quality (*e.g.*, salinity, temperature) has not been tested, and the effect of the fishery on commercial sponges and allied species have never been studied. We are conducting a series of field and laboratory studies to better understand: (a) the community structure of hard-bottom in the Florida Keys, (b) the basic population dynamics of commercial sponges, (c) the impact of the sponge fishery on sponge communities, and (d) the effect of changes in salinity on sponge and octocoral populations. My presentation will highlight the key findings of each of these studies, which in general suggest that hard-bottom communities are likely to be quite sensitive to the kinds of changes in salinity that might result from Everglades restoration, but less so to current rates of commercial sponge exploitation.

The influence of the sea urchin *Echinometra lucunter* in the local distribution of *Darwinella* sp. (Porifera: Haplosclerida) in Arraial do Cabo, RJ, Brazil

Emiliano Nicolas Calderon{ XE "Calderon, Emiliano Nicolas" }; Paulo Cesar de Paiva{ XE "Paiva, Paulo Cesar de" }

Laboratório de Polychaeta, Departamento de Zoologia, Instituto de Biologia, Centro de Ciências da Saúde.
Ilha do Fundão s/n., Rio de Janeiro, RJ. Cep:21941-590, Brazil. encalderon@yahoo.com.br;
ppoliqueta@hotmail.com

In rocky substrates competition for space and predation act as biotic determinant of sessile marine invertebrates distribution. Sea urchins are known to control algae populations, leading to an increase in substrate accessibility for many organisms, thus having a fundamental role in the maintenance of diversity in those habitats. In Arraial do Cabo, RJ, Brazil, sponge communities seem to have a large spatial variation in scales of a few meters to kilometers. However, the mechanisms responsible for the observed distribution patterns have not been studied. *Darwinella* sp. is a sponge common at several sites in Arraial do Cabo, but factors determining its patchiness pattern are still unknown. The goal of the present study is to establish a the role of *Echinometra lucunter* densities in the algal turf cover and consequently in the density of *Darwinella* sp.. The research was conducted at Porcos Island, Arraial do Cabo. Eight sites on the rocky substrate were chosen (4-6m depth). *E. lucunter* density was visually estimated for each site as "High" or "Low". After that in each site, a 5m transect line was placed 25cm above and parallel to the bottom from were 5-6 random points were chosen. In each point, two quadrats (39x29cm) were photographed, one above and the other below the transect. Quadrats above the transect were used to quantify *Darwinella*, alga turf and *E. lucunter* densities, while quadrats below were only used to quantify *E. lucunter*, since *Darwinella* was almost inexistent. *Darwinella* and algal turf's percent cover were calculated with a 60 point grid system. One-way ANOVAs were conducted within transects to determine if there were significant differences among transects according to the High and Low prior classification. A T-test was used to verify if portions considered "Low" and "High" were significantly different. A Mann-Whitney test tested if *Darwinella* cover was different between "Low" and "High" portions. Additionally, Spearman Rank Correlations were used to determine if there was a relationship between sea urchin density, *Darwinella* and algal turf cover. Results showed that *E. lucunter* density was significantly smaller in "Low" (10 ind./m²) than in "High" (63 ind./m²) portions. Areas with "High" urchin density had a significantly higher *Darwinella* cover (mean 18.0%) than areas with "Low" density (mean 0.1%). The correlation between sea urchin and *Darwinella* was positively significant, while between sea urchin and algal turf was significantly negative. Correlation between *Darwinella* and algal turf was negatively significant, indicating that they might be competing for space. These results suggest that the presence of *E. lucunter* may be affecting positively *Darwinella* cover by decreasing algae turf cover. Thus, *E. lucunter* seems to have an important ecological role by increasing substratum availability for *Darwinella*, as usually happens with other sessile marine invertebrates.

Diversity patterns in sponges assemblages from the Northern Pacific Ocean: The influence of physical disturbances
(invited lecture)

José Luis Carballo{ XE "Carballo, José Luis" }

Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México. Avenida Joel Montes Camarena s/n. Apartado Postal 811, 82040 Mazatlán, Sin., México, jcarballo@ola.icmyl.unam.mx

Attempts to explain spatial and temporal patterns of diversity in nature have been and continue to be a very timely theme of ecological research. Many physical and biological processes have been linked with patterns of diversity and community structure. In addition, human activities also shape marine ecosystems. However, the interaction between physical disturbance such as increases in sand inundation or substrate movement and diversity has been one of the most debated issues, and is postulated to have strong influences on shallow benthic communities in many habitats around the world. Since several years ago, we are studying the structure of the sponge communities in different habitats from the Pacific Mexican coast (Mexico). We present the general spatial and temporal diversity patterns and give an overview of the physical processes affecting the sponge assemblages in consolidated (rocky habitat) and non-consolidated substrates (boulder field habitats). The major form of disturbance occurs by sand inundation on rocky habitat and when waves, generated by winter storms, overturn boulders. The largest impacts on the assemblages were the reduction of the diversity, losses and substitution of species, and a shift from a relatively mature and stable community to another more unstable, less diverse and dominated by encrusting species more adapted to the local environmental conditions. The surface of the rocks were subjected to an extremely turbulent flow regime with the increase in wave exposure affecting mostly to sponges of massive and branching morphology. Thus, our observations are also consistent with studies which show that morphological diversity of sponge assemblages decreases when perturbation increases. However, although many species showed a rapid decline in abundance, our study also showed that the recovery of some species was relatively fast (less than 1 year). Thus, the results suggest that sponge assemblages are more dynamic than previously thought, and clearly contrast with observations that the structure and dynamics of sponge communities exhibit an extremely slow rate of change even on a long-term scale. Our findings are also consistent with others in northern Pacific Ocean that show that competition plays a minimal role in the dynamics of rocky sublittoral communities, and that the physical features of habitat are sufficient to explain the biological patterns. In conclusion, the dynamics of sponge assemblages in North Pacific ocean are inextricably linked to oceanographic processes that can vary across enormous spatial and temporal scales.

Life history of the symbiotic association *Haliclona caerulea* (Porifera: Haplosclerida) and *Jania adherens* (Rhodophyta: Corallinales) in the Bay of Mazatlán

José Luis Carballo{ XE "Carballo, José Luis" }^(1*); Enrique Ávila{ XE "Ávila, Enrique" }⁽¹⁾;
Susana Enríquez{ XE "Enríquez, Susana" }⁽²⁾

(1) Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México. Avenida Joel Montes Camarena s/n. Apartado Postal 811, 82040 Mazatlán, Sin., México, jcarballo@ola.icmyl.unam.mx;

(2) Instituto de Ciencias del Mar y Limnología, UNAM. Apto. Postal 1152, 77500 Cancún, Q. Roo, México

The sponge *Haliclona caerulea* and the calcareous alga *Jania adherens* form a symbiotic relationship on shallow rocky ecosystems from the Bay of Mazatlán (Mexican, Pacific Ocean). The association consists of a massive and compact form, where the sponge covers the alga and fills the spaces between the algal branches. This association is mainly distributed from 2 to 5 m depth, where it is not possible to find the two species living in isolation, even though *J. adherens* lives in isolation in the intertidal zone. The abundance of the association is higher in spring and lower in summer-autumn, and it is correlated negatively with water temperature. There is no clear relationship between the period of higher abundance and the period of sexual reproduction of the sponge, which indicates that the association reproduces mainly by fragmentation. The growth coincides with the increase in water temperature (spring-summer), and later, when water temperature decreases, a shrinkage period is presented (autumn-winter). The populations can losses more than 50% of the biomass in one month, which is attributed to the fragmentation or detachment of the specimens caused mainly by strong water movement during the period of tropical storms of this region. It has been suggested that the main factor that limits the distribution of the association toward shallower water is the wave force, which diminishes the possibility of new recruits adhering to the substratum. We have also made laboratory experiments with the sponge larva that have demonstrated that they can actively select *J. adherens*, rejecting other substrata types. When living in association with *J. adherens*, *H. caerulea* can replace more than 27% of its skeleton with the alga's structure, obtaining a significant energy saving in the construction of its own skeleton, and also a bigger tissue resistance. Both properties allow the sponge to colonize a hydrodynamic environment that is suboptimal for the fragile tissue in the isolated form. On the other hand, *J. adherens* (in association with the sponge) has the physiologic capacity to photoacclimate to deeper and less illuminated areas of the bay, changing the growth form by expanding the branches of their canopy and reducing the self-shading of their pigments. The associated form of *J. adherens* forms taller individuals that are branched in a wider canopy, and are able to survive the high hydrodynamism in the Bay of Mazatlán, due to the protection that the sponge exercises on the thin and fragile branches of their canopy. Although it is not still clear how and when this association was originated, it is possible that more favorable environmental conditions could have existed in the past, so that both species could cohabit in isolated form in the same interval of depth. It seems clear that under the current environmental conditions of the Bay of Mazatlán, this association is an obligatory symbiosis with enormous ecological success.

Diversity and distribution of boring sponges from the Mexican Pacific coast: Excavating patterns and abundance in coral reefs ecosystems

José Luis Carballo{ XE "Carballo, José Luis" }^(*); Héctor Nava{ XE "Nava, Héctor" }; Eric Bautista{ XE "Bautista, Eric" }; José Antonio Cruz-Barraza{ XE "Cruz-Barraza, José Antonio" }

Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México. Avenida Joel Montes Camarena s/n. Apartado Postal 811, 82040 Mazatlán, Sinaloa, México,
jcarballo@ola.icmyl.unam.mx

Boring sponges constitute a major group that causes considerable destruction to marine calcareous marine structures. However, little is known about the taxonomy, distribution, and natural levels of sponge bioerosion in the eastern Pacific Ocean, and more particularly in the Mexican Pacific coast. In this work, twenty-five excavating sponges belonging to five genera were identified from 59 localities from the Mexican Pacific coast. Twenty three species belong to Clionidae: *Cliona papillae*, *C. vallartense*, *C. californiana*, *C. raromicrosclera*, *C. amplicavata*, *C. flavifodina*, *C. euryphylla*, *C. vermifera*, *C. microstrongylata*, *C. aff. caribbea*, five non-described *Cliona*-species, *Cliotheosa* aff. *hancokii*, *Pione carpenteri*, *P. mazatlanensis*, *Thoosa calpulli*, *T. mismalolli*, *T. sp.*, *Sphaciospongia incrustans* and *S. ruetzleri*. The family Phloeodictyidae (order Haplosclerida) is represented by two non-described species of the genus *Aka*. All species were found excavating different kinds of calcareous substrates: Thirteen species were exclusively found excavating coralline substrates (*C. vallartense*, *C. aff. caribbea*, five non-described clionids, *C. aff. hancokii*, *T. calpulli*, *T. sp.*, *S. incrustans*, and two species of *Aka*). Five species were found excavating only non-coralline structures such as bivalve shells, tubes of polychaetes and calcareous algae (*C. papillae*, *C. raromicrosclera*, *C. euryphylla*, *C. microstrongylata*, *S. ruetzleri*), and seven species were found excavating both coralline and non-coralline structures (*C. californiana*, *C. amplicavata*, *C. flavifodina*, *C. vermifera*, *P. carpenteri*, *P. mazatlanensis* and *T. mismalolli*). A description of spicule features, skeletal arrangement and distribution is given. The frequency of boring was also evaluated in different reef systems, and other aspects like intensity of boring and excavating patterns in coral species of the genera *Pavona* and *Pocillopora* are also briefly discussed. For this, more than 2000 coral samplings were collected randomly and examined for boring sponges in five different coral reef ecosystems 1000 km apart. 44% of the samples showed evidence of invasion. *Cliona vermifera* was the most abundant species (60% of the pieces collected), followed by *Cliona* sp. (6.7% of the pieces collected). Attached dead coral substrata had the highest invasion frequency (60%), followed by live coral (44.7%) and rubble (40.8%). The coral reef community from Isabel Island presented the highest diversity of boring sponges (9 species) and invasion frequency (58% of the samples collected in this locality), despite having one of the best conserved coral communities in the Mexican Pacific coast. Entrega's reef community had the lowest sponge diversity (5 species), and presented the second highest frequency of invasion (48.67%). Punta de Mita had the lowest sponge incidence (22.6%), despite suffering the most harmful effects of the 1998 ENSO event, when 96% of its live coral cover became dead coral substrata.

Overview on sponges for studying environmental perturbations

Emma Cebrian{ XE "Cebrian, Emma" }⁽¹⁾; Thierry Pérez{ XE "Pérez, Thierry" }^(2*)

(1) Centre d'Estudis Avançats de Blanes, CSIC, C/Accés a la Cala St. Francesc s/n, 17300 Blanes, Girona, Spain; (2) UMR CNRS 6540 DIMAR, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France, perez@com.univ-mrs.fr

Marine ecosystems are subject to a wide range of perturbations and it's currently accepted that only a combination of different biological tools from the ecosystem level to the molecular one, with a range of species representing various trophic levels and dwelling in different habitats can give an overall view of the pressures on marine ecosystems. However the monitoring programs implemented in temperate waters, and especially in the Mediterranean Sea, rarely consider the usefulness of a panel of bioindicators. Although harboring a huge percentage of the temperate marine biodiversity, sub-tidal rocky communities for example are not taken into account in European Monitoring programs. The aim of this work is to underline the relevance of sponges thanks to several Mediterranean examples. During the last three decades, it has been proven that sponges have some fundamental characteristics of good bioindicators. The simplification of assemblages has led to the proposition of resistant species as indicators. The Clionaidae are among the taxa frequently proposed, their abundance and variable growth forms being related to environmental conditions. In the 80's and the 90's, several experimental works using sponge ability to uptake very fine particles showed the usefulness of sponges as biomonitors of pollutant bioavailability. Wide inter-specific variations can be observed, even for species inhabiting the same habitat. However, we demonstrated that using sponges in local programs can be complementary to filter-feeders approaches traditionally used in monitoring networks. For instance, the improvement of the quality of some rocky communities after the implementation of waste-water policies has been shown using *Spongia officinalis*. Experimental and field studies also provided evidences of this species biotransformation or biodegradation ability of pollutants. Sponges are suitable models for evaluating sub-lethal responses to stress. The stake of the so-called biomarkers is identifying causal relationships, evaluate and predict the consequences on populations. Abundant molecular approaches to assess pollution risks and ecosystem health were developed using a panel of sponge species. There are number of studies assessing the genotoxicity of contaminants. Stress proteins expression, such as Metallothioneins or Heat Shock Proteins which are involved in maintaining homeostasis, were also investigated through experimental and field studies on pollution gradients. Hypothesising that HSP expression must present finer variations than previously known, we recently tested them to assess their relevance in the global warming context. Moreover, first attempts have been made correlating the expression of these biomarkers with effects on the metabolism, reproduction or growth. The time has come for a "Sponge Watch"! The only thing that we still need is baselines on a wide geographic scale for the most promising taxa and approaches.

Sponges as bioaccumulators of heavy metals: Spatial and temporal variations

Emma Cebrian{ XE "Cebrian, Emma" }^(1*); Maria-J. Uriz{ XE "Uriz, Maria-J." }⁽¹⁾; Xavier Turon{ XE "Turon, Xavier" }⁽²⁾

(1) Centre d'Estudis Avançats de Blanes (CEAB). CSIC Accés Cala St Francesc, 14. 17330 Blanes (Girona). Spain, emma@ceab.csic.es; (2) Department of Animal Biology (Invertebrates), University of Barcelona, 645 Diagonal Av. E-08028 Barcelona, Spain

Contamination by metals has increased drastically in coastal Mediterranean areas during the last 20 years. The present study analyses the variation in metal accumulation in sponge tissues at temporal and spatial scales. The widespread Mediterranean sponge *Crambe crambe* was sampled from 14 relatively clean localities along the Costa Brava (NW Mediterranean). Cu, Pb, Cd, Hg and V were analyzed but only Cu and Pb were present in the sponge tissues. All the individuals accumulated copper to some extent but the sponges living in marinas presented very high levels of copper, probably related with the use of copper-based antifouling paints for boats. When a monthly sampling on *C. crambe* was conducted in one of the target localities, a clear seasonality was found. Lead accumulated three times more in winter than in summer, while copper was accumulated twice more in summer (probably related to a more intense nautical activity). A particular study in the most contaminated zones (marinas) was performed to compare accumulation by different species (*Dysidea avara*, *Phorbas tenacior*, *Crambe crambe* and *Chondrosia reniformis*). A strong inter-species variation in metal accumulation was observed: *D. avara* accumulated significantly higher amounts of copper to be followed by *P. tenacior*, *C. crambe* and *C. reniformis*. In contrast, *C. reniformis* accumulated significantly more lead than the other three species. It can be concluded that metal accumulation depended on both the species and the metal considered. When comparing metal concentration in sediments and sponge tissues, we found a significantly positive correlation for copper and lead, although the sponges presented a higher concentration than sediments only for copper, which indicates an active accumulation of this metal. Sponges seem to be good monitors for relatively low metal contamination due to their capacity to accumulate metals, in particular copper without showing conspicuous adverse effects.

**Observations of reef coral undermining by the Caribbean excavating sponge
Cliona delitrix (Demospongiae, Hadromerida)**

Andia Chaves-Fonnegra{ XE "Chaves-Fonnegra, Andia" }; Sven Zea{ XE "Zea, Sven" }(*)

Departamento de Biología and Centro de Estudios en Ciencias del Mar - CECIMAR, Universidad Nacional de Colombia, INVEMAR, Cerro Punta de Betín, Santa Marta, Colombia, andiecita@hotmail.com; szea@invemar.org.co

Among sessile organisms inhabiting calcareous hard bottoms, sponges which simultaneously encrust and excavate the substratum, successfully compete for space by undermining the basal support of their neighbors. *Cliona delitrix* is an excavating and encrusting Caribbean reef sponge that colonizes massive corals, aggressively displacing their live tissue, until the whole coral head is totally encrusted and deeply burrowed. To establish with some detail the process of coral colonization and undermining by this sponge, at San Andrés Island (SW Caribbean, Colombia) we carried out observations of unmarked and marked sponge-colonized corals, and obtained samples of the frontiers of interaction for microscopical observation in cut blocks and ground and polished sections. The sponge, sending under the surface a front of tissue with pioneer filaments, excavates the skeleton underneath the coral polyps, and forms a band of death coral around it. But as the polypar tissue and its supporting skeleton are so strongly interwoven, the extent of sponge undermining appears not to be enough to produce detachment and sloughing off of the polyps. Thus, it appears that the coral death band is the product of other mechanism(s) acting at the tissue or cell level. Coral and sponge tissue close to each other or in contact show accumulation of granulous cells and what appears to be coral cell debris. The sponge erodes away the upper 5 mm of the coral skeleton; its tissue first fills in and then expands the coral's skeletal spaces, opening wide deep crevices in oscular areas, but elsewhere maintaining a 5 mm thick crust at the surface regularly perforated at the corallites' cavities, where inhalant papillae are located. The coral death band is quickly colonized by filamentous algae, which apparently help consolidate or expand it, with the aid of damselfish, of the long-spined sea urchin *Diadema antillarum*, and possibly of coral pathogens. The sponge ramifies internally, reaching the surface beyond the main body, a mechanism that allows it to advance and overpower more quickly a coral colony. Its advance and penetration continues until the whole coral head is taken.

Population ecology of conspicuous sponges in Florida Keys coral reef and hard-bottom habitats

Mark Chiappone{ XE "Chiappone, Mark" }^(1*); Leanne M. Rutten{ XE "Rutten, Leanne M." }⁽¹⁾; Steven L. Miller{ XE "Miller, Steven L." }⁽¹⁾; Dione W. Swanson{ XE "Swanson, Dione W." }⁽²⁾

(1) Center for Marine Science, University of North Carolina at Wilmington, 515 Caribbean Drive, Key Largo, FL 33037, Tel: 305 451 0233, Fax: 305 453 9719. USA, chiapponem@uncw.edu; (2) Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149. USA

Despite intensive assessment and monitoring efforts of coral reefs in the southeast Florida region, little is known concerning the population ecology of conspicuous reef-associated sponges. We report here for the first time results of a comprehensive survey concerning the distribution, abundance, and volume measurements of a targeted group of sponges commonly found in the Florida Keys. To quantify habitat distribution patterns of sponge density and size (volume), a stratified random sampling design was employed during May-October 2005 that incorporated unique habitat types across the southeast Florida shelf and regional sectors hypothesized to represent spatial variations in water quality regimes. Replicate 8-m x 1-m transects were surveyed at 195 sites spanning 200 km of the Florida Keys from the northern end of the Florida Reef Tract to the Marquesas Keys and included a spectrum of habitat types varying in depth (2-27 m), structural complexity, and benthic community structure. Habitat types sampled included patch reefs, high-relief spur and groove, shallow and deeper low-relief hard-bottom, shallow and deeper low-relief spur and groove, and the deeper fore reef slope from 15-20 m and from 25-27 m depth. Transects were used to quantify the number of targeted sponges and measurements were made of sponge dimensions to estimate the volume per individual. Targeted species represented some of the more abundant species as determined from historical data: *Callyspongia vaginalis* (tube), *Ircinia campana* (vase) *I. felix* (encrusting to amorphous) *I. strobilina* (amorphous), *Niphates digitalis* f. *digitalis* (tube or vase) and *Xestospongia muta* (massive). Surveys of the 195 sites (3,120 m²) yielded 4,107 individuals of the targeted sponges, of which *I. felix* (1,631 individuals, 40%) and *N. digitalis* (813 individuals, 20%) were the most abundant. The sponges surveyed tended to exhibit species-specific patterns in density and volume distribution among the habitat types sampled. Four of the six species had greater mean densities (no. individuals/m²) on habitat types closest to shore (patch reefs), with relatively low densities (< 0.1) on the most wave-exposed habitat types along the reef tract. Densities of *N. digitalis* were generally higher offshore below 6 m depth, independent of structural complexity, while densities of *X. muta* increased with depth. All six sponges exhibited relatively high densities from 15-27 m depth, especially *X. muta* (0.39-0.56). Comparisons of density and volume measurements indicated that *C. vaginalis*, *N. digitalis*, and *X. muta* exhibited the greatest average volume in habitats closest to shore or those that were deepest on the fore reef slope. In contrast, each species of *Ircinia* exhibited relatively similar average individual volumes among most of the habitat types, indicating that differences in volume per transect were due to density differences and not variations in size. While many of the patterns in abundance and size documented can be at least partially explained by differences in wave energy across the south Florida shelf, results from this survey comprise a baseline from which other studies may discern how variations in recruitment, predation, recent storm damage, and water quality affect the population ecology of sponges in the Florida Keys.

**Assessment of invasiveness of the orange keyhole sponge *Mycale armata* in
Kāneʻohe Bay, Oʻahu, Hawaiʻi**

Steve L. Coles{ XE "Coles, Steve L." }^(*); Holly Bolick{ XE "Bolick, Holly" }

Department of Natural Science, Bishop Museum, 1525 Bernice St., Honolulu, Hawaii, 96817, USA

The introduced Orange Keyhole Sponge *Mycale armata* has been considered to be highly invasive on coral reefs in Kāneʻohe Bay, Oʻahu, Hawaiʻi, where it can overgrow native, habitat-forming, hard corals. In order to determine the impact and invasiveness of the sponge a study was conducted in 2004-2005 to determine its distribution, abundance and growth rates throughout the bay and whether mechanical removal would be an effective management technique for its control. Results from 190 manta board surveys and paired 25 m belt transects using photo quadrats on 19 reefs indicated that the sponge had maximal coverage in the south-central part of the bay, in the vicinity of Coconut Island. Despite the visual dominance of this conspicuous sponge on many reefs, its maximum coverage on any transect was 9.2% of available area, with a mean of two transects at this site of 6.5%. Sponge coverage on reefs decreased to less than 1% on reefs at 1-3 km from this zone of maximum abundance, and mean coverage for all 19 reef sites was 1.7%. Quarterly measurements of changes on sponge coverage on permanent photo quadrats indicated a mean increase of 12.7% in the area covered by sponge during one year on undisturbed control quadrats, with a mean decrease in coral cover of 16.% on the same quadrats. By comparison, sponge coverage on quadrats where all feasible sponge had been mechanically removed at the beginning of the year showed similar a sponge mean increase of 10.6% and a mean decreased coral cover of 6.3%. These results indicate that mechanical removal would not be an effective method of controlling the sponge over the long term, especially considering that removal required an equivalent of 11-22 hours per sq. m. Studies are continuing in 2006 to determine if the distribution and coverage of the sponge is expanding in the the bay and if the rates of increase on both control and removal quadrats continues to occur.

Spicule armament and defense in temperate zone sponges: A transcontinental comparison

Andy Davis{ XE "Davis, Andy" }⁽¹⁾; Adrian Ferguson{ XE "Ferguson, Adrian" }⁽¹⁾; John Himmelman{ XE "Himmelman, John" }⁽²⁾

(1) School of Biological Sciences, University of Wollongong, NSW 2517 Australia; (2) Department de Biologie, Université Laval, Quebec G1K7P4 Canada

We tested the hypothesis that siliceous spicules from temperate zone sponges were an effective defense against common rocky-reef sea-urchins. We added spicules to palatable agar discs and determined the amount consumed by *Strongylocentrotus droebachiensis* in the northern Gulf of St. Lawrence and *Centrostephanus rodgersii* in southeastern Australia. The energy and protein values of the agar discs were matched to that of the sponges tested. Of the five sponge species examined in Australia, three dissuaded local urchins from feeding while spicules were an effective defense for two of the three sponge species examined in Canada. For several sponge species the effectiveness of spicules as a defense was dramatic, despite modest sample sizes. Generally megascleres were an effective defense, but surprisingly microscleres defended *Chondrilla australiensis*. In contrast to our findings with 'local' sponge spicules fed to 'local' urchins, spicules from the other continent were surprisingly ineffective. For example, Australian sponge spicules failed to dissuade Canadian urchins from feeding. Our findings confirm that spicules play more than a role in skeletal support, but may underestimate the defensive capabilities of sponges as we have not considered chemical defense. We are aware that some of the sponges we have examined exhibit chemical defense against urchin feeding. Importantly, our data indicate that physical defense in sponges may be tightly co-evolved within a continent.

Foraminifera associated to the sponge *Mycale (Carmia) microsigmatosa* (Mycalidae, Poecilosclerida, Demospongiae) in Niterói, Brazil

Mirna Helena M. Dias{ XE "Dias, Mirna Helena M." }⁽¹⁾; Suzi M. Ribeiro{ XE "Ribeiro, Suzi M." }^(2*); Patrícia O. Silva{ XE "Silva, Patrícia O." }⁽³⁾

(1) Faculdades Integradas Maria Thereza. Bacharelado em Biologia Marinha. Niterói, RJ, Brazil; (2) Pós-graduação em Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense. P.O. box 100.644, 24001-970, Niterói, RJ, Brazil, suzi@acd.ufrj.br; (3) Programa de Geoquímica, Instituto de Química, Universidade Federal Fluminense, 24020-150, Niterói, RJ, Brazil

Ecological relationships (*e.g.* mutualism, comensalism and parasitism) between sponges and polychaetes, crustaceans, algae, protozoans, and other marine organisms have been studied. However, there are few studies on the ecological relations between sponges and foraminifera. The main goal of this study was to relate the occurrence of benthic foraminifera associated to ten individuals of the sponge *Mycale (Carmia) microsigmatosa* collected at Itaipu Beach, Niterói, Rio de Janeiro state, Brazil. Prior to sampling, sponges were covered with a plastic bag to reduce loss of foraminifera. The seawater in bag was filtered using a sieve with a mesh size of 0.062 mm, and the sponges with their associated fauna were fixed in 70% ethanol. A total of 26 foraminifera species were found living inside *M. microsigmatosa*. Among them, *Lobatula aff. lobatula*, *Rosalina globularis*, and *Rosalina floridana* were the most frequent species found. The latter was the most representative species in abundance and frequency. Foraminifera species inside the sponges were found in various ontogenetic stages, suggesting the occurrence of comensalism as they use this microhabitat for nutrition, and reproduction purposes.

Sponge biodiversity and abundance as indicators of mangrove epibenthic community health
(invited lecture)

Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }(1, 2*); Klaus Rützler{ XE "Rützler, Klaus" }(1)

(1) Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0163, USA, ruetzler@si.edu; (2) Museo Marino de Margarita, Boulevard El Paseo, Boca del Rio, Peninsula de Macanao, Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com

In the Caribbean Sea, sponges are diverse and common colonizers of subtidal mangrove substrates. Comparing Porifera species richness and composition across various Caribbean systems (Panamá, Belize, and Venezuela) we find two general trends: (1) few species are common, while the majority is rare, and (2) there is a high natural variability within and between sub-systems (islands, lagoons, canals). Therefore we conclude that sponge species richness can only serve as a bioindicator of subtidal health if there are baseline data for one location to determine its variation over time, not to compare geographically separate mangroves. We are evaluating changes over time in the occurrence, size, and abundance of common and widely distributed mangrove species, such as *Haliclona manglaris*, *H. curacaoensis*, *H. implexiformis*, *Chalinula molibta*, *Mycale magniraphidiphora*, *Clathria venosa*, *Geodia papyracea*, *Terpios manglaris*, and *Halicarca* sp. In addition, we monitor more habitat-generalist species, particularly, *Tedania ignis*, *Niphates erecta*, *Hyrtios proteus*, *Spongia tubulifera*, *Chondrilla nucula*, *Chondrosia collectrix*, *Mycale microsigmatosa*, and *Scopalina ruetzleri*. Results indicate a highly dynamic system with a large variation in relative abundance of major taxa (algae, sponges, ascidians), and divergent population dynamic strategies among sponge species. Some of the most common mangrove species are being investigated for their suitability as bio-indicators of mangrove health by evaluating changes in their population dynamics and responses to natural and anthropogenic ecological stress conditions.

Spatial variability in community structure of Dictyoceratid sponges across Torres Strait, Australia

Alan R. Duckworth{ XE "Duckworth, Alan R." }^(1*); Carsten Wolff{ XE "Wolff, Carsten" }⁽¹⁾;
Elizabeth Evans-Illidge{ XE "Evans-Illidge, Elizabeth" }⁽¹⁾; Stephen Whalan{ XE "Whalan,
Stephen" }⁽²⁾; Stanley Lui{ XE "Lui, Stanley" }⁽³⁾

(1) Australian Institute of Marine Science, PMB No 3 Townsville, QLD 4810, Australia; (2) James Cook University, Townsville, QLD 4811, Australia; (3) Queensland Department of Primary Industries and Fisheries, Cairns, QLD 4870, Australia

Spatial variability in community structure of dictyoceratid sponges (Class Demospongiae; Order Dictyoceratida) was examined on coral reefs in Torres Strait, an archipelago of 70 small islands between northern Queensland, Australia, and Papua New Guinea. Dictyoceratid sponge abundances and environmental parameters were recorded at 4 locations, separated by 50-220 km. Each location was subdivided into 5-7 sites, each ~2 km apart. At each site four 50x2 m belt transects were quantitatively surveyed, recording dictyoceratid numbers, substrate type (rock, rubble and sand), water clarity, degree of reef slope and depth. Dictyoceratid abundance was similar among locations, averaging 15.5 individuals per 100 m², but varied significantly among sites. Twenty five dictyoceratid species were recorded in Torres Strait, with approximately half (12/25) found in only one location. Canonical Correspondence Analysis determined that the measured environmental factors explained only 26% of the spatial variation. Cluster analysis revealed a complex dictyoceratid community structure with consistent patterns among neighbouring sites and among sites separated by hundreds of kilometers. Conversely the dictyoceratid community could vary greatly between neighbouring sites 2 km apart and on the same reef complex. The results of this study suggest that spatial variability of dictyoceratid sponges is influenced by a combination of environmental, biological and stochastic processes.

Nickel biomagnification in marine sponges

Ana Esteves{ XE "Esteves, Ana" }^(1*); Marise Almeida{ XE "Almeida, Marise" }^(1,2); Angela Serafim{ XE "Serafim, Angela" }⁽³⁾; Maria João Bebianno{ XE "Bebianno, Maria João" }⁽³⁾; Liliana P. Ferreira{ XE "Ferreira, Liliana P." }^(4,5); Madalena Humanes{ XE "Humanes, Madalena" }⁽¹⁾

(1) Centro de Química e Bioquímica - Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal, aiesteves@fc.ul.pt; (2) Instituto de Tecnologia Biomédica, Faculdade de Medicina Dentária da Universidade de Lisboa, 1649-003 Lisboa, Portugal; (3) Centro de Investigação Marinha e Ambiental, Faculdade de Ciências da Mar e do Ambiente, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; (4) Departamento de Física, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, 3004-516 Coimbra, Portugal; (5) Centro de Física da Matéria Condensada, Departamento de Física, Faculdade de Ciências da Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal

Nickel has been reported as a “toxic” and “less metabolically essential” metal, or classified as essential but with no further reference to its metabolic role. It is not unequivocally considered essential to aquatic organisms mainly because its requirements for many species have not been studied. Nickel’s essentiality can only be confirmed for plants and (cyano)bacteria due to its documented role in urease and hydrogenase metabolism. This essentiality to aquatic animals, invertebrate as well as vertebrate species, remains unknown, as a nickel-containing biological molecule has yet to be recovered from their tissues. Nickel, along with other divalent cations, although essential in living organisms, can become potentially toxic in high concentrations, due to its low oxidation potential and its ability to form highly reactive species damaging to the cell. Thus, it is surprising that some organisms are able to accumulate this metal in levels that are much higher than those in the surrounding environment. In previous works, several marine sponges from the Berlengas Natural Reserve and the Arrábida Natural Park have been studied for their content in several metals, using energy dispersive x-ray fluorescence (EDXRF). One species, *Cliona viridis*, has shown to accumulate nickel at c.a. 3000 ppm, a value up to 50 times higher than that of the sediments of the site of capture. In this work, we report the first known nickel biomagnification in marine sponges, particularly in the species *Cliona viridis* and *Cliona varians*. The molecule responsible for this nickel accumulation has been identified and successfully isolated using differential pulse polarography, gel filtration chromatography and SDS-PAGE. It is a very soluble and thermally stable small molecule, with a molecular weight of 4032 Da, as determined by MALDI-TOF Mass Spectrometry. The molecule presents a high content of glycids, of which 54% are hexoses, and a small peptide, with the most abundant aminoacid being phenylalanine. Atomic absorption spectroscopy studies gave us a ratio of 30 nickel atoms per molecule. This molecule is paramagnetic and the first magnetic experiments (in Superconducting Quantum Interference Device - SQUID) suggest that both Ni (II) and Ni (III) are present. This last form of nickel is quite unusual, especially in natural systems. This technique and the electron paramagnetic resonance will also provide us information about the geometry and local symmetry of the nickel atoms. Polarographic studies revealed the presence of thiol groups, indicating that the nickel is probably coordinated through S-Ni bonds. Further characterization steps are undergoing to determine the protein sequence and structure as well as its location within the sponge/associated bacteria consortium and its eventual role in the sponge metabolism.

Allelopathic effects of the Caribbean sponge *Svenzea zeai* on the coral *Montastraea annularis*

Deborah Gochfeld{ XE "Gochfeld, Deborah" }^(1*); Lucy Harrison{ XE "Harrison, Lucy" }⁽²⁾; Julie Olson{ XE "Olson, Julie" }⁽³⁾; Michael P. Lesser{ XE "Lesser, Michael P." }⁽⁴⁾; Sridevi Ankisetty{ XE "Ankisetty, Sridevi" }⁽²⁾; Marc Slattery{ XE "Slattery, Marc" }⁽²⁾

(1) National Center for Natural Products Research, University of Mississippi, P.O. Box 1848, Oxford, MS 38677-1848, USA, gochfeld@olemiss.edu; (2) Department of Pharmacognosy, University of Mississippi, P.O. Box 1848, Oxford, MS 38677-1848, USA, harrisonlucy@hotmail.com; (3) Department of Biological Sciences, Box 870344, University of Alabama, Tuscaloosa, AL 35487, USA, jolson@bama.ua.edu; (4) Department of Zoology and Center for Marine Biology, 46 College Rd., University of New Hampshire, Durham, NH 03824, USA, mpl@unh.edu

Allelopathic interactions among sessile marine invertebrates have been frequently proposed, but rarely investigated. In this study, we demonstrate that the encrusting sponge *Svenzea zeai* is able to overgrow the massive reef-building coral *Montastraea annularis* by secretion of allelochemicals that kill the underlying coral tissue. In the Bahamas, *M. annularis* is a dominant reef-building species, yet on certain reefs, large colonies of this coral are overgrown by *S. zeai*. To determine the mechanism by which *S. zeai* is able to overgrow *M. annularis*, a series of field experiments were conducted by attaching screens containing various treatments onto live *M. annularis* tissue on the affected reefs. Treatment effects were measured with digital image analysis and by use of pulse amplitude modulated fluorescence (PAM). Pieces of *S. zeai* attached to living coral tissue caused rapid mortality, with no remaining living tissue after 7 days. Shading alone did not appear to be the cause of this mortality, as opaque black strips attached to living coral tissue did not cause changes to the underlying tissue that differed from controls. To determine whether mortality caused by the sponge was due to allelochemicals, the crude extract of *S. zeai* was incorporated into agar strips and attached to the coral tissue alongside control strips. The crude extract and subsequent organic fractions caused mortality in tissues of *M. annularis*. Our results indicate that allelopathic compounds from the sponge *Svenzea zeai* may be important in its ability to dominate substrate through competitive interference.

***Aplysina* red band syndrome: A new condition affecting Caribbean sponges**

Deborah Gochfeld{ XE "Gochfeld, Deborah" }^(1*); Marc Slaterry{ XE "Slaterry, Marc" }⁽²⁾; Julie Olson{ XE "Olson, Julie" }⁽³⁾

(1) National Center for Natural Products Research, P.O. Box 1848, University of Mississippi, University, Mississippi 38677; (2) Department of Pharmacognosy, P.O. Box 1848, University of Mississippi, University, Mississippi 38677; (3) Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487

A substantial and increasing number of reports have documented dramatic changes and continuing declines in Caribbean coral reef communities over the past twenty years. To date, the majority of disease reports have focused on scleractinian corals, whereas sponge diseases have been less frequently documented. In this report, we describe *Aplysina* red band syndrome (ARBS) affecting Caribbean rope sponges of the genus *Aplysina*, observed on shallow reefs in the Bahamas and Panama. Visible signs of disease presence included one or more rust-colored leading edges, with or without a trailing area of necrotic tissue, such that the lesion formed a contiguous band around part or all of the sponge branch. Microscopic examination of the leading edge of the disease margin indicated that a cyanobacterium was consistently responsible for the coloration. Although the presence of this distinctive coloration was used to characterize the diseased state, it is not yet known whether this cyano bacterium is directly responsible for disease causation. The prevalence of ARBS declined significantly from July to October 2004 before increasing to above July levels in January 2005. Transmission studies in the laboratory and field demonstrated that contact with the leading edge of an active lesion was sufficient to spread ARBS to a previously healthy sponge, suggesting that the etiologic agent, currently undescribed, is contagious. Studies to elucidate the etiologic agent of ARBS are ongoing. Sponges are an essential component of coral reef communities and emerging sponge diseases clearly have the potential to impact benthic community structure on coral reefs.

Color variability in the sponge *Halichondria panicea* (Demospongiae) in the Northeastern Pacific Ocean

Jason E. Hall{ XE "Hall, Jason E." }(1*); Ann L. Knowlton{ XE "Knowlton, Ann L." }(2)

(1) Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA, 98112 USA, Jason.Hall@noaa.gov; (2) Notlwonk Science, PO Box 80036, Cornish, UT, 84308 USA, gypsea2003@hotmail.com

The color of the sponge *Halichondria panicea* (Demospongiae), a common rocky intertidal and subtidal sponge, varies along a gradient from orange to yellow to green. The color variability of this sponge occurring in the northeastern Pacific Ocean was investigated. The results of field surveys and manipulative laboratory experiments show a clear relationship between sponge color and light exposure, with sponge color changing along a gradient from orange to yellow to green with increasing light exposure. In addition, increased growth rates of sponge tissues were observed in response to reduced light exposure in lab-based light exclusion experiments. Analyses of sponge pigments confirm the presence of photosynthetic organisms within the sponge matrix although the results do not support the hypothesis that these endobionts are responsible for the observed color variability of this sponge. Alternatively, the data presented here support the hypothesis that *H. panicea* color variability may be caused by blue carotenoproteins produced by sponge tissues.

Diversity and chemical ecology of shallow water sponge communities in the Chinese Yellow Sea: Status and perspectives

Dorte Janussen{ XE "Janussen, Dorte" }^(1*); Wei Zhang{ XE "Zhang, Wei" }⁽²⁾; Daniela Henkel{ XE "Henkel, Daniela" }⁽³⁾

(1) Marine Evertebraten I, Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany, Dorte.Janussen@senckenberg.de; (2) Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China, WeiZhang@dicp.ac.cn; (3) Marine Evertebraten I, Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany, Daniela.Henkel@senckenberg.de

With a large total area and climatic range, the Chinese oceans are among the richest in the World in terms of biodiversity, this is true not least to the sponges. The number of sponge taxa, particularly from South China Sea, is estimated to be exceptionally high with over registered 1500 species and many more waiting to be described, some of which contain valuable natural products. However, the industrial use of these sponges is severely hampered by the lack of knowledge on their taxonomy and ecology. Most Chinese sponges described so far were collected in the tropical South China Sea and East China Sea. But in the Yellow Sea and Bohai Sea, less than 10 sponge species have so far been recorded, most of which are only preliminarily determined, biochemical investigations have been performed on 2 species. All together, the sponge fauna of the North-Eastern China Seas is heavily under-explored. Preliminary investigations done by snorkelling at different localities in and around Dalian (Chinese Yellow Sea) revealed that the biotopes are strongly impacted by the wide-spread aquaculture, especially of brown algae which dominate the underwater landscape at shallow depths. However, the diversity of sessile organisms (sponges and ascidians) is comparably high, and it is possible to observe a distinct sponge zonation even within the upper 10 m. This is especially clear in the probably less eutrophic Dalian Bay where the intertidal community is characterized by 3 spp of demosponges (e.g. the very common *Hymenacidon perleve* and *Reniochalina* n.sp.). The shallow subtidal community is characterized by 4 distinct spp, which appear to be especially common below 5 m depth. Diversity of the benthic fauna increases with depth, a tendency which we expect to hold true below 10 m, where algae populations cease. Both Chinese Yellow and Bohai Sea represent an oceanic area with great temperature variability (between 37° C and -17° C, summer and winter, respectively). We hypothesize that environmental stress on the sponges is high, and sponges from this region are expected to biosynthesize unique bioactive metabolites, although the general chemical diversity is probably lower than in tropical seas. To test this working hypothesis, our future investigations include: 1. Extensive mapping of sponge distribution and diversity at chosen locations in the Yellow and Bohai Seas by SCUBA diving, including a detailed record of the sponge species and description of the expected new taxa. Definition of key sponge associations for different environmental settings and comparison with other known faunas (e. g. from Korea). 2. Investigation of sponge-associated infauna, especially the meio-infauna. 3. Analysis of bacteria associated with the sponge species by both cultivation-independent molecular methods and cultivation-based methods. 4. Bioactivity screening to elucidate the biochemical diversity of the sponge

A new species of *Scottocheres* (Copepoda: Siphonostomatoida) associated with *Craniella quirimure* Peixinho *et al*

Rodrigo Johnsson{ XE "Johnsson, Rodrigo" }^(*); Elizabeth Neves{ XE "Neves, Elizabeth" };
Bruno Cosme{ XE "Cosme, Bruno" }

Dept. Zoologia, Inst. Biologia, Universidade Federal da Bahia, Campus de Ondina, CEP: 40210-170,
Salvador, BA, Brazil

Craniella quirimure Peixinho *et al.* is a recently described species whose type locality is in Itaparica Channel located west of Itaparica Island in Todos os Santos Bay (Bahia state). The freshwater input on the bay is less than half the volume of seawater and salinity is higher than 36.4 nearly all over, characterizing a true marine environment. However the sampling location has salinity varying from 3 to 31/1000 and annual mean around 25/1000. The area is surrounded by mangroves, characterized by a muddy-sand substrate, where *C. quirimure* attaches by its rhizoidal projections reaching densities of approximately 12 individuals per square meter. *Craniella quirimure* has a nearly perfect radial arrangement, varying externally from barrel-shaped to almost spherical and reaching a maximum of 23 mm in diameter and 25 mm in height. Although it is well known that many copepods, chiefly siphonostomatoids, are found in association with sponges, the morphological display of *C. quirimure* apparently does not provide great harbor to such tiny creatures. However an amazing rich siphonostomatoid fauna was found in association, probably living on the surface of the host instead of inhabiting its channels. *Scottocheres* is a very slender siphonostomatoid characterized by a long siphon, usually half the length of the body, mandibular palp absent and a leaf-like free segment of leg 5 armed with 2 or 3 distal setae. So far eight species of the genus are known and three of them have been registered in Brazil. *Scottocheres elongatus* (Scott & Scott) and *S. laubieri* Stock, two sibling species previously known from the Mediterranean have been recorded in Picinguaba (São Paulo state) associated with an unidentified sponge and *S. youngi* was described to California Reefs (Abrolhos Region, Bahia state) in association with *Monanchora*. The new species shows many reductions on the leg setation and therefore differs completely from any other known species.

Seasonal and geographic variability of organic content in *Halichondria panicea* (Demospongiae) and its effects on *Archidoris montereyensis* (Archidorididae)

Ann L. Knowlton{ XE "Knowlton, Ann L." }^(1*); Jason E. Hall{ XE "Hall, Jason E." }⁽²⁾

(1) Notlwonk Science, PO Box 80036 Cornish, UT 84308, USA, gypsea2003@hotmail.com; (2) Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112, USA, Jason.Hall@noaa.gov

Organic content of *Halichondria panicea* was investigated at sites in the Puget Sound and outer coast regions of Washington state in the northeastern Pacific Ocean. Small seasonal variations in total organic fraction were observed, but with little variation between geographic locations. Lab-based feeding experiments indicate that color variability in *H. panicea* impacts the growth of the nudibranch *Archidoris montereyensis*, a primary predator of this sponge, with higher growth rates being observed under a yellow sponge diet as compared to a green sponge diet. In addition, sponge color variability may influence the reproductive output of *A. montereyensis* although the results of these analyses are inconclusive. The results of these experiments do not indicate that the organic content of the sponge diet is the driving factor for the observed differences in nudibranch growth and reproduction given that no significant differences in organic content ($P > 0.05$) were observed in the sponge diet.

Intertidal diversity of sponges and ecological conditions in and around Mumbai coast (west coast of India)

Balasaheb Govind Kulkarni{ XE "Kulkarni, Balasaheb Govind" }^(1*); Neetash D'cruz{ XE "D'cruz, Neetash" }⁽²⁾; Ashok Jaiswar{ XE "Jaiswar, Ashok" }⁽³⁾

(1) Department of Zoology, The Institute of Science, 15 Madam Cama Road, 400 032, Mumbai, India, balasahebkb@yahoo.com; (3) Department of Biology, Central Institute of Fisheries Research Education, Andheri, Mumbai, India

Mumbai, one of the most urbanized and industrialized cities in India, is located on the West coast, which is encircled by the Arabian Sea. The Coast of Mumbai receives plenty of untreated and partially treated domestic sewage and industrial effluent. Therefore, most of the intertidal area in and around Mumbai is under deterioration. In spite of such detrimental conditions, at some places of Mumbai coast still, diversity of macrobenthos is recorded recently. Among the macrobenthos plentiful diversity of sponges is recorded at Nariman Point coastal area and TIFR coast. Three species of sponges were recorded at Nariman Point coastal area whereas at TIFR shore only two species were recorded. Density and biomass of the sponges were also higher at Nariman Point coast. Analysis of water quality surrounding the sponges revealed higher concentration of phosphate and nitrate above their background level. To analyze stress response to polluted water, respiratory rates of the sponge were measured. Furthermore, work on extraction of bioactive compounds from the sponge species is in progress.

Sessile epifauna of Ceara's Shelf - high dominance of sponges

Tito M.C. Lotufo{ XE "Lotufo, Tito M.C." }^(1*); Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽²⁾;
Cristina P. Santos{ XE "Santos, Cristina P." }⁽²⁾; Célio A. Ribeiro{ XE "Ribeiro, Célio A." }⁽¹⁾;
Israel R. Joca{ XE "Joca, Israel R." }⁽¹⁾; Flávio F. da Silva{ XE "Silva, Flávio F. da" }⁽¹⁾; Márcia
F. Rocha{ XE "Rocha, Márcia F." }⁽³⁾; Guarani H. Cavalcanti{ XE "Cavalcanti, Guarani H." }⁽³⁾

(1) Departamento de Engenharia de Pesca, Universidade Federal do Ceará, CP 12168, CEP 60455-760, Fortaleza, CE, tmlotufo@ufc.br; (2) Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, Rio de Janeiro, RJ, hajdu@acd.uff.br; (3) Centro de Pesquisas e Desenvolvimento Leopoldo A. Miguez de Mello (CENPES), Petróleo Brasileiro S.A. (PETROBRAS), Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ, guaranihc@petrobras.com.br

Brazil has the largest typically tropical coast in the world, with a varied set of ecosystems, including coral reefs, mangroves and beaches. Due to its extension and lack of people and financial support, most of the continental shelf is still ignored in terms of its biodiversity. The Ceara's Shelf, located in the northeast region, is moderately narrow and shallow, with a dominance of soft bottoms. In order to describe the benthic epifauna from Ceará's Shelf, a collection of material from 12 regularly spaced stations was performed with an otter trawl net. The material was sorted in higher taxa aboard the ship and immediately fixed in alcohol 70% or formaldehyde in seawater at 4%. In situations when a large biomass of the same taxon was obtained, the total amount was weighted and a sub-sample was collected for further determination. The animals were divided into sessile or motile in order to analyze the data. Sessile epifauna was obtained from 10 out of the 12 stations, comprising 51 species and 204 kg of total wet weight. The results showed a high degree of heterogeneity in composition and abundance along the sampled stations. The dominant taxon was Porifera, with 90% of the total wet weight (184Kg), with special account for *Agelas dispar*, *Ircinia strobilina*, *Leucascus* sp. and *Monanchora* group *arbuscula*. Ascidians were the second most abundant group, with 5% of the total wet weight represented by the species *Stomozoa gigantea*. The fauna of stations in depths below 30m was more abundant and diverse. Despite the fact that trawl nets are inadequate for sampling sessile organisms, these results improve considerably the knowledge on the benthic epifauna of Ceara's Shelf.

This work is part of the "Ceará Basin Environmental Assessment Project", coordinated by CENPES/PETROBRAS.

Biomarkers in marine sponges - metallothioneins and acetylcholinesterase in the sponge *Cliona celata*

Daniela Marques{ XE "Marques, Daniela" }⁽¹⁾; Marise Almeida{ XE "Almeida, Marise" }⁽¹⁾;
Joana Xavier{ XE "Xavier, Joana" }⁽²⁾; Madalena Humanes{ XE "Humanes, Madalena" }^(1*)

(1) Centro de Química e Bioquímica - Departamento de Química e Bioquímica da Faculdade de Ciências da Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal, mmhumanes@fc.ul.pt; (2)

Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Mauritskade 57, 1092 AD Amsterdam, The Netherlands and Departamento de Biologia, Universidade dos Açores, Rua Mãe de Deus, Apartado 1422, 9501-855 Ponta Delgada, Portugal

The monitoring of environmental quality of the marine milieu is a fundamental task to guarantee a sustainable use of the sea. Changes in different pollutant levels in the sea water should be assessed before they cause irreparable damage to the communities. To accomplish these tasks is fundamental: (i) an assessment of pollution risks; (ii) the estimation of the health of animals in their marine environment. Sponges, ubiquitously occurring organisms, seem to be ideal to establish biomarkers for the estimation of different types of pollution in the sea. To contribute to this global aim, we studied the variation of well known biomarkers in the cosmopolitan sponge *Cliona celata*: - metallothioneins for heavy metal response; - acetylcholinesterase for organic aromatics response; along with general oxidative stress response biomarkers such as superoxide dismutase and the glutathione system. It is very difficult to keep sponges in an aquarium and, even if we could keep them alive, the representativity of results is not granted. So, we choose some of the collection sites in the least polluted areas we could find - Berlengas Natural Reserve - and Arrábida Natural Park where some anthropogenic influences can occur in some of the sites and Ria de Ferrol, a kind of bay with the normal activities, where sponges were collected near the open ocean. With this study we just want to: check if these biomarkers are present in the sponge; determine the mean value and the variation interval; check for eventual differences in the collection sites. Standard analytical methods for these types of determinations were used - Ellman's method for the determination of metallothioneins and the production of 5-thio-2-nitrobenzoic acid for the determination of acetylcholinesterase. Twenty one samples collected in nine different sites of three locations were evaluated for the presence of these two biomarkers. The presence of the metallothionein was detected and measured in all the samples. The mean value for this set was 17.00 µg/mL and the variation interval was determined as 3.77 - 26.4 µg/mL. No remarkable differences were found between collection sites. Concerning the acetylcholinesterase activity two samples, one from Arrábida and another from Berlengas did not present any activity of this type. The mean value for the remaining set was 2.87×10^{-8} U/g/mg protein and the variation interval was $0-1.10 \times 10^{-7}$ U/g/mg protein. The differences between collection sites are not relevant, but the samples preserved for longer time have smaller values. When we choose the collection sites we tried to find the least polluted environments; the results we got seem to indicate that there is not a significant difference between them if the sponge *C. celata* is responding to these kinds of stressors. It is not possible to exclude that the organism either is not responding to possible variations or the disturbance is too small to be measured.

Coral mortality and bioerosion by encrusting excavating sponges: Do fish play a role?

Juan C. Márquez{ XE "Márquez, Juan C." }; Sven Zea{ XE "Zea, Sven" }^(*)

Instituto de Investigaciones Marinas y Costeras - INVEMAR, Cerro Punta Betín, A.A. 1016, Santa Marta, Colombia, jcmárquez@invemar.org.co; szea@invemar.org.co

Excavating and encrusting sponges undermine live coral tissue, displacing it at rates of centimeters per year, eroding the skeletal surface. Corallivore and herbivore fishes feed on the coral-sponge boundary. To establish if these fishes mediate in the advance into corals by the Caribbean sponges *Cliona tenuis* (Islas del Rosario) and *Cliona aprica* (San Andrés Island), we experimentally prevented, with plastic mesh, their access to the boundary. After six months, lateral advance of the sponges was measured, comparing it between enclosed and open portions. It was found that fishes contribute to the erosion of the coral surface; when their access was prevented, the sponge advanced at a level shallower than normal. In *C. tenuis*, the mediating effect of corallivorous fish varied with coral species. Its advance was equally faster in enclosed and open portions of *D. strigosa*, and significantly slower in the enclosed portions of *Siderastrea siderea*. Because of the low relief and apparent hardness of *S. siderea* superficial skeleton, parrotfish bite denude the margin confronting the sponge, thus facilitating its advance. The sinuous high-relief and apparently less hard superficial skeleton of *D. strigosa*, facilitates advance whether fish do or do not bite at the boundary. The role of herbivorous fish in mediating sponge advance seems to be small, because algae are transient at the boundary. Experiments with *C. aprica* were not conclusive because of its low rates of advance.

Patterns of recruitment and the influence of disturbance on intertidal sponges

Justin I. McDonald { XE "McDonald, Justin I." }^(1*); Keith A. McGuinness { XE "McGuinness, Keith A." }⁽²⁾

(1) School of Plant Biology (M090), Faculty of Natural & Agricultural Sciences, University of Western Australia, 35 Stirling Highway Crawley, 6009 Western Australia, justinmc@cyllene.uwa.edu.au; (2) School of Science, Charles Darwin University, Darwin, NT 0909, Australia

Recruitment in two species of intertidal sponge was investigated and the periodicity and spatial patterns of recruits were examined. We tested the influence disturbance plays upon sponge recruits through scouring and clearing the reef surface. Recruitment of *Cinachyrella australiensis* onto intertidal reefs was not random, with clumped distribution patterns recorded at 15 cm scales. There was also significantly more clumping of *C. australiensis* recruits within sheltered microhabitats (East Point mean $207 \pm 25 \text{ m}^2$; Channel Island mean $10 \pm 6 \text{ m}^2$), such as ridges and depressions than in areas of open reef flat. There were greater survival rates of *C. australiensis* in sheltered microhabitats (controls 83; 'all cleared' 46; 'sponge removed' 57%), compared to those in exposed regions (controls 50; 'all cleared' 26; 'sponge removed' 36%), indicating that sheltering may enhance persistence of this species in the inter-tidal environment. Recruitment patterns in the *Spongia* species revealed that recruits were randomly distributed and that despite the large numbers of larvae produced within the sponge (mean $432 \pm 39 \text{ SD}$ based on # eggs/larvae from 5 sponges 10 brood chambers per sponge) there was limited recruitment observed on the reef (East Point mean $48 \pm 14 \text{ m}^2$; Channel Island mean $10 \pm 6 \text{ m}^2$). The low number of recruits recorded prevented definitive conclusions about microhabitat occupation being made. Disturbance, via removal of algae and/or sponge clearance, had varying influence upon recruitment in the two species. Clearance had a strong positive influence on *Cinachyrella australiensis* with significantly higher recruitment in cleared versus non-cleared areas (controls $10.6 \pm 2.7 \text{ SD}$; 'all cleared' $19 \pm 10.5 \text{ SD}$; 'sponge removed' $24.6 \pm 6.1 \text{ m}^2$). The influence of disturbance on *Spongia* sp. recruitment was not as clear again due to the low numbers of recruits (controls $1 \pm 0.7 \text{ SD}$; 'all cleared' $0.66 \pm 0.57 \text{ SD}$; 'sponge removed' $2.33 \pm 4.0 \text{ m}^2$).

Overgrowth of gorgonians by various sponge species

Elizabeth Layli McLean{ XE "McLean, Elizabeth Layli" }⁽¹⁾; Paul Yoshioka{ XE "Yoshioka, Paul" }⁽²⁾

(1) University of Puerto Rico - Mayaguez Department of Marine Sciences Magueyes Island Lajas, 00667 Telephone:(787) 899 2048 ext. 228; Puerto Rico, elmclean@sbcglobal.net; (2) Paul M. Yoshioka University of Puerto Rico - Mayaguez Department of Marine Sciences Magueyes Island, La Parguera Lajas, 00667. Telephone:(787) 899 2048 ext. 228; Puerto Rico, p_yoshioka@cima.uprm.edu

The biodiversity of the sessile marine invertebrates of coral reefs may be highly dependent upon competitive interactions for space. Overgrowth by sponges occurs by different mechanism which may involve differential growth rates, aggressive behavior and the production of allelochemicals. An initial survey was done in La Parguera, South West coast of Puerto Rico to estimate the diversity of sponges that overgrow gorgonians and the interactions with the gorgonian surface.

Sponge distribution in São Pedro & São Paulo Archipelago, Brazil

Leandro C. Monteiro{ XE "Monteiro, Leandro C." }^(*); Fernando Moraes{ XE "Moraes, Fernando" }; Máira V. de Oliveira{ XE "Oliveira, Máira V. de" }; Guilherme Muricy{ XE "Muricy, Guilherme" }

Departamento de Invertebrados, Museu Nacional/UF RJ. Quinta da Boa Vista, s/n, São Cristóvão, 20940-040, Rio de Janeiro, RJ, Brazil, lcmonteiro@mn.uffj.br

The São Pedro & São Paulo Archipelago (former St. Paul's Rocks) is one of the smallest and most isolated in the world, with 400 m of extension, and located 1010 km NE from the Northeast coast of Brazil (0°55' N - 29°21' W). A few studies on sponge taxonomy had been done in the archipelago, but none described the structure of the sponge community in this site. Here we describe the abundance and distribution of sponges in four depths from a horizontal cove to a vertical wall of São Pedro & São Paulo. Samples were taken by SCUBA diving in August 2005 in 12 quadrats of 0.25 m² in each depth: 5, 10, 20, and 30 m. Cluster analysis using Bray-Curtis coefficient allowed distinction of two groups: shallow-water samples (5 and 10 m depth) and deep-water samples (20 and 30 m depth). Sponge abundance was greater in 5 and 20 m depth (73 ind.m⁻² and 69 ind.m⁻² respectively) which were dominated by *Clathria (Microciona) calla* (15%) and *Scopalina ruetzleri* (29%) respectively. Sponge abundance was reduced in 30 m (61 ind.m⁻²), dominated by *Scopalina ruetzleri* (30%), and 10 m (49 ind.m⁻²) dominated by *Chondrosia collectrix* (20%). Shannon's diversity index was high showing only small differences between habitats: 5 m (3.8 bits.ind⁻¹), 20 m (3.5 bits.ind⁻¹), 10 m (3.3 bits.ind⁻¹) and 30 m (3.2 bits.ind⁻¹). These results are opposed to the usual trend of increase of diversity with depth. The presence of large groups of the algae *Caulerpa* sp. in shallow waters could explain the high abundance and diversity of sponges in this habitat. Sponges were common on the bottom among the roots of *Caulerpa* sp., and these algae probably protect the sponges from light and predation. *Caulerpa* sp. is very common between 3 and 15 m depth, rare in 20 m and absent in 30 m. In 10 m depth, the abundance of sponges can be reduced due to competition with large colonies of the cnidarian *Palythoa caribaeorum*, common in this depth.

Sponge allelopathy detection using mathematical models

Gregory Nishiyama{ XE "Nishiyama, Gregory" }

Department of Biology, College of the Canyons, 26455 Rockwell Canyon Road 91387, Santa Clarita,
California, USA, nishiyamag@sbcglobal.net

The predictive values of mathematical models to detect sponge allelopathy to various substratum competitors were determined. Both contact and near-contact models, Markov chain analysis, and autocorrelation models were used in the analysis. Data from sponge-substratum competitor interactions in both marine tropical (Philippines) and temperate (California, USA) benthic habitats as well as results from settlement studies were used in the assessment. Bioassays established the toxicity of the sponge allelochemicals to substratum competitors. The models demonstrated that the spatial arrangements of sponges to substratum competitors correlated with sponge allelochemical toxicity. Although the predictive values in identifying sponge allelopathy varied between the different models and substratum competitors, the study demonstrated that a combination of mathematical models can be used in the preliminary detection of sponge allelopathy.

Distribution and abundance of coral reefs sponges at Cienaga de Ocumare de la Costa Bay, Venezuela

Sheila M. Pauls{ XE "Pauls, Sheila M." }

Instituto de Zoología Tropical, Universidad Central de Venezuela, A.Postal 47058, Caracas 1041-A, Venezuela

Sponges are an important component of the Caribbean coral reefs. Despite of this importance, the study of the community structure of sponges are poorly known in Venezuela. The quantitative distribution of reef sponges was studied at Cienaga de Ocumare de la Costa Bay, located in the central coast of Venezuela, at 67°48'30"W - 10°28'15' - 10°29'20"N. Surveys were made at 17 sites throughout the bay along gradients from the surface to 20 m depth. Sponges diversity and percent cover were estimated at 5 m intervals along 100 m transects perpendicular to the coast using 1 m² quadrats. A total of 23 species of sponges (Porifera) were recorded. In the depth gradient, 10 species occurred in shallow waters and the highest diversity was recorded between 6 and 15 m. The wave-action and turbulence appear to limit the occurrence of sponges in the exposed sites and shallow depths. The sponge assemblage observed in the bay is similar from other Caribbean reef sponges communities. *Scopalina ruetzleri*, *Niphates erecta*, *Dysidea etheria* and *Ircinia felix* were the most frequent species and occurred along the entire depth gradient. *Aplysina archeri*, *Iotrochota birotulata*, *Clathria venosa* and *Aiolochoia crassa* were restricted to the deeper zones below 6 m. Sponge cover varied between 0.1 and 3.1% of all reefs coverage and increased in the inner sheltered sites. Those sites are subjected to freshwater inflow and nutrient input. The most abundant sponges were *S. ruetzleri* (18.6%), *C. venosa* (17.5%), *N. erecta* (13.7%), *I. felix* (13.4%) and *I. strobilina* (12.7%), that constituted more than 75% of all sponge cover. The low diversity and cover of sponges inside the bay probably reflect environmental conditions: lower levels of nutrients and high physical turbulence.

Community structure of marine sponges associated to red mangrove (*Rhizophora mangle*) aerial roots from “Laguna de la Restinga” National Park, Venezuela

Adriana Pérez{ XE "Pérez, Adriana" }⁽¹⁾; Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }⁽²⁾;
Luis León{ XE "León, Luis" }^(1*)

(1) Escuela de Ciencias Aplicadas del Mar, Boca del Río, Universidad de Oriente Nueva Esparta, Venezuela, adripeva@yahoo.com; lbleon@ne.udo.edu.ve; (2) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com

A study on the ecological structure of sponge populations living on red mangrove aerial roots, from the National Park “Laguna de La Restinga” was carried out between august 2004 and august 2005. Our objective was to understand the spatial and temporal variation of species richness, diversity and relative abundant of sponge species in this rich and productive marine ecosystem. Three localities were chosen within the Park: “La Entrada”, “Caño Viejo”, and “El Manamo”. Each presents a distinct regime of temperature, salinity, and particulate organic matter content. At each locality, 2 line transects (15 m long each) were laid along the mangrove fringe; both transect lines were separated by 15 m. Within each transect, all species of sponges on each root, on the fringe, were accounted for. Area coverage of sponge species and other major taxa was estimated on 15 roots (separated by 1 m each) along each transect. Approximately 50 sponge species were encountered, with the order Poecilosclerida and Haplosclerida being the most diversely represented. Four species are new reports for Venezuela: *Haliclona smithsae*, *H. magnifica*, *Terpios manglaris*, and *Halisarca* n.sp. The most abundant species were *S. ruetzleri* (1319 individuals), and *Chalinula molitba* (454 individuals). Diversity (H') levels range between 2,719 (Caño Viejo) - 3,211 (La Entrada) bits/ind, with “Manamo” presenting intermediate values (3.02 bits/ind). Spatial and temporal changes of sponge species richness and relative abundance within the year studied are shown. Species richness between stations ranged from 36 species (Caño Viejo) to 50 species (La Entrada). The community at “La Entrada” station presented the highest diversity and species richness probably due to the more favorable physicochemical conditions there.

Biodiversity and distribution of sponge fauna in Madeira Island (NE Atlantic Ocean)

Rosa Pestana { XE "Pestana, Rosa" }⁽¹⁾; Nicole Boury-Esnault { XE "Boury-Esnault, Nicole" }⁽²⁾;
Manuel Biscoito { XE "Biscoito, Manuel" }⁽¹⁾

(1) Estação de Biologia Marinha do Funchal, Cais do Carvão, Promenade da Orla Marítima, Gorgulho, 9000-107 Funchal; (2) Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille

This contribution presents the results of the first quantitative survey of the sponge fauna in the South coast of the island of Madeira, Portugal. In order to study the systematic and distribution patterns of species according to depth, 3 sampling stations (Santa Cruz, Baixa do Carneiro and Ponta da Cruz) were established and 3 transects/station were done on rocky wall substrates near the coast, between 0 and 12 meters depth. Samples were collected by scuba diving from January to July 2001. A total of 14 species were collected: Desmospongiae - *Chondrosia reniformis* Nardo, 1833, *Aplysilla sulphurea* Schulze, 1878, *Ircinia dendroides* (Schmidt, 1862), *Suberites carnosus* (Johnston, 1842), *Aaptos aaptos* (Schmidt, 1864), *Cliona celata* Grant, 1826, *Batzella inops* (Topsent, 1891), *Haliclona* sp., *Phorbas fictitius* (Bowerbank, 1866), *Clathria coralloides* (Olivi, 1792), *Mycale macilenta* (Bowerbank, 1866), *Eurypon lacazei* (Topsent, 1891), *Tedania anhelans* (Lieberkühn, 1859) and *Aplysina aerophoba* Schmidt, 1862. The encrusting sponges were dominant (80%). As a result of this survey, *A. sulphurea*, *C. celata*, *Haliclona* sp., *P. fictitius*, *M. macilenta*, *E. lacazei*, *T. anhelans* and *C. coralloides* are recorded for the first time from the Archipelago of Madeira. The most common species found were *Batzella inops* and *Phorbas fictitius*, having a vast and continuous distribution (1-12 m). These two species were the only ones which were common in 8 of the 9 transects studied and therefore seem not to be habitat-specific. Only in *B. inops*, the covering area increased slightly with depth at Ponta da Cruz. Diversity indexes were low in the 3 sampling stations. The presence/absence of sediments and currents were two factors that seemed to influence the presence of species in certain areas.

An integrated study of excavating sponges from Laucala Bay and Suva Barrier Reef, Suva, Fiji Islands

Susanne M.L. Pohler{ XE "Pohler, Susanne M.L." }^(1*); Klaus Feussner{ XE "Feussner, Klaus" }⁽²⁾; Jioji Tabudravu{ XE "Tabudravu, Jioji" }⁽²⁾

(1) Marine Studies Programme, University of the South Pacific, Suva, Fiji, pohler_s@usp.ac.fj; (2) Institute of Applied Science, University of the South Pacific, Suva, Fiji, feussner_k@usp.ac.fj; tabudravu_j@usp.ac.fj

A study is underway to investigate the excavating sponges along a transect from a nearshore intertidal flat (Nasese) to a Barrier reef complex (Sosoikula Reef) seaward of Suva City, Fiji. The objectives of the study are threefold: (1) Determination of the different genera and species of excavating sponges present in order to assess biodiversity and to contrast the number of cosmopolitan species versus indigenous Southwest Pacific species; (2) Investigation of the role of excavating sponges in carbonate degradation at the various sites; (3) Determination of the heavy metal uptake of large excavating sponges *Spherospongia* spp. in the polluted site (close to Suva Harbour) versus a pristine site (Natadola Reef) to assess the ability of the sponges to protect themselves from heavy metal poisoning. To date excavating sponges belonging to the genera *Aka*, *Cervicornia*, *Cliona*, *Cliothosa*, *Dotona*, *Pione*, and *Spherospongia* were found along the transect in clearly defined environments. Three different species of *Aka* De Laubenfels can be distinguished, two are limited to the reef front (12-15 m depth) with a third occurring on the reef flat. All three species are quite large measuring up to 10 cm in diameter with 5 cm long fistules. One species of *Cervicornia*? Ruetzler & Hooper is dominating the nearshore intertidal zone with large colonies of which 2/3 are buried in sediment. There are at least seven species of *Cliona* Grant distributed along the transect including *C. mucronata* Sollas, *C. vermifera* Hancock and a species close to *C. celata* Grant. *Cliothosa* Topsent is present with two species, one occurs in the intertidal nearshore zone, the other, *C. hancocki* (Topsent) was found only on the reef flat. One species of *Dotona* Carter was found at the reef front and remains to date the only representative of the Alecionidae from the transect. Three (or more?) different species of *Pione* Gray can be distinguished: *P. cf. vastifica* (Hancock) is very abundant in the intertidal nearshore zone infesting 85% of coral rubble and dead shells. *Pione* sp. 2 is common on the reef flat and characterized by spiny styles in addition to spiny and smooth microxemas. A single specimen of *Pione*? sp. 3 was found in a sample from the reef front with smooth microxemas and spiny microrhabds reminiscent of *Archaeocliona* Czerniavsky. Several purple boring sponges from the reef front possess smooth microxemas, spiny microrhabds and spirasters. Their generic assignment to *Pione* is tentative due to scarcity and smoothness of microxemas and unusual morphology of microrhabds. *Spherospongia vagabunda* (Ridley) is a common sponge on the lagoonal side of the barrier reef. Several of the excavating sponges found so far are cosmopolitan species, however, further study may reveal that some of the as yet undetermined species are endemic to the South Pacific or Fiji.

Chemical defense of marine sponge *Hymeniacidon heliophila* against predation

Suzi M. Ribeiro{ XE "Ribeiro, Suzi M." }^(1*); Éverson M. Bianco{ XE "Bianco, Éverson M." }⁽²⁾;
Renato C. Pereira{ XE "Pereira, Renato C." }⁽¹⁾

(1) Pós-graduação em Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, P.O. box 100.644, Zip Code 24001-970. Niterói, RJ - Brazil, suzi@acd.ufrj.br; (2) Pós-graduação em Química Orgânica, Instituto de Química, Universidade Federal Fluminense, Zip Code 24020-150. Niterói, RJ - Brazil

Sponges are primitive metazoans that have been living in world's oceans for more than a billion years. Because they are sessile and soft-bodied organisms, they are easily targeted by some predators. To defend themselves they produce a great array of chemical compounds. The genus *Hymeniacidon* is reported to produce peptides, alkaloids and steroids, but the ecological role of these compounds remains unclear. The goal of this work was therefore to investigate defense properties of crude extract of the sponge *H. heliophila* against the hermit crab *Calcinus tibicen*. Chemical extraction was performed with three organic solvents: extract 1 was obtained with hexane, extract 2 was obtained using ethyl acetate, and extract 3 was obtained with a mixture of acetone-methanol 1:1. Aquarium assays were performed by utilizing natural concentrations of crude extracts, which were added to artificial foods (control: without extract, and treated: with extract) that were offered simultaneously to every hermit crab placed in individual containers. Artificial foods with extracts EB1 and EB2 were consumed significantly less than their respective controls. It is indicative of chemical defense against the consumption by *C. tibicen*. The extract EB3 (hydrophilic) was strongly deterrent. The literature corroborates this functionality also against fishes. Detailed chemical studies will be made to isolate and identify substances responsible for antipredation activity.

Antipredation defenses of sponge *Tethya* sp. against hermit crab

Suzi M. Ribeiro{ XE "Ribeiro, Suzi M." }^(*); Renato C. Pereira{ XE "Pereira, Renato C." };
Valéria L. Teixeira{ XE "Teixeira, Valéria L." }

Pós-graduação em Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, P.O. box
100.644, Zip Code 24001-970, Niterói, RJ - Brazil, suzi@acd.ufrj.br

Chemical mediation is widespread in the marine environment. Several organisms, such as algae, corals, and sponges, use secondary metabolites, which play distinct ecologic roles (e.g. allelopathy, antipredation defenses, antifouling). Chemicals from several sponges of the genus *Tethya* have important pharmacologic activities, such as cytolytic, antiviral and antifungal. However, the ecological roles of its substances remain unknown. The goal of this study was to investigate chemical and physics defense properties of *Tethya* sp. against the hermit crab *Calcinus tibicen*. Chemical extraction was performed with acetone. Spicules were isolated from sponge with nitric acid or sodium hipoclorite. Aquarium assays were done utilizing natural concentrations of crude extracts, and spicules were added to artificial foods. Natural concentrations of extract and spicule were simultaneous, but separately offered to *C. tibicen* and also offered together to this crab. The artificial foods (control and treated) were offered simultaneously to every hermit crab placed in individual containers. In the assays, both crude extract and spicules of *Tethya* sp. were significantly less consumed than their respective controls ($p < 0.001$, $n=20$ and $p < 0.00001$, $n=21$, respectively). The combination of *Tethya* spicules and crude chemical extract deterred predation ($p < 0.00001$, $n=31$) to a greater extent than that observed for both these potential defense mechanism separately, probably indicating probably an additive mechanism of defense used by this sponge species. Preliminary chemical investigation found sterols as major compounds in this sponge species. Chemical and structural deterrents may be a common to co-occur as defensive strategy against multiple predators. Future studies are necessary to evaluate this possibility.

Effect of anthropogenic disturbance on sponge community structure and disease incidence

Carmen Schloeder{ XE "Schloeder, Carmen" }⁽¹⁾; Robert W. Thacker{ XE "Thacker, Robert W." }⁽²⁾; Deborah Gochfeld{ XE "Gochfeld, Deborah" }^(3*)

(1) Smithsonian Tropical Research Institute, Unit 0948, APO AA, 34002-9948, USA, schloederc@si.edu;
(2) Department of Biology, University of Alabama at Birmingham, 109 Campbell Hall, 1300 University Blvd., Birmingham, AL 35294-1170, USA, thacker@uab.edu; (3) National Center for Natural Products Research, University of Mississippi, P.O. Box 1848, University, MS 38677-1848, USA, gochfeld@olemiss.edu

Sponges are sessile, filter-feeding organisms that are sensitive to both biotic and abiotic components of their environment and are therefore likely to be impacted by environmental stressors. For this reason, sponges are often useful as bioindicators of changing environmental conditions. Earlier studies suggested that domestic waste and sedimentation can alter sponge community structure. The present study tested the hypothesis that sponge diversity and abundance decrease and disease incidence increases with increasing anthropogenic disturbance. Sponge diversity and abundance were characterized from 15 quadrats on each of three reefs in Bocas del Toro, Panama. Disease incidence was assessed from three band transects on each reef. The reefs were similar in general characteristics, however, one site was located just offshore of a village where "black water" outflow (sewage, road pollution, and solid waste dumping) is known to occur. The other two sites were upstream from the impacted site. Overall, 51 species and 2532 individual sponges were identified from the surveyed reefs. Cluster analysis indicates significant differences in sponge community structure between the three sites. The impacted site had significantly fewer species per quadrat, although total number of individuals and number of individuals per quadrat were similar between the impacted site and the more distant upstream site. Evenness (J) and diversity (H') were significantly reduced at the impacted site, as was the slope of the species-area (rarefaction) curve. Dominant species also differed among sites, with three of the five most abundant species at the impact site considered rare at the upstream sites. Only *Niphates erecta* was among the five most dominant species at all three sites, whereas *Aplysina fulva* and *Chondrilla nucula*, which dominated the upstream sites and are known to be sensitive to stress, were absent at the impacted site. *Aplysina* red band syndrome (ARBS) was present at all three sites, but incidence was higher and more variable at the impacted site. These data suggest that sponge community structure, including diversity, evenness, and species composition, can provide an index of anthropogenic impacts on coral reef ecosystems.

**Population dynamics of sponges at Stetson Bank, Northwestern Gulf of Mexico,
derived from annual photographic monitoring**

George P. Schmahl{ XE "Schmahl, George P." }^(*); Emma L. Hickerson{ XE "Hickerson, Emma L." }; Emily Platzer{ XE "Platzer, Emily" }

NOAA, Flower Garden Banks National Marine Sanctuary, 1200 Briarcrest Dr., Suite 4000, Bryan, Texas 77802, USA, george.schmahl@noaa.gov; emma.hickerson@noaa.gov; emilyplatzer@yahoo.com

Aspects of the population dynamics of three species of sponges were investigated at Stetson Bank, a topographic feature located in the northwestern Gulf of Mexico. Annual repetitive photographs of 44 permanently marked stations, taken between 1993 and 2005, were analyzed to determine growth and mortality of *Ircinia strobilina*, *Chondrilla nucula* and *Agelas clathrodes*. Sponges are a dominant component of the benthic community at Stetson Bank, with over 25 species comprising an average of 33% of the substrate coverage of the exposed reef ridges and pinnacles, within the depth range of 17 to 30 meters. The primary non-sponge component of the benthic community is the encrusting hydrozoan *Millepora alcicornis* (fire coral), which accounts for up to 31% of the substrate cover. Photographic monitoring stations were established in 1993 as part of a long term monitoring program to evaluate the health and condition of biological resources associated with this feature. Each monitoring photograph covers approximately two square meters of reef area. Analysis of this data indicates that the marine communities are healthy and have remained relatively stable through recent years. One of the most popular dive sites in the region, Stetson Bank was added to the Flower Garden Banks National Marine Sanctuary in 1996. The sponge species selected for study are three of the most common at Stetson Bank, yet exhibit quite different life history strategies. Individual specimens of the targeted species were identified and followed throughout the course of the 12-year monitoring record. Mortality was determined through the persistence or disappearance of individuals over time. Growth was determined by two-dimensional measurement of projected area, including fusion and fragmentation of individual colonies. Mortality was low for all species, with overall persistence of individuals over the study period. *Ircinia strobilina* accounts for about 8% of the total substrate cover. Growth was moderate for this species, especially in the smaller specimens. Some mortality was noted, with some large specimens showing indication of senescence and tissue decay. *Chondrilla nucula* is the most abundant sponge species, accounting for up to 17% of substrate cover. Growth patterns in this species are dynamic with numerous incidents of fusion and fragmentation noted throughout the data record, and mortality was moderate. *Agelas clathrodes*, which accounts for about 2% of the substrate cover, is the most stable of the three species investigated. Growth and mortality were very low for this species.

Ecology and bioactivity of deep-reef sponge communities in the Bahamas

Marc Slattery{ XE "Slattery, Marc" }^(1*); Deborah Gochfeld{ XE "Gochfeld, Deborah" }⁽²⁾;
Michael P. Lesser{ XE "Lesser, Michael P." }⁽³⁾

(1) University of Mississippi, Department of Pharmacognosy and the National Institute for Undersea Science and Technology, P.O. Box 1848, Oxford, MS 38677-1848, USA, slattery@olemiss.edu; (2) National Center for Natural Products Research, University of Mississippi, P.O. Box 1848, Oxford, MS 38677-1848, USA, gochfeld@olemiss.edu; (3) Department of Zoology and Center for Marine Biology, University of New Hampshire, Durham NH 03824, USA, mpl@cisunix.unh.edu

The transition between shallow coral reef communities and aphotic deep-water communities in the tropics (= 50m to 150m) is often called the "twilight zone", and it is characterized by reduced light and consequently photosynthesis. But while reef-building coral diversity decreases with increasing depth, many sponges, soft corals, and even low-light acclimatized primary producers (*e.g.*, corals) are abundant. Thus, this is a zone of potentially incredible ecological significance, unusual physiological adaptations, and unique biodiversity. This is also one of the least studied ecosystems in the marine environment since it exceeds the limits of traditional SCUBA, but is considered too shallow to warrant costly submersible time. We have examined the Twilight Zone off the Exuma Sound Shelf, Bahamas, over the last three years using technical mixed gas diving. Biodiversity data indicate a rich sponge community consisting of 92 species and approximately 15% endemism. Sponge diversity and density increased with depth as picoplankton food abundance increased. Sponges were also larger and faster growing at depth than in shallow-water conspecific populations. This unique biodiversity represents an important biotechnological resource as well; two thirds of the sponges exhibit promising biomedical activity compared to less than one third of the shallow reef sponge species.

Association between sponges and macroalgae on rhodoliths in the Abrolhos marine national park, Bahia, Brazil

Fabiana A. de Souza{ XE "Souza, Fabiana A. de" }^(1, 2); Marcia A. de O. Figueiredo{ XE "Figueiredo, Marcia A. de O." }⁽¹⁾; Guilherme Muricy{ XE "Muricy, Guilherme" }⁽²⁾

(1) Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão 915, Rio de Janeiro, RJ, 22460-03, Brasil; (2) Museu Nacional / UFRJ, Quinta da Boa Vista s/n, Rio de Janeiro, RJ, 20940-040, Brasil

The Abrolhos Archipelago is part of the only two Marine National Parks of Brazil and it is important for its coral reef formations and calcareous algae banks built by rhodoliths. When these free living algae dominate the environment they become a substratum and shelter for diverse organisms, such as sponges and other macroalgae. Sponges are sessile and filter-feeder animals, with a few burrowing species. Previous studies have identified sponges as epibionts or perforating the calcareous algae. The studied area is a rhodolith-seagrass bank located around 5m depth between the Siriba and Redonda Islands. The aim of this project is to study the composition and abundance of the epibiontic algae and sponges on the calcareous algae that build the rhodoliths in Abrolhos. Samples of rhodoliths with sponges were collected through SCUBA diving following 30 random points on a 30m-long transect. Sponges were photographed in the field. The rhodoliths collected were fixed and taken to the laboratory to be separated in morphological groups with the aid of a magnifying glass. For sponge identification, histological sections and spicule preparations were made through classical methods. The epiphytic algae were separated in morphological groups and identified using a stereomicroscope and optical microscope. Most of the calcareous algae of rhodoliths were species of *Mesophyllum erubescens* and *Mesophyllum* sp. The rhodoliths presented volumes between 2 and 252 ml and mass ranging from 1.6 to 171 g. There was a positive significant relationship between mass and volume of the rhodoliths ($R^2 = 0.5441$; $n = 27$; $p < 0.005$). Among epiphytic algae 45 taxons were identified, being the most abundant *Dictyota cervicornis*, *Dictyopteris delicatula*, *D. plagiogramma* and *Halitilon cubense*. Eight new records for the Abrolhos Bank were found: *Boodleopsis pusilla*, *Caulerpa pusilla*, *Gelidiopsis planicaulis*, *Gracilaria cf. paucirramosa*, *Halitilon cubense*, *Polysiphonia sphaerocarpa*, *Sargassum ramifolium* and *Spyridia hypnoides*. The macroalgae species richness varied between 1 and 14 per rhodolith. Twenty-one taxons of sponges were identified. *Tedania ignis*, *Clathria (Microciona)* sp. and *Cliona varians* were the most abundant epiphytic sponges on rhodoliths. Most sponges were found only upon living calcareous algae, but *Cliona varians* was also found within rhodoliths thus confirming its perforating ability. The species richness of sponges varied between 1 and 5 per rhodolith. In contrast to macroalgae, sponges did not show preferences for a given rhodolith size. There was no significant relationships between the abundance of epiphytic sponges and macroalgae ($R^2 = -0.15317$, $n = 18$, $p > 0.05$), which indicates a co-existence of these two organisms in the studied area

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Long term evaluation of sponge population recovery following a widespread mortality: Will we ever know when recovery has occurred? Is restoration necessary?

John Stevely{ XE "Stevely, John" }^(1*); Donald Sweat{ XE "Sweat, Donald" }⁽²⁾; Robert Wasno{ XE "Wasno, Robert" }⁽³⁾; Charles Adams{ XE "Adams, Charles" }⁽⁴⁾

(1) Florida Sea Grant Extension Program, 1303 17th St. W., Palmetto, FL 34221, USA, jmstevely@ifas.ufl.edu; (2) Florida Sea Grant Extension Program, 830 First St. C., St. Petersburg, FL 33710 USA, dsweat@seas.marine.usf.edu; (3) Florida Sea Grant Extension Program, 3406 Palm Beach Blvd., Ft. Myers, FL 33916, USA, wasnorm@leegov.com; (4) Florida Sea Grant Extension Program, 1193 McCarty Hall, Gainesville, FL 32611-0240. USA, cmadams@ifas.ufl.edu

One of the most dramatic manifestations of the perceived deterioration of the Florida Bay ecosystem has been widespread sponge mortalities caused by cyanobacteria blooms. During 1991 and 1992 widespread sponge mortalities significantly impacted sponge populations in the Florida Keys, USA. The extent of the impacted areas was estimated to be approximately 1,000 square kilometers. The work described here (initiated prior to the mortalities) documented a highly significant reduction (>90%) in sponge community volumetric biomass. Sponge numerical abundance data has been collected annually since 1991, allowing for the development of a unique, truly long-term (1991-2005) picture of sponge population dynamics following the mortalities. As the project has evolved into a long-term picture of sponge population recovery, it is now possible to sort out natural sponge population abundance variability. The data identified several sponge species that are short-lived and that widely fluctuate in abundance (*Adocia* sp., *Cinachyra* sp., *Halichondria melanadocia*, *Haliclona molitba*, *Hyrtilos* sp., *Niphates erecta* and *Tedania ignis*). It may be impossible to conclude that these species have recovered because their abundance is probably constantly changing. On the other hand, the data indicate that there are several long-lived sponge species that show gradual consistent recruitment. These species dominate sponge community biomass in the study area. Two species, the loggerhead sponge (*Spherospongia vesparia*) and vase sponge (*Ircinia campana*) represented 59% of the sponge community biomass prior to the mortalities. It is apparent that only a few species are important from a resource management perspective because they constitute the majority of sponge habitat. If these long-lived species successfully recruit, then sponge population can be considered complete as the abundance of the other short-lived species will continue to fluctuate. After eleven years there has been significant recovery of sponge populations. Certain key species (in terms of biomass) have not recovered fully, but their reestablishment appears to have begun. In this case, recovery of sponge community biomass, unaided by human intervention, appears to be a decades long process.

Specificity of zoanthid-sponge symbioses in the Central Western Atlantic region

Timothy D. Swain{ XE "Swain, Timothy D." }

Department of Biological Science Florida State University Tallahassee, Florida, 32306-1100, USA,
swain@bio.fsu.edu

Sponges form symbioses with a vast array of taxa, but many interactions are only known from a few exemplar species and remain poorly understood. Determining the range of species combinations of hosts and symbionts establishes the foundation for further investigation, defines the specificity of the interactions, and provides hints about what the adaptive significance of the interactions might be. Symbioses between coral reef sponges and zoanthids of the genera *Parazoanthus* and *Epizoanthus* are widespread (from Brazil to Bermuda, and Belize to Barbados), common (able to colonize all host individuals in a population), and have been known in a few systematically disparate but numerically abundant sponge host species for more than a century. However, the extent of the diversity of species interactions on a region-wide scale had not been examined. To explore the diversity of symbiotic combinations, I have compiled sponge-zoanthid species combinations as 1) reported in the sponge and zoanthid taxonomic literature; 2) captured in the collections of the United States National Museum of Natural History; and 3) observed in field surveys conducted in Panama, the United States (Florida, Georgia, and Navassa Island), Dominica, Barbados, and Tobago. The results indicate that more than 70 sponge species (representing 7 orders) host at least 7 species of zoanthid across the central western Atlantic region. The patterns of sponge-zoanthid species combinations demonstrate that 1) sponges are highly specific to zoanthid species, 2) zoanthids are not specific to sponge species, however 3) zoanthids are specific to sponge higher taxa.

Christiane Sym{ XE "Sym, Christiane" }^(1*); Guilherme Muricy{ XE "Muricy, Guilherme" }⁽²⁾;
Karina Garcia{ XE "Garcia, Karina" }⁽¹⁾; Emmanoel Silva-Filho{ XE "Silva-Filho, Emmanoel" }⁽¹⁾

Sponge communities or indicator sponge species have frequently been used to evaluate environmental impacts in temperate and in tropical ecosystems. Sponges are sessile filter feeders, widely distributed in various habitats, mainly on rocky bottoms of coastal ecosystems, which are the most susceptible to pollution. Due to their feeding habits, with no selection of the nutrient particles besides the size of the inhalant pores, they can collect and concentrate a wide range of pollutants from both suspended and dissolved phases. Some studies have shown that sponges may be used as bioindicators of metal pollution. *Mycale microsigmatosa* Arndt, 1927 is abundant at both polluted and clean sites, from 0-20 m depth. It is an opportunist species, reported as tolerant to oil and domestic wastes. These are some of fundamental characteristics of the ideal biomonitor. The objectives of this work are: (i) to quantify trace metal (Co, Cr, Fe and Zn) concentrations in *Mycale microsigmatosa* from Rio de Janeiro, Brazil, and (ii) to evaluate the use of this sponge as a bioindicator in monitoring programs of coastal ecosystems. The specimens were collected through free diving between 2 and 3m depth. All symbiontes (bivalves, polychaetes, and others) were removed. The samples were frozen at 4°C soon after sampling and subsequently lyophilized at the laboratory. A total of 0,4 g of each freeze-dried sample was mineralized in PTFE bombs using 20ml of nitric acid 65% in a closed system. The extract obtained was analyzed for metal determination by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). Experimental procedure was evaluated through analyses of certified material of reference (DORM-2, Institute for Environmental Chemistry, Canada). Good to excellent recoveries (90-129%) were obtained for all metals studied. Heavy metals concentrations found in *Mycale microsigmatosa* were Co: 0,03 - 0,06 mg.g⁻¹; Cr: 0,6 - 1,0 mg.g⁻¹; Fe: 152,8 - 304,5 mg.g⁻¹ and Zn: 31,0 - 597,7 mg.g⁻¹. Among all metals studied, Fe and Zn showed higher variability. Although Guanabara Bay has been reported as a contaminated environment by untreated domestic and industrial wastes, the reducing conditions of sediment may cause metal immobilization as sulphides. Therefore, low concentrations of these metals found in the studied organisms certainly reflect the low bioavailability of them in this ecosystem.

Activated chemical defense in *Aplysinella rhax*Carsten Thoms{ XE "Thoms, Carsten" }^(*); Peter J. Schupp{ XE "Schupp, Peter J." }University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96923, USA,
cthoms@guam.uog.edu; pschupp@guam.uog.edu

The bromotyrosine metabolites psammaplin A-sulfate (1) and psammaplin A (2) were previously reported as major compounds of the sponge *Aplysinella rhax*. However, an in depth investigation on the chemical profile of this sponge revealed that intact specimens are almost free of psammaplin A (2), whereas psammaplin A-sulfate (1) dominates the organic crude extract, accounting for about 7% of the dry weight of the sponge. Upon mechanical injury of the sponge tissue, we observed a pronounced decrease of psammaplin A-sulfate. Concomitantly, psammaplin A was formed at high concentrations. The extent of this shift is directly correlated to the intensity of wounding and the reactions take place within less than 30 sec, indicating an enzymatic bioconversion (*i.e.*, the hydrolysis of a sulfuric ester group) of (1) into (2). The observation that artificially added psammaplin A-sulfate (1) is only converted to psammaplin A (2) when ground together with fresh tissue of *A. rhax*, but remains unaffected when ground together with tissue of other sponge species further corroborates the assumption that the reaction is enzyme-catalyzed. Moreover, the conversion also takes place when water is added to ground lyophilized tissue of *A. rhax*, but fails to appear, when hot water is added instead, indicating inhibition of the enzyme by heat. The extract from both, intact and injured *A. rhax* showed pronounced deterrence in fish feeding assays when tested at volumetric concentrations as found in the respective sponge tissue. Direct comparison of the two extracts revealed that the latter (consisting mainly of psammaplin A (2)) has a significantly stronger deterrent effect than the former (consisting mainly of psammaplin A-sulfate (1)), indicating that the injury-induced reaction in *A. rhax* is paralleled by an intensified protection of the sponge against predatory fishes. Based on these findings we propose that the observed formation of psammaplin A in *A. rhax* following tissue wounding is an activated defense mechanism of the sponge against potential predators.

Large skeletal structures do not always accompany high tensile strength in resistant morphologies of the North Atlantic intertidal encrusting demosponge, *Halichondria panicea*

Yvonne R. Vaillancourt{ XE "Vaillancourt, Yvonne R." }

Biology Department, University of Massachusetts at Boston 100 Morrissey Blvd., Boston MA 02125 USA,
yvonne.vaillancourt@umb.edu

Intertidal sponges can respond plastically to changes in the environment, often producing resistant morphologies with high tissue tensile strength in high wave-energy environments. Larger skeletal structures often accompany higher tensile strength. I compare tensile strength and various aspects of the skeleton between sponges from an exposed and protected shore. Sponge morphology differs between sites. Tissue tensile strength was greater on the exposed shore but was not always accompanied by wider skeletal structures, higher total inorganic material or greater tissue density. Mean spicule width changes with season, but tensile strength does not. These results suggest other factors are involved in the composition of the higher tensile strength of resistant morphologies. This challenges the traditional view of what primarily contributes to tensile strength in sponge tissue of this species.

Sponge diversity and composition across a disturbance gradient in the Thousands Islands Reef Complex off Jakarta, Indonesia

Nicole J. de Voogd{ XE "Voogd, Nicole J. de" }^(1*); Daniel F.R. Cleary{ XE "Cleary, Daniel F.R." }⁽²⁾

(1) National Museum of Natural History, 'Naturalis', P.O. Box 9517, 2300 RA Leiden, The Netherlands, voogd@naturalis.nnm.nl; (2) Institute for Biodiversity and Ecosystem Dynamics (Zoological Museum), University of Amsterdam, P.O. Box 94766, 1090 GT, Amsterdam, The Netherlands

The growth of human populations and increasing urbanisation are expected to have an important impact on global biodiversity. Highly urbanised terrestrial environments, such as Singapore, have for example, already lost much of their original diversity. Relatively little is, however, known about the impact of human conurbations on coral reef ecosystems. At present very few coral reefs are located close enough to large cities to study the influence of large urban populations on reef assemblages. An exception is the Thousands Islands reef complex just to the north of Jakarta, the capital city of Indonesia, with more than 10 million inhabitants. Extensive coral surveys have been done since the beginning of the last century, which make it one of the better-explored regions within Indonesia. Here we present data from a detailed survey where sponge assemblages were assessed at 30 patch reefs associated with islands in five zones along an in-to-offshore gradient. The zones are based on geomorphology, oceanography and distance from Jakarta. Sponge assemblages are described at three taxonomic levels of detail (species, genus and family level) and we furthermore relate assemblage turnover to spatial and environmental variables in order to assess the relative contribution of both to structuring sponge assemblages. Furthermore, we assess whether the spatial variation in species composition is related to variation in a suite of environmental parameters including temperature, salinity and live coral cover. We recorded a total of 35 families, 65 genera and 135 sponge species. *Aaptos suberitoides*, *Clathria reinwardti*, *Petrosia nigricans* and *Xestospongia testudinaria* were the most common species among all zones. Some sponges that are commonly found elsewhere in Indonesia, such as *Haliclona fascigera*, *Niphates olemda* and *Ianthella basta* were absent in the Thousand Islands. We found a pronounced difference in species composition among zones with the most pronounced difference in the inshore zone. The environment in this zone was characterised by very poor water transparency and low live coral cover with the dominant substrate composed of silt and sand. Environmental conditions and sponge diversity increased offshore although most areas showed evidence of adverse human impact. These results indicate that human conurbations can have a highly adverse effect on proximate marine assemblages and should heighten the need for further research.

Managing the impacts of translocation decisions for sponge aquaculture using reproductive biology

Stephen Whalan{ XE "Whalan, Stephen" }⁽¹⁾; Christopher Battershill{ XE "Battershill, Christopher" }⁽²⁾; Piers Ettinger-Epstein{ XE "Ettinger-Epstein, Piers" }⁽¹⁾; Alan R. Duckworth{ XE "Duckworth, Alan R." }⁽²⁾; Carsten Wolff{ XE "Wolff, Carsten" }⁽²⁾; Elizabeth Evans-Illidge{ XE "Evans-Illidge, Elizabeth" }⁽²⁾; Rocky de Nys{ XE "Nys, Rocky de" }⁽¹⁾

(1) School of Marine Biology & Aquaculture, James Cook University, Townsville, QLD, Australia, stephen.whalan@jcu.edu.au; (2) Australian Institute of Marine Science, Townsville, QLD, Australia

Translocating wild stock to suitable culture sites can pose potential environmental impacts from genetic pollution to local populations if translocated explants become reproductive during their culture life. Additionally, aquaculture enterprises can be impacted through policy decisions restricting distances over which wild stock can be translocated. Farming trials of the dictyoceratid sponges *Rhopaloeides odorabile* and *Coscinoderma* sp. are being undertaken in the Palm Islands, Australia. Both sponges show aquaculture potential, growing from explants (9cm³) to market size (1000cm³) within 18 months. Another dictyoceratid sponge, *Luffariella variabilis* shows potential for biomedical compounds. All sponges are subject to translocation restrictions of 500m. Sponge explants of both *R. odorabile* and *Coscinoderma* sp. were seeded onto culture long lines and left to recover and grow. *Luffariella variabilis* explants were placed into oyster culture baskets and left to recover and grow on the benthos. We sampled *R. odorabile* and *Coscinoderma* sp. sponge explants at 6 months and two years and *L. variabilis* explants at 6 months for reproductive potential. Standard histological techniques were used to assess if explants were reproductive. At six months explants from both *R. odorabile* and *Coscinoderma* sp. had recovered from explanting however no samples showed any sign of sexual reproduction. In contrast 20% of *L. variabilis* explants samples were reproductive. *L. variabilis* is a simultaneous hermaphrodite that broods larvae and each reproductive sample contained combinations of spermatocytes, embryos and/or larvae. Two year old explants of *R. odorabile* and *Coscinoderma* sp. were reproductive; 20% of *R. odorabile* and 25% of *Coscinoderma* sp. had reproductive propagules. Both species are gonochoristic with males making up 100% and 50% of reproductive samples for *R. odorabile* and *Coscinoderma* sp. respectively. This study demonstrates that fundamental knowledge of reproductive life history traits can be a contributing factor to the design of best practise methods for culture and management of the risks associated with translocation.

Morphological strategies of sponges: Resistance vs. recovery vs. recruitment
(invited lecture)

Janie L. Wulff{ XE "Wulff, Janie L." }

Department of Biological Science Florida State University Tallahassee, FL 32306-1100 USA

An inverse association between ability to regenerate and structural complexity, illustrated by comparisons among animal phyla, suggests a dichotomy between suites of morphological traits that promote recovery from damage vs. resistance to damage. The phylum Porifera is particularly interesting for exploration of these relationships. Sponges are noted for their structural simplicity and great facility at regeneration, relative to members of all other animal phyla; but sponge species vary widely in ability to recover from damage. Growth forms that range from thin crusts to enormous barrels to branching arrays of tubes, and skeletal materials with properties as divergent as siliceous spicules and spongin fibers, combine in a diversity of morphologies. High within-habitat species and morphological diversity of sponges allows comparisons among individuals with different suites of traits that are subject to the same environmental circumstances. To see how morphology is related to resistance to damage and recovery in coral reef sponges, the amount and type of damage was recorded for 576 individual sponges of 67 species at two coral reef sites directly after a major hurricane in Jamaica. Sponge species were divided into 5 morphological categories, amount of damage into 4 categories, and type of damage into 3 categories. For the subsequent 5 weeks, each individual was monitored for continued mortality or recovery. Patterns of recovery from injuries and reattachment of loose fragments demonstrated an inverse relationship between extent of injuries dealt by the storm and recovery success. Small-based sponges, such as erect branching and vase or tube-shaped species, were damaged the most, and were highly successful in regeneration of injuries; whereas tough massive species and encrusting species were damaged significantly less, but also recovered significantly less. The predominant type of damage differed among growth forms and influenced success of recovery. These data provide a framework within which to compile and evaluate data relating to susceptibility to damage, capacity to recover, and recruitment by sexually produced larvae. The combined data, compiled from previous studies of natural injuries, experimental regeneration in the field and lab, transplantation or reattachment in the field, and recruitment to field populations, focus attention on the question: Do sets of traits conferring resistance to damage vs. efficient recovery from damage vs. population-level recovery by larval recruitment represent opposing strategies for sponges? A trade-off between morphological strategies that promote resistance to damage vs. those that promote efficient recovery could result in the disproportionate loss of resistant species, as these poor recoverers are increasingly overwhelmed by the multiple challenges affecting coral reefs and other coastal marine ecosystems.

**Competition for space between the Caribbean encrusting and excavating sponge
Cliona delitrix and reef corals**

Sven Zea { XE "Zea, Sven" }; Andia Chaves-Fonnegra { XE "Chaves-Fonnegra, Andia" }

Departamento de Biología and Centro de Estudios en Ciencias del Mar - CECIMAR, Universidad Nacional de Colombia, INVEMAR, Cerro Punta de Betín, Santa Marta, Colombia, szea@invemar.org.co; andiecita@hotmail.com

The Caribbean excavating sponge *Cliona delitrix* is one of the most destructive agents of reef substratum, colonizing, amply encrusting, and deeply excavating coral colonies. As the sponge advances into live coral, its tissue is preceded by a band of death coral. To establish the circumstances and the speed at which this sponge advances laterally into live coral, and the coral retreat, at San Andrés Island (SW Caribbean, Colombia), 44 sponge-colonized coral colonies were marked and followed for 13 months. Steel nails were driven at the sponge-coral frontier, and used as landmarks to estimate rates of advance and retreat. Fragments of the sponge were implanted into or near to live coral, to evaluate their survivorship and their ability to take root and grow, in relation to the degree of aggressiveness of the host coral species. When death coral bands around the sponge were narrow (up to about 2.5 cm), the sponge was responsible for the coral tissue death and retreat, being both organisms in direct competition for space. At greater distances, the coral retreat was independent of the sponge advance, being indirectly dependent on other conditions that tended to accelerate it. Although quite variable, differences in sponge rates of advance seemed to depend on the coral's skeletal density. When the sponge advanced at its greatest speed into the coral *Siderastrea siderea*, its surface tissue quickly covered the spaces denuded of live coral; at slower speeds, the death band was colonized by algae and other organisms. Biting by the long-spined sea urchin *Diadema antillarum* allowed the sponge to advance slightly faster than when the death band was covered by algae or other organisms. The presence of damselfish territories at the death band or the frequency of occurrence of *D. antillarum* around the coral colony did not have a consistent effect on sponge rates of lateral advance. Contrary to other encrusting and excavating sponges, transplanted fragments of *C. delitrix* showed low survivorship and did not easily take root, independently of the host's coral degree of aggressiveness. Thus, fragmentation appears not to be a viable asexual dispersion mechanism in this sponge.

Taxonomy and ecology of Caribbean sponges: Effective training for new investigators

Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }⁽¹⁾; Robert W. Thacker{ XE "Thacker, Robert W." }^(2*); Guilherme Muricy{ XE "Muricy, Guilherme" }⁽³⁾; Rachel Collin{ XE "Collin, Rachel" }⁽⁴⁾

(1) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com; (2) Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA, thacker@uab.edu; (3) Depto. de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/no., São Cristóvão 20940-040 Rio de Janeiro, RJ, Brasil, muricy@acd.ufjr.br; (4) Smithsonian Tropical Research Institute, Panama, Apartado Postal 0843-03092, Balboa, Ancon, Republic of Panama, collinr@si.edu

In August 2005, the Smithsonian Tropical Research Institute sponsored a course on the taxonomy and ecology of Caribbean sponges, with a goal of training new investigators in sponge biology. Participants (3 lecturers and 13 students) represented 8 countries in North, Central, and South America and the Caribbean. Lecture material included discussions of the current taxonomy of marine sponges, an overview of the morphological characters that differentiate sponge families, recent developments of cytological characters, and applications of molecular systematics to questions in sponge taxonomy. Students learned to identify sponges using spicule preparations and histological sections in the laboratory, as well as external morphology in the field. Field surveys of mangrove and reef communities allowed students to gain further experience identifying unfamiliar species and using several survey techniques. Students observed 32 species in mangrove habitats and 57 species on shallow reefs. Laboratory exercises examined the role of microbial symbionts in sponge metabolism. Students demonstrated significant symbiont photosynthesis and nitrate accumulation in *Chondrilla nucula* and *Xestospongia proxima*, but found no evidence of these processes in *Niphates erecta*. Students presented the results of independent projects at the end of the course, including studies of taxonomy (sponge fauna under coral rubble, chimeric species, and sibling species) and ecology (anthropogenic disturbance affecting sponge communities, larval biology, sponge diseases, and photosymbionts).

Report on two cases of human skin injuries caused by sponge spicules at the Amazon

A. Magalhães{ XE "Magalhães, A." }⁽¹⁾; Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }⁽²⁾; José F.M. Barcellos{ XE "Barcellos, José F.M." }⁽¹⁾; J.C. Cardoso{ XE "Cardoso, J.C." }⁽³⁾; M. Dos-Santos{ XE "Dos-Santos, M." }⁽⁴⁾

(1) Lab. de Histologia, Instituto de Ciências Biológicas, UFAM, Av. Gen. Rodrigo Octávio Jordão Ramos, 3000, Coroadó, 69.077-000, Manaus, AM, attazoom@yahoo.com.br; f.marques123@ig.com.br; (2) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, CP 1188, 90001-970, Porto Alegre, Brazil, cvolkmer@fzb.rs.gov.br; (3) Hospital Vital Brazil, Instituto Butantan, Av. Vital Brasil, 1500, Butantã, 05503-900, São Paulo, SP, jlcardoso@butantan.gov.br; (4) Lab. de Imunologia, Instituto de Ciências Biológicas, UFAM, Av. Gen. Rodrigo Octávio Jordão Ramos, 3000, Coroadó, 69.077-000, Manaus, AM, mcsantos@ufam.edu.br

Freshwater sponges are recognized throughout the Brazilian Amazonia as “cauixi”. At the low water season their crusty or bulbous specimens are easily seen covering whatever hard substrates are available at river bottoms or river banks such as roots, trunks and leaves of the flooded forests. When the raining season begins, the rivers flush against the dried sponge skeletons taking the thinner spicule fibers and the spare ones to the river water. These fragments also settle at the sediments of the lakes left behind the banks by the descending waters. The native people long acquainted with the waters favoring the “cauixi” production do not enter such waters or walk around their marginal areas. The two cases of skin injury now reported were caused by ignorance and took place at the Pará state, one at river Ituqui and the other at the beach of Aramaná, by the Tapajós River. River Ituqui is in fact a tiny arm of the large Amazon setting apart a small island with the same name and subject of the Ituqui Project, a Global Tropical Forestry Project focusing on Lake Reserves and Community Based Management. Santa Helena is one such community and there the first case took place. L.M.C., a 20 years aged woman, during 2005 low water season took a can to splash the river water over her dressed body. After 15 hours, puncture itching wounds appeared in her body at the places where the underwearing had been pressed to the skin (i. e. around the breast and ankle). A few hours afterwards the punctures turned into blisters 1,0 cm wide. Of the four family members who had been bathing with this girl she was the only one to come out with skin reaction. The point is that the very splashing and her dressing acted to press and retain the spicules in the river water against the skin thus facilitating their penetration. The same reaction was observed at the Aramaná beach where J.M.S., a 15 years old girl, who, with other family members picked the sandy stretch under the shadow of the beach trees to lay around. The fact is that such trees are substrate to the most abundant dry sponge outcrop one can think of. The girl did not get into the river but layed on her bathing towel placed on the sand. At some moment she turned the towel upside down and layed on it again what was followed by skin stinging and burning. The sting progressed as in the upper reported case. Of the six family members who gathered at that place, only the youngest one did not present stinging reaction. She was the one who kept bathing at the river margin (150 m distance). A preliminary sponge surveying took place at the two just described areas, plus water sampling at Ituqui. At Aramaná the species are herein listed in order of decreasing abundance: *Spongilla spoliata*, *Drulia uruguaiensis*, *Drulia cristata*, *Corvospongilla sekti*, *Trochospongilla paulula* and *Oncosclera navicella*. At Ituqui sponges were rare but the river water contained a good amount of spicules.

The influence of Sponge Bob Square Pants™ cartoon in natural science teaching to kids between ten and twelve years old

Meiryelen Vieira da Silva{ XE "Silva, Meiryelen Vieira da" }^(*); Leonardo Luis Marques Assumpção{ XE "Assumpção, Leonardo Luis Marques" }; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }

Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524 - PHLC, sala 205, Maracanã, 20550-013, Rio de Janeiro, RJ, Brasil

The mass communication represents an essential factor in the development of modern societies, their cultural and educative aspects. The technological development stimulates the exponential growth of human knowledge and demands teachers a different behavior to help student to learn-how-to-learn. TV media plays a huge influence in day-by-day of students and reflects its power in classrooms. A cartoon created by Stephen Hillenburg in 1999 and broadcasted by Nickelodeon, tells the tale of a square yellow sea sponge named Sponge Bob Square Pants™ and had been interfering in Natural Sciences teaching. Sponge Bob lives in a pineapple deep down in the Pacific Ocean in the city of Bikini Bottom, loves his job as a fry cook at the Krusty Krab Fast-Food Restaurant, and has a knack for getting into trouble without really trying. When he is not getting on the nerves of his cranky neighbor Squidward, Sponge Bob is usually smack in the middle of a strange situation with his best starfish buddy, Patrick. Learning is a process where an individual appropriates knowledge produced by the society, and it is also an active procedure that induces man transformation. Students have unconventional conceptions over several themes before they start formal learning at basic school. The aim of this work is to evaluate the influence of Sponge Bob Square Pants™ cartoon at the alternative understanding about Porifera life history before and after the first year of contact with the subject at school. Five public and five private schools received a quiz form applied to students at the fifth and sixth year of basic school, corresponding to ten to twelve years old kids. First results revealed confusion in invertebrate biology concepts at the fifth year, when the subject was not yet being introduced to students. However, the badly elaborated notions persist in their minds even after formal introduction of the subject matter. After analysis of resulting data, the scientific staff returned to schools to confer a workshop on invertebrate biology named Sponge Bob Study Group. The workshop promotes the get together of students with technical characteristics of sponges and other marine invertebrates shown at the cartoon, supervising the correct fixation of animal biology comprehension. In this way, this work is not a criticism to the cartoon, neither a proposition to correct its concepts, instead a suggestion to use the media vehicle to improve the teaching tools of basic school teachers.

Perplexing distribution of compounds in haplosclerid sponges

Leontine E. Becking{ XE "Becking, Leontine E." }^(1*,2), Nicole J. de Voogd{ XE "Voogd, Nicole J. de" }⁽²⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽¹⁾; Nobuhiro Fusetani{ XE "Fusetani, Nobuhiro" }⁽³⁾; Shigeki Matsunaga{ XE "Matsunaga, Shigeki" }⁽³⁾

(1) Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box. 94766, 1090 GT Amsterdam, The Netherlands, lebecking@gmail.com or becking@naturalis.nnm.nl; (2) National Museum of Natural History, Invertebrates Department, P.O. Box 9517, 2300 RA Leiden, The Netherlands; (3) Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

The present classification of sponges is mainly based on morphology and life history characteristics. The classification of sponges can at times be ambiguous, there fore any additional traits or markers may be useful in verifying phylogenies. Since the 1970's numerous natural product laboratories have extracted, isolated, and elucidated the structures of sponge secondary metabolites. Approximately a decade ago, these records were reviewed to discuss the utility of secondary metabolites as systematic tools, concluding that 3-alkylpyridinium alkaloids may be useful markers for the Order Haplosclerida. This field called chemotaxonomy has yet to manifest itself. Since the mentioned reviews many more publications of compounds extracted from sponges have appeared, which induced us to once again review the distribution of 3-alkylpyridinium derivatives among sponge taxa. We furthermore selected a particular 3-alkylpyridinium alkaloid, and traced its distribution in several genera to establish whether such a compound could be viewed as a genuine chemotaxonomic marker. In our review of 3-alkylpyridinium derivatives, after having made the necessary amendments on sponge taxonomy and nomenclature, we determined that this group of compounds has been solely extracted from sponges of the Order Haplosclerida, and was recorded from all eleven families; however it has not (yet) been recorded from all 24 genera. We determined that the use of more specific compound structures/groups, rather than the relatively broad nominator "3-alkylpyridinium derivatives", ought to be the next step in supporting classifications based on compounds. For instance, previous work suggested that the closely related 3-alkylpyridinium alkaloids halitoxin and amphitoxin may be markers for the family Niphatidae. Our review showed a concentration of these types of compounds in not only Niphatidae, but also Callyspongiidae. Thus we set out to further test the utility of these compounds as a true marker for these families. In the present study, we examined the methanolic extracts of one species of *Amphimedon* from southern Japan as well as two species of the genus *Callyspongia*. *Callyspongia biru* collected in Indonesia from which amphitoxin is expected to be the source of its bio-activity and *Callyspongia truncata*, collected in Japan and from which a large amount of bio-active poly-acetylenic derivatives have been extracted, but to date no hali- or amphitoxins. With the exception of *C. biru*, the extracts of the sponges examined contained neither hali- and/or amphitoxins nor other types of 3-alkylpyridinium alkaloids. Our results diminish the value of both 3-alkylpyridinium derivatives and hali/amphitoxins as monophyletic markers. This study may, however, prove of some value in the ongoing debate on the classification of the poriferan phylum and chemotaxonomy.

Mitogenic activity of ACL-I lectin from the marine sponge *Axinella cf. corrugata*

Roger Remy Dresch{ XE "Dresch, Roger Remy" }^(1*); Gilberto Dolejal Zanetti{ XE "Zanetti, Gilberto Dolejal" }⁽²⁾; Rodrigo Pestana Lopes{ XE "Lopes, Rodrigo Pestana" }⁽³⁾; Moises Evandro Bauer{ XE "Bauer, Moises Evandro" }⁽³⁾; Beatriz Mothes{ XE "Mothes, Beatriz" }⁽⁴⁾; Cléa Lerner{ XE "Lerner, Cléa" }⁽⁴⁾; Vera Treis Trindade{ XE "Trindade, Vera Treis" }⁽²⁾; Amélia Teresinha Henriques{ XE "Henriques, Amélia Teresinha" }⁽¹⁾; Magdolna Maria Vozári-Hampe{ XE "Vozári-Hampe, Magdolna Maria" }⁽²⁾

(1) Pharmaceutical Sciences Pos-graduate Program, Pharmacy School, UFRGS, Av. Ipiranga, 2752, 90610-000, Porto Alegre, RS, Brazil, rogdresch@yahoo.com.br; (2) Department of Biochemistry, Institute of Health Basic Sciences, Federal University of Rio Grande do Sul, Rua Ramiro Barcelos, 2600, 90035-003, Porto Alegre, RS, Brazil, hampe@orion.ufrgs.br; (3) Laboratory of Cellular and Molecular Immunology - Pontifical Catholic University of Rio Grande do Sul, Av. Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brazil, rpestlopes@gmail.com; (4) Zoobotanic Foundation, Rua Dr. Salvador França, 1427, 90690-000, Porto Alegre, RS, Brazil, bmothes@fzb.rs.gov.br

Lectins are proteins that possess at least one domain able to recognize and to bind in a reversible manner and with certain specificity carbohydrates. The binding of lectins to the carbohydrates of specific receptors of cell surface triggers an array of biological responses such as cell aggregation, cytotoxicity, mitogenic stimulation of lymphocytes, induction of interleukines secretion and neutrophil migration. The biological properties of these proteins make them valuable tools in many areas of biology and medicine. Lectins are widely distributed in plants, microorganisms and animals, including sponges. The physiological role attributed to them in the sponges is to be defense proteins against pathogens and predators attack. In this work we purified a lectin (ACL-I) from *Axinella cf. corrugata* collected at the South Atlantic coast of Brazil. The proteins of the sponges were extracted with phosphate buffered saline, pH 7.2 (PBS). Isolation of ACL-I was achieved by preparative gel filtration on Ultrogel ACA 44 column, followed by affinity chromatography on a rabbit stroma coupled to polyacrylamide gel column and by gel filtration on Sephadex G-200. The purification was monitored by SDS-PAGE and the proteins were evaluated by the Bradford method. Hemagglutinating activity and its inhibition by carbohydrates were done by the double dilution method in microtitre plates using 2% rabbit erythrocytes suspension. Mitogenic activity was tested by the MTT method of Sladowski, using human peripheral blood mononuclear cells cultivated in microculture plates. Each microculture contained 1.5×10^5 cells in 0.2 mL of medium and was incubated at 37°C under 5% CO₂ atmosphere. The results showed that N-acetyl-D-galactosamine, N-acetyl-D-mannosamine, N-acetyl-D-glucosamine and N,N,N'-triacyetyl-chitotriose inhibited the hemagglutinating activity of ACL-I. N-acetyl-D-glucosamine also inhibited the mitogenic effect of the lectin. PHA was used as positive control. Our results suggest that ACL-I can be used as tool in immunology and in carbohydrate recognition cell surface studies.

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Effect of marine sponges on Pdr5p catalytic activity of *Saccharomyces cerevisiae*

Patrícia Fernandes Ferreira{ XE "Ferreira, Patrícia Fernandes" }^(1*); Luciana Pereira Rangel{ XE "Rangel, Luciana Pereira" }⁽¹⁾; Gustavo Nazaré Livramento{ XE "Livramento, Gustavo Nazaré" }⁽¹⁾; Guilherme Muricy{ XE "Muricy, Guilherme" }⁽²⁾; Andre Goffeau{ XE "Goffeau, Andre" }⁽³⁾; Antônio Ferreira Pereira{ XE "Pereira, Antônio Ferreira" }⁽¹⁾

(1) Laboratório de Estudos Integrados em Bioquímica Microbiana, Departamento de Microbiologia Geral, IMPPG, UFRJ; (2) Departamento de Invertebrados, MN, UFRJ; (3) Université Catholique de Louvain, Louvain-la-Neuve, Belgium

The search for new compounds that can act as multidrug resistance inhibitors is a very promising approach to improve chemotherapy efficacy. This kind of resistance is often promoted by ABC transporters, for example, P-glycoprotein from mammalian cells, decoded by the gene ABCB1 (MDR1). In *Saccharomyces cerevisiae*, homologous genes were detected, such as PDR5. The product of this gene, the protein Pdr5p, confers resistance to several unrelated drugs, as the same used by P-glycoprotein. Recent studies demonstrate that marine sponges can be a great source of new natural products with interesting biological activities. The use on chemotherapy of many of these compounds from sponges is under investigation. In this study, we have evaluated the effect of several sponge extracts on Pdr5p catalytic activity. Aqueous and organic extracts obtained from sponges collected in the coast of Fernando de Noronha and Rio de Janeiro (Brazil) were tested against Pdr5p ATPase activity in a fixed concentration of 200 µg/mL. Ethanolic extracts of *Petromica citrina* and *Agelas* sp had an inhibitory effect higher than 50% and were selected for further dose-dependence studies. These results showed that these extracts probably contain new compounds with modulatory effects on multidrug resistance transporters. Future assays aiming the identification of the compound responsible for the inhibition will be done.

How complex is sponge genome? Example: The demosponge *Suberites domuncula*

Vera Gamulin{ XE "Gamulin, Vera" }^(*); Helena Cetkovic{ XE "Cetkovic, Helena" }; Lada Lukic-Bilela{ XE "Lukic-Bilela, Lada" }; Matija Harcet{ XE "Harcet, Matija" }; Drago Perina{ XE "Perina, Drago" }

Department of Molecular Biology, Rudjer Boskovic Institute, Bijenicka cesta 54, Box 170, 10002 Zagreb, Croatia, gamulin@irb.hr

During the last 15 years a large amount of data about the structure and function of genes/proteins from different sponges (Porifera) has been accumulated. The majority of molecular genetic data were collected from the marine demosponge *Suberites domuncula*. Based on these data, supported in many cases by cell biological studies, it is now well established that all metazoan phyla evolved from one ancestor, the hypothetical Urmetazoa. Although sponges are the simplest living multicellular animals, their genomes are far from being simple. Based on the information obtained from thousands of partial cDNA sequences (ESTs), hundreds of complete cDNA sequences and dozens of gene sequences from *S. domuncula*, we can only conclude that the genome of (at least) this demosponge is unexpectedly complex. It encodes thousands of proteins, including many sophisticated proteins involved in higher order processes and biological functions found so far exclusively in higher animals. These proteins include, for example, numerous receptor and cytoplasmic tyrosine kinases and Ras family small GTPases, molecules involved in cell-cell interactions, morphogenesis, immune response, apoptosis, skeleton formation etc. In addition, many genes/proteins from *S. domuncula* with clear orthologs in human are not encoded in *Caenorhabditis elegans* or *Drosophila melanogaster* genomes. Another unexpected finding came from the detailed analysis of sponge genes/proteins. The majority of *S. domuncula* genes and proteins are more related to their orthologs in vertebrates/mammals/human than to either *D. melanogaster* or *C. elegans* counterparts: 1) intron positions in sponge and mammalian orthologous genes are highly conserved, what is often not true for orthologs from insects and worms; 2) sponge proteins show on average highest degree of sequence similarity with proteins from vertebrates (including mammals). In addition, when orthologs (or even whole protein families like ribosomal proteins) from *S. domuncula*, *D. melanogaster*, *C. elegans* and mammals are compared, the highest degree of sequence conservation is often found between sponge and mammalian protein pairs. Both model invertebrate organisms experienced recently accelerated evolution. Significant number of *D. melanogaster* and *C. elegans* genes are highly modified and nothing is known about the extent of gene loss in these model organisms. Therefore, sponge genes/proteins very probably better reflect the structure and complexity of the ancestral metazoan genome (Urmetazoa), which obviously already encoded huge number of proteins, including many metazoan "novelties". These ancient genes/proteins, common to all Metazoa, changed to the less extent during the evolution of Deuterostomia (and sponges) than during the evolution of two model invertebrates. The complete information about gene content and complexity of sponge genome ("Sponge sequencing project") will be of extreme importance for the elucidation of metazoan evolution.

Study of the structure and organization of the gene family of silicatein proteins in Baikal sponges

Oksana V. Kaluzhnaya{ XE "Kaluzhnaya, Oksana V." }^(1*); Sergey Belikov{ XE "Belikov, Sergey" }⁽¹⁾; Anatoli Krasko{ XE "Krasko, Anatoli" }⁽²⁾; Heinz C. Schröder{ XE "Schröder, Heinz C." }⁽²⁾; Werner E.G. Müller{ XE "Müller, Werner E.G." }^(2, 1)

(1) Limnological Institute of the Siberian Branch of Russian Academy of Sciences, Ulan-Batorskaya 3, RUS-664033 Irkutsk, Russia, oksana@lin.irk.ru; (2) Institut für Physiologische Chemie, Abteilung Angewandte Molekularbiologie, Universität, Duesbergweg 6, D-55099 Mainz, Germany, wmueller@mail.uni-mainz.de

Lake Baikal (East Siberia) harbors the largest diversity of sponge species among all freshwater biotopes. Currently over 18 species have been described from this lake, 13 of them are endemic. In the lake they form the endemic family of Lubomirskiidae. These sponges are able to accumulate inorganic silicon from the environment forming the biogenic siliceous skeleton (spicules). Silica polycondensation takes place around the organic axial filament which consists of special proteins - silicateins, members of the papain-like cysteine proteases family. In this study the existence of four genes of silicatein- α ($-\alpha 1$, $-\alpha 2$, $-\alpha 3$, $-\alpha 4$) in the fresh-water Baikal sponge *Lubomirskia baicalensis* is shown for the first time. Such polymorphism of genes is unique among the investigated sponges and can testify to special conditions of evolution of sponges in Lake Baikal. We assume that duplications of this gene took place and resulted in the formation of closely related family of genes which were mutated independently from each other. The intron-exon structure of the full-size silicatein- $\alpha 1$ gene was also determined. This gene has total length of 1988 bp and includes 6 introns (71-378 bp) and 7 exons (102-231 bp). Sizes of introns (1007 bp) and exons (981 bp) are approximately equal. On the basis of the sequence data obtained primers for further analysis of silicateins from different species of Baikal sponges were designed. Partial nucleotide sequences of two genes, belonging to the endemic species *Baikalospongia intermedia* were obtained. It was found that these sequences are related with already known sequences from *Lubomirskia baicalensis*. The size of the coding part of all genes remains constants whereas the size of the introns greatly varies. A number of differences between silicatein genes from various species of sponges allows the development of identification markers for closely related Baikal sponge species and, probably, also to estimate the heterogeneity of their populations.

Construction and screening of cDNA library from a marine sponge *Chondrosia reniformis*

Anne Kuuskalu{ XE "Kuuskalu, Anne" }⁽¹⁾; Tõnu Reintamm{ XE "Reintamm, Tõnu" }⁽¹⁾;
Madis Metsis{ XE "Metsis, Madis" }⁽²⁾; Merike Kelve{ XE "Kelve, Merike" }^(1*, 2)

(1) Department of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia, kuuskal@kbfi.ee; tonu@kbfi.ee; merike@kbfi.ee; (2) Institute of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 19086 Tallinn, Estonia

Sponges are evolutionarily the most ancient and simplest multicellular animals. Though the molecular studies of recent years have demonstrated a surprising similarity between sponges and mammals, our knowledge about the sponge genome is still limited. Our previous study has demonstrated the presence of the 2'-5'-oligoadenylate synthetase (OAS) enzymatic activity, characteristic for mammals, in a variety of marine sponges. In mammals this enzyme is a part of a biochemical pathway of innate immunity. OAS cDNAs have been cloned from two marine sponges - *Geodia cydonium* and *Suberites domuncula*. The sponge OAS have limited homology to each other and particularly to the vertebrate one. However, the motifs essential for the OAS activity are conserved. The action mechanism of the sponge enzyme seems to be different from that in mammals and very little is known about its role in sponges. This study was directed to the construction of a cDNA library from the marine sponge *Chondrosia reniformis* and screening it with OAS cDNA from *G. cydonium*. Specimens of *C. reniformis* were collected from the Aegean Sea. The presence of specific mRNA was verified by northern blot analysis. 1.1 kb DNA fragment from coding region of OAS from *G. cydonium* served as a probe. The cDNA library was constructed using ZAP Express system (Stratagene). The duplicate plaque lifts were hybridized in low stringency conditions to *G. cydonium* probe. The plaques of interest were subjected to in vivo excision. The insert length was determined and end-sequenced. Homology searches were performed using BLAST and sequence motifs were studied with MEME motif discovery tool. Northern blot analysis of *C. reniformis* mRNA revealed a specific 1.5 kb mRNA signal. The constructed cDNA library contained approximately 90 000 individual clones. The length of the inserts ranged from 0.7 to 3kb (average 1.6 kb) and random sequencing of clones revealed that the majority of them contained full-length sequences as compared to homologous genes. The low stringency screening of the library resulted in 96 individual positive clones. Their end sequences were determined. Preliminary analysis of end-sequencing data did not directly assign any of these sequences to the OAS family. We suggest that this results from low homology between sequences. Several clones contained sequence motifs present also in *G. cydonium* probe and are subjects for the continuing analysis. Presently assigned sequenced cDNA clones represented evolutionarily conserved sequences coding for various functional classes of proteins: enzymes (helicase, acyl-CoA synthetase, chondroitin synthase, cysteine dioxygenase, protein phosphatase RAS-like small GTPase etc.) and structural proteins (actin, cortactin, actinin, dynactin, collagen, myosin). The MEME motif discovery tool analysis has revealed several common motifs in these sequences, the functional meaning of which will be further studied.

Characterization of matrix proteins in the coralline Demosponge *Astrosclera willeyana*

Luciana Macis{ XE "Macis, Luciana" }; Joachim Reitner{ XE "Reitner, Joachim" }; Gert Wörheide{ XE "Wörheide, Gert" }^(*)

Geoscience Centre Göttingen, Dept. of Geobiology, Goldschmidtstr. 3, D-37077 Göttingen, Germany,
gert.woerheide@geo.uni-goettingen.de

In contrast to most soft-bodied sponges, coralline sponges (also called 'sclerosponges') secrete a secondary rigid calcium carbonate skeleton. Here, we studied biocalcification processes from the cellular to the proteomic/genetic level in the 'stromatoporoid' taxon *Astrosclera*. Generally, the calcareous basal skeletons of coralline sponges, as members of the most ancestral metazoan clade, represent the simplest biologically controlled mineralization, intermediate between biologically induced- (e.g. organomineralization) and the fully enzymatically controlled mineralization of higher Metazoa. The secondary basal skeleton of *Astrosclera* consists of aragonitic spherulites, which are made by biologically controlled and matrix-mediated biocalcification processes in two steps. First, the spherulites are built intracellularly and second, they fuse together by epitaxial growth. Soluble matrices were extracted separately from spherulites and growing skeleton and separated by SDS-gel electrophoresis. Soluble matrix from spherulites was characterized by a prominent large (~60 kDa) band, whereas the epitaxially grown basal skeleton showed three bands around 35 kDa. Initially, we focused our investigations on the latter smaller bands. Sequencing of their N-terminus showed high similarity among them. A cDNA library was screened with the aim to fully characterize matrix proteins and full-length cDNA clones were obtained. Analysis of the deduced amino acid sequence revealed homology to carbonic anhydrase, which is thought to play an important role in the calcification process. Investigation of genes and regulatory mechanisms involved in poriferan biocalcification and their phylogenetic relationships promises to provide a roadmap for our understanding of the evolution and diversification of metazoan calcium carbonate biomineralization.

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Evolution of multicellularity in Porifera via molecular self-assembly of glyconectin carbohydrates

Gradimir N. Misevic{ XE "Misevic, Gradimir N." }^(1*); Yann Guerardel{ XE "Guerardel, Yann" }⁽²⁾; Maurice Demarty{ XE "Demarty, Maurice" }⁽¹⁾; Camille Ripoll{ XE "Ripoll, Camille" }⁽¹⁾; Vic Norris{ XE "Norris, Vic" }⁽¹⁾; Pascal Ballet{ XE "Ballet, Pascal" }⁽³⁾; Yannis Karamanos{ XE "Karamanos, Yannis" }⁽⁴⁾; Emmanuel Maes{ XE "Maes, Emmanuel" }⁽²⁾; Lazar T. Sumanovski{ XE "Sumanovski, Lazar T." }⁽⁵⁾; Octavian Popescu{ XE "Popescu, Octavian" }⁽⁶⁾; Gerard Strecker{ XE "Strecker, Gerard" }⁽²⁾

(1) Assemblages Moléculaire: Modelisation et Imageries SIMS, University of Rouen, 76821 Mont St Aignan Cedex, France; (2) Unité de Glycobiologie Structurale et Fonctionnelle, Université des Sciences et Technologies de Lille, UMR 8576 CNRS, 59655 Villeneuve D'Ascq, France; (3) University of Brest, France; (4) Laboratoire de Biochimie Moléculaire et Cellulaire, Université d'Artois, Faculté J Perrin, rue J. Souvraz, SP18, 62307 Lens, France; (5) Department of Research, University Hospital of Basel, CH-4058 Basel, Switzerland; (6) Molecular Biology Center and Institute for Interdisciplinary Experimental Research, Babes-Bolyai University, 400006 Cluj-Napoca, Rumania

Research done in the last century on Porifera has provided insights into the molecular mechanism of the biological processes of cell adhesion, innate immunity, and self-recognition. Evidence that this mechanism is based on glyconectin self-assembly is shown by the structure to function relationships deduced from studies of carbohydrates isolated from three different sponge species. The structural studies were performed on purified glyconectin carbohydrates from *Microciona prolifera*, *Halichondria panicea* and *Cliona celata* using NMR and mass spectrometry. Seventeen, novel, species-specific, carbohydrate sequences were revealed that belong to the Porifera glyconectin family. The functional, cell recognition analyses of carbohydrate self-association were performed by measuring binding forces between individual glycan molecules under physiological conditions; the results show that the association strength between homotypic pairs of glycans (400 pN) are higher than those between heterotypic pairs (20 pN). This difference is sufficient to explain the species-specific separation of glycan-coated beads *in vitro* and the sorting of cells in nature. We propose that the glyconectin carbohydrates, which are the constituents on the cell surface that are the most exposed to the environment, were responsible for the molecular recognition processes that underpinned the emergence of multicellularity.

Porifera as model systems for early metazoans: The genetic complexity of siliceous sponges

(invited lecture)

Werner E.G. Müller{ XE "Müller, Werner E.G." }

Institut für Physiologische Chemie, Abteilung Angewandte Molekularbiologie, Universität, Duesbergweg 6, 55099 Mainz, Germany. Tel.: +49-6131-392-5910; Fax.: +49-6131-392-5243, wmueller@uni-mainz.de

In a long and painful scientific process the most enigmatic metazoan phylum, the Porifera, was positioned phylogenetically correct among the eukaryotes in general and the multicellular animals in particular. Molecular biological techniques allowed to prove the monophyly of the Porifera with the other metazoan phyla, justifying a unification of all multicellular animals to only one kingdom, the Metazoa. Here we give evolutionary novelties of the Porifera (primarily proved with the demosponges *Suberites domuncula* and *Geodia cydonium*), that very likely were also present in the Urmetazoa: (i) Molecules involved in cell-cell interaction: The extracellular aggregation factor that promotes species-specific aggregation of cells. The isolation of a galectin sequence as the first cell-cell adhesion molecule made clear that sponges contain molecules known to promote adhesion in Protostomia and Deuterostomia. (ii) Molecules involved in cell-substrate interaction: The dominant molecule in the extracellular matrix of sponges is collagen. Corresponding genes were cloned and functionally analyzed. (iii) Membrane receptors: e.g. integrin and neuronal receptors. (iv) Transcription factors: e.g. T-box, Forkhead and Homeobox genes, a LIM/homeobox protein and the putative Iroquois transcription factor. (v) Genes indicative for Wnt signaling: Example Frizzled receptor. (vi) Molecules involved in skeleton formation: Recently, evidence has been presented that the formation of spicules is mediated by the enzyme silicatein. (vii) Moreover, basic parts of the potent immune system and the apoptotic system in *S. domuncula* have been identified. It is a fortune that the phylogenetic oldest metazoan phylum (according to the fossil records), the Porifera, did not become extinct during the last 800 million years, since it is now obvious that they are provided with most of the metabolic pathways and genetic elements required for the construction of a metazoan bauplan. It should also be stressed that the potential commercial value of sponge biodiversity is of unpredictable scale. A new application field which very likely will have a considerable impact in biotechnology is the elucidation and the synthesis of bioactive, low-molecular weight compounds from sponges in a recombinant manner. To reach this goal gene clusters required for the synthesis of such compounds from the sponge host as well as the associated symbiotic bacteria have to be isolated and expressed in a heterologous system.

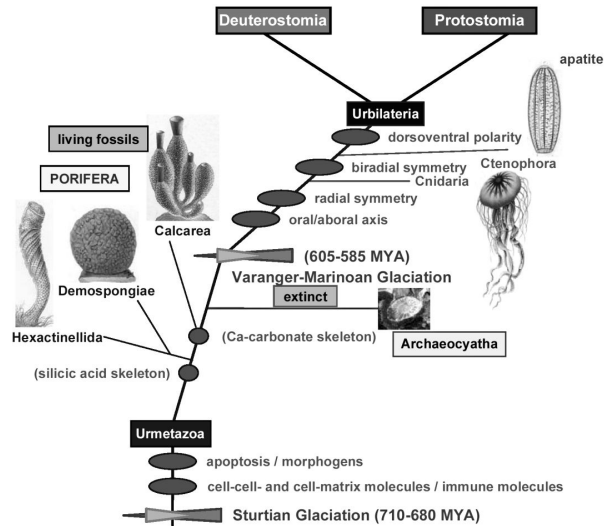


Figure. Phylogenetic position of the Porifera between the Urmatazoa and the Urbilateria. Very likely the Urmatazoa emerged between the two major “snowball earth events”, the Sturtian glaciation (710 to 680 MYA) and the Varanger-Marinoan ice ages (605 to 585 MYA). The major evolutionary novelties which have to be attributed to the Urmatazoa are those molecules which mediate apoptosis, and control morphogenesis, the immune molecules and primarily the cell adhesion molecules. First, the siliceous sponges with the two classes Hexactinellida and Demospongiae emerged and finally the Calcarea, which possess a calcareous skeleton. These three classes of Porifera are living fossils that provide a reservoir for molecular biological studies. The Calcarea are very likely a sister group of the Cnidaria. From the latter phylum the Ctenophora evolved which comprise not only an oral/aboral polarity but also a biradial symmetry. Finally the Urbilateria emerged from which the Protostomia and the Deuterostomia originated.

Halogenases from the marine sponge *Erylus discophorus*

Marisa Nicolai{ XE "Nicolai, Marisa" }^(*); Ana Esteves{ XE "Esteves, Ana" }; Gisela Gonçalves{ XE "Gonçalves, Gisela" }; Marise Almeida{ XE "Almeida, Marise" }; Madalena Humanes{ XE "Humanes, Madalena" }

Centro de Química e Bioquímica - Departamento de Química e Bioquímica da Faculdade de Ciências da Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal

Marine sponges are ubiquitous animals showing a wide array of metabolites with several biological activities. Although, the presence of halogenated compounds are well documented in the Porifera phylum little is known about the biosynthetic pathways leading to their production. The studies with sponge enzymes are scarce and particularly, those involving halogenating enzymes are practically non-existent. Our group has been interested in the investigation of halogenating enzymes in marine sponges. Preliminary results with sponge crude and partially purified extracts showed haloperoxidase activity. Several attempts were made to isolate this enzyme - a very difficult procedure due to the loss of activity. We report, by the first time, the complete purification procedure and partial characterization of a halogenase from the marine sponge *Erylus discophorus*. The enzyme is a glycoprotein with a molecular mass around 200 kDa and was purified using different types of chromatographies (an anionic exchange chromatography followed by a gel filtration step). The enzyme catalysed the oxidation of iodide to triiodide and the formation of monochloromonobromodimedone, in the presence of hydrogen peroxide and the corresponding halide ion. The formation of moniodotyrosine and diiodotyrosine, compounds, well recognized as secondary metabolites in marine sponges, are also catalyzed by this enzyme. The nature of the active site is still not known, but our results exclude the presence of a heme or a vanadium cofactor.

Influence of rock substrata on 3D sponge cell culture development. A molecular biology study

Marina Pozzolini{ XE "Pozzolini, Marina" }⁽¹⁾; Laura Valisano{ XE "Valisano, Laura" }⁽²⁾; Carlo Cerrano{ XE "Cerrano, Carlo" }⁽²⁾; Raffaella Risso{ XE "Risso, Raffaella" }⁽¹⁾; Mattia Menta{ XE "Menta, Mattia" }⁽¹⁾; Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽³⁾; Umberto Benatti{ XE "Benatti, Umberto" }⁽¹⁾; Marco Giovine{ XE "Giovine, Marco" }^(4*)

(1) Dipartimento di Medicina Sperimentale-Sez. Biochimica, Università degli Studi di Genova, Genova, Italy; (2) Dipartimento per lo studio del territorio e delle sue risorse, Università degli Studi di Genova, Italy;

(3) Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Ancona, Italy; (4) Dipartimento di Biologia, Università degli Studi di Genova, Viale Benedetto XV, 5 16132 Genova

Several marine organisms live attached to rocks or on sandy bottom during all their life period or in some specific phase of their development. Our research group is studying the influence of rock substrata on sponges' cells growth. The experimental procedure is based on the use of a 3D sponge cell culture model better known as "primmorphs". More in details, we have tested the primmorph development rate on quartz, calcium carbonate, glass and cell culture plastic dishes. Our preliminary results suggest that the typology of substrata strongly affects the growth rate and the morphology of primmorphs and molecular biology studies using Real Time PCR showed a remarkable influence on sponge genes expression determined by the chemical and/or mineralogical composition of rocks. In particular, we have evaluated the expression of silicatein and HSP70 genes in primmorphs obtained from the marine sponge *Petrosia ficiformis* growth on different materials.

Partial purification and preliminary characterization of ATP N-glycosidase from a marine sponge *Axinella polypoides*

Tõnu Reintamm{ XE "Reintamm, Tõnu" }^(1*); Merike Kelve{ XE "Kelve, Merike" }^(1, 2)

(1) Department of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia, tonu@kbfi.ee; merike@kbfi.ee; (2) Institute of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 19086 Tallinn, Estonia

Novel enzymatic activity, ATP N-glycosidase (ATPNG), releasing adenine from high-energy adenosine nucleotides has been described in a crude extract of a marine sponge *Axinella polypoides*. Here we report our current results in ATPNG isolation and characterization. The sponge material was collected near the island Kalymnos (Greece). D3 fragment of 28S RNA gene was amplified by PCR and subjected to direct sequencing to distinguish *Axinella polypoides* (AF051438) and *Axinella verrucosa* (AY319312) growing at this location. During the purification procedure the ATPNG was monitored in parallel with ADP ribosyl cyclase (ADPRC) which had been characterized earlier in *Axinella polypoides*. The initial extract was a micro-heterogenous suspension from which the ATPNG tended to coprecipitate and/or adsorb with an unknown nonprotein component. The initial extract in which the ATPNG activity was stable at 4000g, was fractionated using ultracentrifugal devices with 100 kDa and 10 kDa cut-off filters. ATPNG was found only in >100 kDa fraction while ADPRC was present both in 10-100 kDa and >100 kDa fractions. The >100 kDa fraction was possible to concentrate up to 6 times in respect to the initial extract without precipitation of the ATPNG at high-speed centrifugation. >100 kDa fraction and 10-100 kDa fractions were analyzed by several chromatographic methods. ADPRC present in both fractions, eluted from BioSep-Sec-S3000 column as a 28 +/- 4 kDa protein. ATPNG activity present only in >100 kDa fraction, eluted strictly in a void volume of the column (exclusion range for native proteins 5-700 kDa). About half of the loaded ATPNG was eluted, while the rest of the activity was irreversibly adsorbed on the column. ADPRC passed the DEAE column in the flow-through fraction, while the ATPNG eluted in a 0-2 M NaCl gradient. The ATPNG activity was completely retained in a strong anion exchange column ResourceQ even when eluted with a high salt concentration at low pH. The >100 kDa fraction was analyzed by SDS-PAGE electrophoresis with or without heat denaturation at 95°C prior loading. Coomassie staining revealed high-molecular weight aggregates not entering the resolving gel when the sample was applied without denaturation. In case of nondenatured sample it was possible to detect ATPNG activity in the gel but only in the localization of the aggregates. These results suggest that ATPNG is a highly oligomeric acidic protein. The homohexameric structure analogous to purine phosphorylases is one of the possible explanations of our results.

High levels of the stress proteins (Hsp70) accompany quiescent gemmules of the freshwater sponge, *Spongilla lacustris* Linné, 1759

Ralph O. Schill{ XE "Schill, Ralph O." }(*); Martin Pfannkuchen{ XE "Pfannkuchen, Martin" };
Franz Brümmer{ XE "Brümmer, Franz" }

Biological Institute, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany,
ralph.schill@bio.uni-stuttgart.de; martin.pfannkuchen@bio.uni-stuttgart.de; franz.bruegger@bio.uni-stuttgart.de

Freshwater sponges are often directly affected by daily and seasonal water levels or temperature changes. As a reaction to adverse conditions like starvation, desiccation, or low temperatures, they are well known to form reduction bodies so called gemmules in high numbers during reduction, especially during fall and early winter. The metabolism of the enclosed cells during the period of quiescence or diapause is considerably reduced, if not entirely stopped. Hatching can be triggered by a temperature shift and subsequently undifferentiated cells leave the gemmules in an organized, probably pre-determined order. However, the molecular and biochemical mechanisms which enable the gemmules to survive long periods of adverse conditions are so far not known. This study deals with the role of stress gene expression and, accordingly, the formation of stress protein, particularly the well-known protein-stabilizing chaperone Hsp70, in quiescent gemmules and emerging sponge. Gemmules stored at 8°C remained quiescent, however, germination occurred within 24 hours after the water temperature was increased up to 22.5°C. Sponges hatched through the microphyle and subsequently built spicules and a canal system. For the first time we focused on the role of the stress protein Hsp70 and the expression of Hsp70 mRNA in *Spongilla lacustris* during the development of gemmules in the state of quiescence into growing sponges. The found partial sequence of Hsp70 seems to be a true hsp gene since transcription could be clearly enhanced by temperature elevation. The results showed a large pool of cellular Hsp70 and Hsp70 mRNA in quiescent gemmules. Within hours after temperature trigger the Hsp70 level decreased slowly and reached approximately the level of an grown-up sponge. Hsp70 presumably allows gemmules to stabilize their proteins and membranes, and rest from autumn to spring until water temperatures are changing.

Iodoperoxidase activity from sponges of São Miguel Island (Azores)

Henriqueta Veríssimo{ XE "Veríssimo, Henriqueta" }⁽¹⁾; Joana Xavier{ XE "Xavier, Joana" }⁽²⁾.
Ana Costa{ XE "Costa, Ana" }⁽²⁾; Maria do Carmo Barreto{ XE "Barreto, Maria do Carmo" }^(1, 3*)

(1) D.C.T.D., Azores University, 9501-801 Ponta Delgada, Portugal; (2) D.B., Azores University, 9501-801 Ponta Delgada, Portugal; (3) CIRN, 9501-801 Ponta Delgada, Portugal, barreto@notes.uac.pt

The existence of halogenated compounds is a common feature of sessile organisms, playing a part in the chemical defense mechanism of these organisms against predation. Many of these compounds display biological activity, and new molecules with pharmacological applications have been discovered. The group Porifera, in particular, has been recognized as a rich source of natural products of potential pharmaceutical value. It is therefore of the utmost interest to gather information about the enzymes responsible for the synthesis of these halogenated molecules, the haloperoxidases. Sponges were collected by scuba diving in São Miguel, in four different sites located on the south of the island: Ilhéu de S. Roque, Ferraria/Mosteiros, Calheta and Ilhéu de Vila Franca. Haloperoxidase activities were determined for whole extracts of seven different species: *Ciocalypa penicillus*, *Halichondria aurantiaca*, *Tedania anhelans*, *Haliclona fistulosa*, *Cliona viridis*, *Petrosia ficiformis* and *Sponginella pulchella*. Iodoperoxidase activity in sponge extracts was determined by spectrophotometrically monitoring the oxidation of iodide ion to molecular iodine in the presence of H₂O₂. The activity of the enzyme varied greatly between different species, but also between different populations of the same organism growing in different sites. Marked differences in the level of iodoperoxidase activity were found, in the case of *Tedania anhelans*, in two samples collected in different areas of the same site. These differences may be correlated with the high plasticity that is characteristic of these organisms, in response to macro and microscale environmental variations.

Antioxidant enzymes from São Miguel Island littoral sponges

Henriqueta Veríssimo{ XE "Veríssimo, Henriqueta" }⁽¹⁾; Joana Xavier{ XE "Xavier, Joana" }⁽²⁾.
Ana Costa{ XE "Costa, Ana" }⁽²⁾; Maria do Carmo Barreto{ XE "Barreto, Maria do Carmo" }^(1, 3)

(1) D.C.T.D., Azores University, 9501-801 Ponta Delgada, Portugal; (2) D.B., Azores University, 9501-801 Ponta Delgada, Portugal; (3) CIRN, 9501-801 Ponta Delgada, Portugal, barreto@notes.uac.pt

Superoxide dismutase and catalase play important roles in the defense of organisms against the harmful effects of reactive oxygen species. Although these enzymes are ubiquitous, few studies have been made concerning marine sponge antioxidant enzymes. Sponges were collected by scuba diving in São Miguel island (Azores), in four different sites located on the south of the island: Ilhéu de S. Roque, Ferraria/Mosteiros, Calheta and Ilhéu de Vila Franca. *Ciocalypta penicillus*, *Halichondria aurantiaca*, *Tedania anhelans*, *Haliclona fistulosa*, *Cliona viridis*, *Petrosia ficiformis* and *Sponginella pulchella* were the species under study. Superoxide dismutase activity was determined for these species by monitoring the inhibition of the autooxidation of adrenaline in carbonate buffer. Catalase activity was determined following the uptake of hydrogen peroxide. The activity of both antioxidant enzymes varied markedly between the species. Differences were also found between organisms from the same species but belonging to different populations.

The role of sulfated polysaccharides in cellular aggregation of marine sponges

Eduardo Vilanova{ XE "Vilanova, Eduardo" }^(1*); Márcio Reis Custódio{ XE "Custódio, Márcio Reis" }⁽²⁾; Paulo Antonio de Souza Mourão{ XE "Mourão, Paulo Antonio de Souza" }⁽¹⁾

(1) Laboratório de Tecido Conjuntivo - HUCFF/UFRJ, Av Brigadeiro Trompowsky s/n, sala 4a01, Rio de Janeiro (RJ), Brazil, CEP 21941-590, evilanova@hucff.ufrj.br; (2) Departamento de Fisiologia - IB/USP, Rua do Matão n.321, Sala 328, São Paulo (SP), Brazil, CEP 05508-900

Species specific cell adhesion in marine sponges is mediated by proteoglycans composed by sulfated polysaccharides attached to a protein core. In the present work we determined the cellular location of sulfated polysaccharides in cells of the marine sponge *Hymeniacidon heliophila* and the effects of different sulfated polysaccharides in the sponge cell culture. Tissues of *H. heliophila* were dissociated using CMFSW + E (calcium magnesium free seawater + EDTA). The cells were stained with Toluidine Blue 1% pH 4.4 for the identification of sulfated polysaccharides by the characteristic metachromasy produced by the dye. All morphologically distinct cell types observed in *H. heliophila* display strong metachromasy in the cell surface and six of them also exhibited metachromatic intracellular vacuoles. Cell suspensions of *H. heliophila* were incubated with different media: FSW only (filtered seawater with antibiotics, sterilized by filtration in 0.2µm cellulose acetate filters. Control); FSW supplemented with purified sulfated polysaccharides from the sponges *H. heliophila* (different concentrations), *Dysidea robusta* (3mg/ml) and *Aplysina fulva* (3mg/ml); and FSW supplemented with Heparin (different concentrations). After 12 hours of incubation, the areas of *H. heliophila* cell aggregates were measured (mean and SD) and compared by the statistical test ANOVA. The aggregates areas in the cell cultures incubated with sulfated polysaccharides from *D. robusta* and *A. fulva* showed no significant differences ($p > 0.05$) with the control ($8.9 \times 10 \mu\text{m}^2$; $11.0 \times 10 \mu\text{m}^2$ and $9.5 \times 10 \mu\text{m}^2$ respectively). However, the addition of sulfated polysaccharide from *H. heliophila* to the culture media produces significantly ($p < 0.05$) smaller aggregation ($1 \text{ mg/ml} = 4.3 \times 10 \mu\text{m}^2$, $2 \text{ mg/ml} = 4.2 \times 10 \mu\text{m}^2$ and $3 \text{ mg/ml} = 2.1 \times 10 \mu\text{m}^2$). Similarly, the cell aggregates area was also reduced by the addition of Heparin ($1 \text{ mg/ml} = 7.2 \times 10 \mu\text{m}^2$, $5 \text{ mg/ml} = 6.0 \times 10 \mu\text{m}^2$ and $10 \text{ mg/ml} = 2.9 \times 10 \mu\text{m}^2$), but only the higher concentration tested (10 mg/ml) showed significantly lower ($p < 0.05$) aggregates than the control. In order to further investigate the affinity between the sulfated polysaccharide of *H. heliophila* and Heparin, we employed an affinity chromatography (HITRAP Heparin) that shows that the *H. heliophila* sulfated polysaccharide interacts with Heparin. Overall, these results indicate that both the homologous sulfated polysaccharide and heparin are able to avoid the sponge cell aggregation. The incapacity of heterologous sulfated polysaccharides to avoid the sponge cell aggregation confirms the species specific interactions between sponge sulfated polysaccharides.

New drug leads and cell biology tools from sponge extracts
(invited lecture)

Raymond J. Andersen{ XE "Andersen, Raymond J." }

Department of Chemistry and Department of Earth and Ocean Sciences, University of British Columbia,
Vancouver, British Columbia, V6T 1Z1 Canada.

The secondary metabolites found in marine invertebrates represent an extremely rich source of novel chemical diversity for academic drug discovery and chemical biology programs. Among the invertebrates, marine sponges continue to be the most prolific source of new marine natural products. Our group at UBC has amassed a sizable library of crude extracts from marine sponges collected in many of the world's oceans. In collaboration with biologists at UBC, this crude extract library has been screened for activity in a variety of cell-based and pure enzyme assays designed to identify promising lead compounds for the development of drugs to treat cancer and inflammation. The cell-based assays invented by our collaborators screen for antimitotic agents, anti invasion/antiangiogenic agents as well as agonists and antagonists of the cannabinoid receptors. Pure enzymes are used to screen for activators/inhibitors of the lipid phosphatase SHIP, PI3 kinase, and inhibitors of hepatitis C, SARS and West Nile viral proteases. Bioassay guided fractionation and spectroscopic analysis is used to identify the structures of compounds active in these assays. Chemical synthesis is used by our group to probe the SAR for new pharmacophores and to provide material for *in vivo* testing in animal models. A number of promising drug leads, including pelorol and the hemiasterlins and cell biology tools, such as the spirastrellolides have been discovered in this program. The lecture will present recent chemical and biological results from our academic drug discovery/chemical biology research involving sponge extracts.

Redox properties of marine sponges collected from the coastline of Santa Catarina, Southern Brazil

Mario Luiz Conte da Frota Junior{ XE "Junior, Mario Luiz Conte da Frota" }⁽¹⁾; Mariana Leivas Muller Hoff{ XE "Hoff, Mariana Leivas Muller" }⁽¹⁾; Guilherme Antônio Behr{ XE "Behr, Guilherme Antônio" }⁽¹⁾; Miriam Anders Apel{ XE "Apel, Miriam Anders" }⁽²⁾; Beatriz Mothes{ XE "Mothes, Beatriz" }⁽³⁾; Cléa Lerner{ XE "Lerner, Cléa" }⁽³⁾; Amélia Teresinha Henriques{ XE "Henriques, Amélia Teresinha" }⁽²⁾; José Cláudio Fonseca Moreira{ XE "Moreira, José Cláudio Fonseca" }⁽¹⁾

(1) Centro de Estudos em Estresse Oxidativo (CEEEO), ICBS - UFRGS, Porto Alegre, Brazil, mlfrotajr@yahoo.com.br; (2) Faculdade de Farmácia, UFRGS, Porto Alegre, Brazil; (3) Fundação Zoobotânica, Museu de Ciências Naturais, Porto Alegre, Brazil

Marine organisms have provided a large proportion of the bioactive natural products reported over the last 20 years. Among the many phyla found in the oceans, the best sources of pharmacologically active compounds are sponges. Reactive oxygen species (ROS) and oxidative stress play an important role in the etiology and progression of major human degenerative diseases. In the search for new bioactive metabolites, relatively few attempts have been made to explore substances that act as antioxidants from sponges. The present study was carried out to evaluate the redox properties of 3 marine sponges collected from the coastline of Santa Catarina, southern Brazil. Aqueous extracts obtained by grinding and maceration for 30 min following freeze-drying, as well as organic solvent extracts (methanol:toluene 3:1 v/v) were prepared from *Polymastia janeirensis*, *Scopalina ruetzleri* and *Haliclona tubifera*. The extracts were concentrated, and subsequently tested at concentrations ranging from 10-100 mg/ml in different systems. Nitric oxide (NO) and hydroxyl radical (\cdot OH) scavenging assays were carried out to evaluate the antioxidant potential of the extracts. As an index of oxidative damage we used the formation of TBARS. Both the aqueous and organic extracts from *P. janeirensis* were able to inhibit \cdot OH production generated by Fenton reaction at higher doses and increase this radical production at lower doses. Furthermore, aqueous extracts from this species inhibited the NO generated from sodium nitroprusside. Interestingly, TBARS was decreased at higher doses of both extracts obtained from *P. janeirensis*. Aqueous extracts from *S. ruetzleri* were able to increase the \cdot OH formation. On the other hand, NO production was decreased. However, the organic extracts diminished these radicals production. TBARS content also was diminished with organic extracts from *S. ruetzleri* and at lower doses of aqueous extracts. For the *H. tubifera*, both aqueous and organic extracts increased \cdot OH production. However, NO production increased only with organic extracts. Moreover, TBARS increased with lower doses, in both aqueous and organic extracts. Our findings have demonstrated the potential of sponges products to act as potent antioxidants or pro oxidants, suggesting that at last in part some of the effects found in the literature may be mediated via ROS. Additional studies are required to understand the exact mechanism by which these extracts works to regulate ROS production, as well as its significance in neoplastic transformation of normal and previously injured cells

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Antifeedant and chemical transformation studies of (S)-(+)-curcuphenol, a sesquiterpene from the marine sponge *Didiscus oxeata*

Helena Gaspar{ XE "Gaspar, Helena" }^(1*); Cristina Moiteiro{ XE "Moiteiro, Cristina" }⁽¹⁾; João Sardinha{ XE "Sardinha, João" }⁽¹⁾; Azucena González-Coloma{ XE "González-Coloma, Azucena" }⁽²⁾; Darío Martín{ XE "Martín, Darío" }⁽²⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽³⁾

(1) INETI- Instituto Nacional de Engenharia, Tecnologia e Inovação I.P., Estrada do Paço do Lumiar, Ed.-F, 1649-038 Lisboa, Portugal, helena.gaspar@ineti.pt; joao.sardinha@gmail.com; cristina.moiteiro@ineti.pt;

(2) Instituto de Ciencias Agrarias-CCMA, CSIC, Serrano 115-dpdo, 2800 Madrid, Spain, azu@ccma.csic.es; (3) Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94766, 1090-GT, The Netherlands, soest@science.uva.nl

The widespread use of synthetic pesticides lead to several environmental problems related with the development of resistance, impact on human health and pollution. The continuous search for more effective and economical methods for controlling a variety of diseases, pest and weeds has turned attention to safe alternatives such as bio-based products derived from natural sources. In the marine environment, sponges (Porifera) are one of the richest sources of biological active secondary metabolites. As part of our general interest in the isolation and characterization of bioactive metabolites from sponges, the sesquiterpene (S)-(+)-curcuphenol was isolated from the Curaçao marine sponge *Didiscus oxeata*. Several authors have studied the biological activities (antimicrobial, anticancer, ichthyotoxic and antifouling) of this metabolite, however there are no reports on the insecticidal activity of (S)-(+)-curcuphenol. The present study is the first report on the insecticidal evaluation of (S)-(+)-curcuphenol and its derivatives. (S)-(+)-Curcuphenol isolated from the sponge methanolic extract was submitted to several chemical transformations (oxygenation and halogenation) producing hemisynthetic sesquiterpenoids. The antifeedant activity of these curcuphenol derivatives and (S)-(+)-curcuphenol was evaluated against several herbivorous insect species (*Spodoptera littoralis*, *Leptinotarsa decemlineata*, *Myzus persicae* and *Ropalosiphum padi*). The results from the biological assays show the insect species behavioral responses vary with structural modifications of the sesquiterpenes.

Chemical investigation of bioactive secondary metabolites of the marine sponges, collected from the Gulf of Thailand

Anake Kijjoa{ XE "Kijjoa, Anake" }^(1*); Júlia Bessa{ XE "Bessa, Júlia" }⁽¹⁾; Paulo Pinho{ XE "Pinho, Paulo" }⁽¹⁾; Rawiwan Watanadilok{ XE "Watanadilok, Rawiwan" }⁽²⁾; Pichai Sonchaeng{ XE "Sonchaeng, Pichai" }⁽³⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽⁴⁾

(1) CIIMAR - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4099-003 Porto, Portugal; (2) Institute of Marine Science, Burapha University, Bangsaen, 20131 Chonburi, Thailand; (3) National Science Museum, Technopolis, Klong Luang, Pathumthai, Thailand; (4) Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, P.O. Box 94766, 1090 GT, Amsterdam, Netherlands

Marine sponges are a rich source of a large number of diverse types of secondary metabolites, many of which possess unique structural features and significant biological properties. In recent years, many secondary metabolites from sponges, which are promising candidates of new drugs, have been discovered. In the past five years, our group has investigated the secondary metabolites from the marine sponges collected from the Gulf of Thailand. Examples of these were *Suberea* aff. *praetensa*, *Pseudoceratina purpurea*, *Tetilla japonica*, *Cliona patera* and *Haliclona cymaeformis*. From these sponges, some interesting novel secondary metabolites have been isolated. Among these, new bromotyrosine derivatives have been isolated from *Suberea* aff. *praetensa* and *Pseudoceratina purpurea*. While 11,17-dideoxyagelarin A and B and subereatensin were new metabolites isolated from *Suberea* aff. *praetensa*, purpuroceratic acids A and B were isolated for the first time from *Pseudoceratina purpurea*. It is interesting to mention also that the NMR spectroscopic method alone was not able to establish conclusively the structure of the two unusual hydroxypyran-2-ones, novel metabolites isolated from *Tetilla japonica*. As these compounds are in equilibrium in the solution, their structures were completely elucidated by the aid of X-ray crystallography. The compounds isolated from these marine sponges were evaluated for their *in vitro* antitumor activity against three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) by sulforhodamine B (SRB) assay. Some of these compounds were found to show moderate antitumor activity. Besides, we have recently identified the homologous series of monoalkyl ethers of glycerol from the extract of *Cliona patera*.

Inhibitors of *Leishmania tarentolae* adenosine phosphoribosyl transferase from marine sponges

Simone Possedente de Lira{ XE "Lira, Simone Possedente de" }⁽¹⁾; Chris A. Gray{ XE "Gray, Chris A." }⁽²⁾; Marcio Silva{ XE "Silva, Marcio" }⁽³⁾; Eli Pimenta{ XE "Pimenta, Eli" }^(1, 3); Otavio Thiemann{ XE "Thiemann, Otavio" }⁽³⁾; Glaucius Oliva{ XE "Oliva, Glaucius" }⁽³⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽⁴⁾; Raymond J. Andersen{ XE "Andersen, Raymond J." }⁽²⁾; Roberto Gomes de Souza Berlinck{ XE "Berlinck, Roberto Gomes de Souza" }^(1*)

(1) Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil, rgsberlinck@iqsc.usp.br; (2) Department of Chemistry and Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, Canada; (3) Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil; (4) Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Leishmaniasis is a public health problem in developing countries in Latin America, Africa and Asia, specially when it is associated with HIV infection. Currently only synthetic antimonials are used as the treatment of leishmaniasis, a highly toxic therapy of low efficacy. Therefore, there is a need of new leads for the development of new anti-leishmaniasis agents. In the present work we have screened 500 extracts of marine invertebrates in an enzyme-based assay using *Leishmania tarentolae* adenosine phosphoribosyl transferase (L-APRT) as a substrate. Only 8 extracts were active, the large majority obtained from marine sponges. Further bioassay-guided fractionation of the active extracts led to the isolation of sulfated sterols from *Petromica cyocaliptoides*, polar long-chain derivatives from *Ptilocaulis walpersi* and of sulfated meroterpenes from *Callyspongia* sp. These are the first marine natural products active against L-APRT, some with very potent inhibitory activity (in the nM range). Previously only plant-derived natural products were known as moderately active L-APRT inhibitors.

Antileishmanial epidioxysterols from isolated sterols of the Colombian marine sponge *Ircinia campana*

Diana Márquez{ XE "Márquez, Diana" }⁽¹⁾; Sara Robledo{ XE "Robledo, Sara" }⁽²⁾; Alejandro Martínez{ XE "Martínez, Alejandro" }^(1*)

(1) Departamento de Farmacia, Universidad de Antioquia, Calle 67 53-108 Bloque 2 Laboratorio 131, Medellín, Apartado Aéreo 1226, Colombia, dmarquez@farmacia.udea.edu.co; amart@farmacia.udea.edu.co; (2) Sede de Investigaciones Universitaria, Universidad de Antioquia, Medellín, Apartado Aéreo 1226, Colombia

Sterols with delta-5, 7-3-hidroxyandrostadiene nuclei were extracted from the Colombian marine sponge *Ircinia campana*. The sterol fraction was oxidized under controlled conditions of light and air for producing 5-alfa,8-alfa-epidioxysterols, as artifacts of oxidation, that were identified by GC/MS. Nine compounds were identified: 5-alfa,8-alfa-epidiox-24-norcholesta-6,22-dien-3β-ol (1); 5-alfa,8-alfa-epidiox-cholesta-6,22-dien-3β-ol (2); 5-alfa,8-alfa-epidioxi-(24)-methylcholesta-6-en-3-β-ol (epimer 1) (3); 5-alfa, 8-alfa-epidiox-cholesta-6-en-3-β-ol (4); 5-alfa,8-alfa-epidiox-(24)-methylcholesta-6-en-3-β-ol (epimer 2) (5); 5-alfa,8-alfa-epidiox-(24)-ethylcholesta-6, 22-dien-3β-ol (6); 5-alfa,8-alfa-epidiox-cholesta-6,9-dien-3β-ol (7); 5-alfa, 8-alfa-epidiox-cholesta-6,9,22-trien-3β-ol (8); and a compound 5-alfa,8-alfa-epidioxsterol with molecular formule C₂₇H₄₂O₄. This fraction showed antileishmanial activity against amastigotes of *Leishmania (V) panamensis*.

Chemical variability within the sponge *Aplysina fulva*

Fabio Renato Pereira{ XE "Pereira, Fabio Renato" }; Suzi de Oliveira Marques{ XE "Marques, Suzi de Oliveira" }; Andréa Mendes do Nascimento{ XE "Nascimento, Andréa Mendes do" }; Kelly Oliveira Santos{ XE "Santos, Kelly Oliveira" }; Érika Virgínia Raphael de Almeida{ XE "Almeida, Érika Virgínia Raphael de" }; Cecília Verónica Nuñez{ XE "Nuñez, Cecília Verónica" }; Roberto Gomes de Souza Berlinck{ XE "Berlinck, Roberto Gomes de Souza" }^(*)

Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil,
rgsberlinck@iqsc.usp.br

Marine sponges belonging to the order Verongida are a rich source of bromotyrosine-derived compounds. Remarkably, a first chemical survey on the Verongid sponge *Aplysina fulva* collected in Brazil carried out in 1979 did not provide any brominated compound, but only sterols. Such an unexpected result prompted us to re-investigate the chemistry of Brazilian specimens of *A. fulva* collected in different regions. Specimens of *A. fulva* were collected at five different sites: São Sebastião (São Paulo state), Angra dos Reis (Rio de Janeiro state), Arraial do Cabo (Rio de Janeiro state), Baía de Todos os Santos (Bahia state) and Mangue de Jiribatuba (Bahia state). All specimens were independently investigated and afforded bromotyrosine derivatives. However, only two compounds were common to the Baía de Todos os Santos and to Arraial do Cabo specimens. The other isolated and identified compounds were different and did not co-occur in distinct specimens. Only the Mangue de Jiribatuba specimen yielded the high molecular weight fistularin-3 derivatives, presenting 3 tyrosine biogenetic precursors. The São Sebastião specimen yielded the medium molecular weight compound aerotionin, which is presumably derived from two tyrosine precursors. The other isolated bromotyrosine-derived compounds were of low molecular weight, presenting only one residue of tyrosine as precursor. Many reasons can explain the chemical variability of Brazilian specimens of *A. fulva*, such as nutrient availability, water temperatures, the co-occurrence of distinct associated microorganisms and the presence of pollutants in sea water.

Bioactive secondary metabolites from *Hippospongia communis* and *Ircinia variabilis*, collected from the Atlantic coast of Morocco

Paulo Pinho{ XE "Pinho, Paulo" }^(1*); Anake Kijjoa{ XE "Kijjoa, Anake" }⁽¹⁾; Júlia Bessa{ XE "Bessa, Júlia" }⁽¹⁾; Saida Rifai{ XE "Rifai, Saida" }⁽²⁾; Aziz Fassouane{ XE "Fassouane, Aziz" }⁽²⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽³⁾

(1) CIIMAR - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4099-003 Porto, Portugal; (2) Faculté des Sciences, Université Chouaib Doukkali, El- Jadida, Morocco; (3) Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, P.O. Box 94766, 1090 GT, Amsterdam, Netherlands

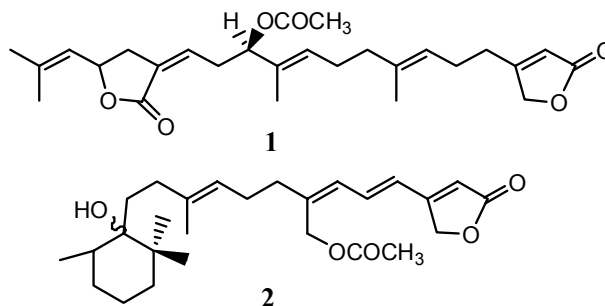
In the marine environment, sponges are one of the richest sources of both biologically active secondary metabolites and chemical diversity. These natural products may play a role in warding off predators, and perhaps they also repel fouling organisms. During our continuing studies on bioactive secondary metabolites from the marine organisms of the Atlantic Coast of Morocco, (-)-untenospongin B has been isolated from the antifungal extract of the marine sponge *Hippospongia communis*, collected from the Atlantic coast of El-Jadida, Morocco. (-)-Untenospongin B was evaluated for its antimicrobial activity against three bacteria, three yeasts and five filamentous fungi. It was found to exhibit antibacterial activity against *Escherichia coli*, *Bacillus subtilis* as well as against the ichthyopathogenic strain *Vibrio anguillarum*. It also showed antifungal activity against *Candida albicans*, *Aspergillus fumigatus* and other fungi. Interestingly, (-)-untenospongin B was found to be even more active than amphotericin B in inhibiting the growth of *Candida tropicalis* and *Fusarium oxysporum*. On the other hand, fasciculatin, a furanosesterterpene isolated from *Ircinia variabilis*, was found to show a moderate cytotoxicity against the three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) but no effect on the mitogenic response of human lymphocytes to phytohemagglutinin.

Two new luffarin derivatives from the sponge *Fasciospongia cavernosa*

Salvatore De Rosa{ XE "Rosa, Salvatore De" }; Sabina Carbonelli{ XE "Carbonelli, Sabina" }

Istituto di Chimica Biomolecolare del C.N.R., via Campi Flegrei, 34; I-80078 Pozzuoli (Napoli) Italy

Marine sponges have provided a large number of sesterterpenoids, possessing novel carbon skeletons different from those present in terrestrial species. Several sesterterpenoids isolated from marine organisms have shown a wide variety of biological activities. In particular, marine sponges belonging to the family Thorectidae, which includes the genus *Luffariella* and *Fasciospongia* are known to be a rich source of new bioactive terpenoids. Many of these compounds show a γ -hydroxybutenolide portion in molecule, such as manoalide, the first sesterterpene isolated from a *Luffariella* sp., or cacospongionolide and its related compounds isolated from several sample of *Fasciospongia cavernosa*. These compounds in addition to demonstrating antibiotic and anti-inflammatory activity are potent inhibitor of phospholipase A2. In the course of our search for marine natural compounds that have biological activities, we isolated and characterized several sesterterpenoids from the Mediterranean horny sponge *F. cavernosa* Schmidt (family Thorectidae). From a specimen of *F. cavernosa* collected in the North Adriatic Sea, together with luffarin V and its isomer (6Z)-luffarin V we isolated two new related sesterterpenes (1-2).



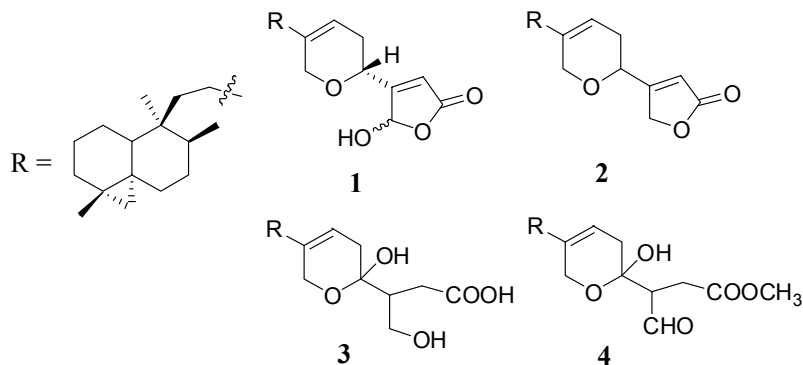
In this communication will be reported the isolation, the structure elucidation and biological activity of the new compounds. The structures of compounds were proposed on the basis of spectroscopic data and chemical transformations. The absolute configuration at C-9 of 9-acetoxy-(6Z)-luffarin V (1) was established using the modified Mosher's method on its deacetyl derivative.

Minor cacospongionolide derivatives from the sponge *Fasciospongia cavernosa*

Salvatore De Rosa{ XE "Rosa, Salvatore De" }; Carmine Iodice{ XE "Iodice, Carmine" }; Sabina Carbonelli{ XE "Carbonelli, Sabina" }

Istituto di Chimica Biomolecolare del C.N.R., via Campi Flegrei, 34; I-80078 Pozzuoli (Napoli) Italy

The Mediterranean sponge *Fasciospongia cavernosa* is a rich source of a new class of sesterterpenoids, named cacospongionolides. Cacospongionolide (1) was the first sesterterpene isolated from the Adriatic Sea sponge *F. cavernosa*, which possesses antimicrobial and antitumoral activity. Subsequently, several related compounds were isolated from the several specimens of *F. cavernosa* collected in the Mediterranean Sea. This class of marine metabolites is inhibitor of phospholipase A2 with potent topical anti-inflammatory profile and they showed high antimicrobial activity against the Gram-positive bacteria *Bacillus subtilis* and *Micrococcus luteus*. Our group has investigated the chemistry of a number of specimens of *F. cavernosa* Schmidt (family Thorectidae) collected in the Mediterranean Sea, to provide sufficient amount of cacospongionolides for exhaustive pharmacological evaluation. From a sample of this sponge collected in the northern Adriatic Sea, together with cacospongionolide we isolated three new related sesterterpenes (2-4).



In this communication will be reported the isolation and the structure elucidation of the new compounds. The structures of compounds were elucidated by spectral data, including 2D-NMR spectroscopy. The isolation of several related constituents from individual specimens of *F. cavernosa* confirms the peculiarity of the sponges belonging to the family Thorectidae.

The sponge biodiversity of the 'Martinique' as a source of bioactive secondary metabolites

Olivier P. Thomas{ XE "Thomas, Olivier P." }^(*); Fabrice Berrue{ XE "Berrue, Fabrice" }; Remi Laville{ XE "Laville, Remi" }; Philippe Amade{ XE "Amade, Philippe" }

Marine Natural Product Chemistry, LCMBA UMR 6001 CNRS, Université de Nice, Faculté des Sciences,
Parc Valrose 06108 NICE Cedex 2, France, olivier.thomas@unice.fr

As part of the French Antilles in the Caribbean, with both influences of the Caribbean sea and the Atlantic ocean, the marine biodiversity of the Martinique Island is known to be extremely rich. However only few studies have been reported to date. To evaluate the bio- and chemiodiversity of the marine invertebrates of this area we organized samplings during an expedition in 2002. We will present the results concerning all the organisms selected and we will focus our attention on three species of sponges very common in this region: *Monanchora unguifera*, *Pachychalina* sp., *Plakortis zyggompha*. These very little studied sponges allowed us to characterize new alkaloids of the batzelladine, crambescidine, and bispyridinium families as well as new original polyketides. Biogenetic hypotheses for these metabolites will be discussed together with their antitumoral and antiinfective activities.

Biostratigraphic and paleogeographic distribution of the fossil registers of sponge and sponge spicules in Argentina, South America
(invited lecture)

Matilde Sylvia Beresi{ XE "Beresi, Matilde Sylvia" }

Cricyt-IANIGLA; A. Ruiz Leal s/n (5500) Mendoza, Argentina, mberesi@lab.cricyt.edu.ar;
www.cricyt.edu.ar/institutos/ianigla

Fossil sponges and sponge spicules have been reported from several regions in Argentina and in strata ranging in age from early Cambrian to Tertiary. Sponges and spicule assemblages have been collected from marine sediments of the Puna, Cordillera Oriental and Sierras Subandinas basins, northern Argentina; Famatina Range; Precordillera terrane, San Rafael block, Neuquén basin and from lacustrine deposits of the Chubut River valley. Knowledge of the sponge fossil record is based on whole relatively rigid skeletons, fragments of skeletal nets and spicules seen in thin sections or recovered from acetic acid-etched residues. Cambrian Porifera and sclerite of chancelloriids are known from shallow carbonate platform facies (upper-Lower to Middle Cambrian) and slope olistostromic facies (allochthonous blocks with Cambrian fossils) in the Precordillera. The autochthonous assemblage from the platform of the San Juan Precordillera consists of sclerites of *Chancelloria* and *Kiwetinokia*. The allochthonous assemblage recovered from carbonate olistoliths consists of hexactines, monaxons and specimens with body preservation of *Diagoniella*? and *Kiwetinokia*. Demosponges have a very limited record in the Cambrian of the Precordillera. Fragment of anthaspidellid sponges had been reported from the platform of San Juan and the carbonate olistoliths of the Mendoza Precordillera. Remains of sponges have been registered in Ordovician rocks of the Puna region and of the Famatina System, western margin of Gondwana. A single specimen of a hexactinellid sponge (Pelicaspongiidae) was found in the Tremadocian volcanoclastic sequence of the Puna. Recently *Protospongia* sp. and hexactinellid mesh were reported for the first time from Upper Cambrian-Lower Ordovician siliciclastic sediments in the Cordillera Oriental and Sierras Subandinas. Fragments of reticulated net (*Protospongia*) also occur in black shales of Lower Tremadocian age in the Famatina System. The most significant Ordovician sponge faunas known from South America are from the carbonate platform (Arenig-Lower Llanvirn) of the San Juan Precordillera and provide an impressive fossil record. Sponge faunas are dominated by orchoclad lithistid demosponge genera: *Archaeoscyphia*, *Rhopalocoelia*, *Calycocoelia*, *Hudsonospongia*, *Aulocopium*, *Patellispongia*, *Anthaspidella*, *Psarodictyum*, *Talacastonia*, and *Protachilleum kayseri*; the megamorinid *Rugospongia*, and the heteractinid *Chilcaia*, among other genera. On this platform, reef-mound structures are characterized by the *Archaeoscyphia-Calathium* association and silicified spicule assemblages of the genera *Dodecaactinella*, *Sardospongia*, *Praephobetractinia* and *Eiffelia*. Hexactinellids and non-lithistid demospongiid triaene and oxeas were described from the Llanvirnian of the Ponón Trehué sequence, San Rafael Block, Mendoza Province. A Late Jurassic carbonate complex was developed on the foreland side of the Neuquén Basin, west-central Argentina. Oxfordian buildup facies contain siliceous sponges with dominance of hexactinellids (*Hexactinosa* and *Lyssakinosa*). *Palaeospongilla chubutensis*, a fresh water sponge, was described from lacustrine Cretaceous deposits of the Chubut River valley. Oxeas and strongyles, belonging to the Family Spongillidae, have been mentioned from Tertiary sediments in the northeastern basin of Argentina.

**On the palaeobiology of two distinctive sponge faunas from the Chatham Islands,
New Zealand**

John S. Buckeridge{ XE "Buckeridge, John S." }⁽¹⁾; Michelle Kelly{ XE "Kelly, Michelle" }^(2*)

(1) Earth and Oceanic Sciences, RMIT University, GPO Box 2476V, Melbourne, VIC 3001, Australia, john.buckeridge@rmit.edu.au; (2) National Centre for Aquatic Biodiversity and Biosecurity (NCABB), National Institute of Water & Atmospheric Research Ltd, Private Bag 109-695, Newmarket, Auckland, New Zealand, m.kelly@niwa.co.nz

Two horizons with remarkably well-preserved sponge body fossils occur in late Palaeocene-early Eocene lithologies of Chatham Island, located some 850 kilometres east of mainland New Zealand. Although both deposits are dominated by hexactinellid, and what are thought to be lithistid sponges, they represent two very different palaeoenvironments. The first, the Tutuiri Greensand, outcrops on the northern coast of the island. In this, the most abundant macrofossils are siliceous poriferans (hexactinellid sponges), although partial opalisation has occurred in some spicules. The Tutuiri Greensand was deposited in mid-shelf conditions during a period of relative geological quiescence, such that very delicate dictyonal frameworks remain, including what have been interpreted as Weltner bodies (mini-sponges) in some frameworks. In contrast, the second more southern lithology, the Red Bluff Tuff, has a very diverse macrofauna, including corals, barnacles, molluscs, vertebrates and bryozoans. The Red Bluff Tuff sponge fossils, thought to be lithistid sponges, are now solid calcite, with the former siliceous skeleton being replaced by calcite during diagenesis. This paper compares the nature of the two lithologies, and uses this to provide a window on the early Tertiary palaeoenvironment of what is now the Southern Pacific.

Sponge spicules in recovered sediments allow for the distinction of 26 kaBP lentic and lotic paleoenvironments at the Paraná basin - Brazil

Mauro Parolin{ XE "Parolin, Mauro" }⁽¹⁾; Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }⁽²⁾; José Cândido Stevaux{ XE "Stevaux, José Cândido" }⁽³⁾

(1) Departamento de Geografia, Faculdade Estadual de Ciências e Letras de Campo Mourão, Av. Comendador Norberto Marcondes, 733. Centro 87300-000 - Campo Mourão, PR. Brazil; (2) Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul. Caixa Postal 1188, 90001-970. Porto Alegre, RS, Brazil, cvolkmer@fzb.rs.gov.br; (3) Laboratório de Geociências, Universidade Guarulhos, Praça Tereza Cristina, 58. Centro 07024070. Guarulhos, SP. Brazil

Four geomorphological units (Porto Rico, Paraná River, Boa Vista Farm and Taquarassu), are recognized at the Porto Rico region (Paraná state/Brazil). The Taquarassu unit is notorious for its large number of lakes with a genesis proposed as stemming from karstic or from a paleo drainage system. The analysis of the Vibro-coring recovered sediments of two such lakes (Samambaia: 22°36'S/53°18'W and Dos-32: 22°27'S/53°13') were TL dated from 4.36 to 32.74 kyBP (5% of mistake considered). For detection of sponge spicules small portions of the differently colored and grained sediments were prepared for microscopic examination and photographing, after nitric acid digestion procedures. Rare to abundant spicules were present as several layers allowing for the taxonomic determination of past assemblies and definition of true spongofacies. At Samambaia Lake gemoscleres of *Dosilia pydanieli*, megascleres of *Radiospongilla amazonensis* and beta megascleres of *Metania spinata* make up an spongofacies indicative of a lentic environmen, whilst megascleres of *Corvospongila sekti* and *Oncosclera petricola* point to a lotic environment, thus confirming the existence of a previous lotic intermitent drainage at this area from upper Pleistocene (around 26 kaBP) up to middle Holocene. During middle Holocene (4,36 KaBP) a large amount of gemoscleres of *D. pydanieli* indicate a period drier than the present one. At the top of this column megascleres of *Metania spinata* and of *Radiospongilla amazonensis* and absence of gemmoscleres are indicative of a wetter period. The reported evidences confirm previous paleoclimatic sequences proposed for this region. At lake Dos-32 from base to top the spicule sequence started with gemmoscleres of *Heterorotula fistula* between 10.2 and 22.2 kyBP, followed by an spongofacies with predominance of gemoscleres and microscleres of *Dosilia pydanieli* between 10.2 and 18.7 kyBP and closed with a top sequence of gemmoscleres of *Dosilia pydanieli* and beta megascleres of *Metania spinata*. This kind of sponge sequence is characteristic of savanna ("cerrado") lakes indicating that the origin of this lake has not been affected by contributions of a previous lotic drainage system and dating its initial stages to the end of the Pleistocene. The results come to emphasize sponge spicules in sediments as an important proxy tool in paleoclimatic and paleohydrologic studies.

Late Eocene fauna of lithistid and soft demosponges from SW Australia: A case of exceptional and highly diversified endemic fauna
(invited lecture)

Andrzej Pisera{ XE "Pisera, Andrzej" }

Institute of Paleobiology, Polish Academy of Sciences, ul. Twarda 51/55, 02-818 Warszawa, Poland,
apis@twarda.pan.pl

Late Eocene sponge rich sediments in SW Australia extend over the distance of 2000 km along its southern edge. These are spiculitic rocks (often silicified), described as the Pallinup Formation, which are locally extremely rich in exceptionally preserved and highly diverse fauna of lithistid sponges, as well as soft demosponges. Some outcrops of these sediments in the Bremmer Basin delivered the most extraordinarily preserved sponges, which have most of the spicules still in opaline form, ectosomal spicules in place, in case of lithistids, and numerous examples of sublithistids and soft demosponges preserved intact. Theonellida, which consists of at least 10 species, dominates the lithistid fauna. Pleromids are very common but only 2-3 species are recognised. Rhizomarine lithistids are less rich in specimens, but rather diversified and have at least 8 species. Corallistidae, which are also common, have at least 5 species. Phymaraphinida, which equals theonellids in specimens number, has only 2 or 3 species. Phymatellidae is also common but only 5 species have been recognised. Among sublithistid/soft demosponges at least 7 species have been recognised: 3 species of pachastrellids, one species of a probable axinellid and at least 3 species of other demosponges with loose spicules. This fauna is also exceptional because it corresponds in composition to that from deep water Norfolk Ridge but all geological data suggested that it inhabited very shallow, at most 20 m deep environment close to shore. At the species level this fauna is highly endemic, with no other Tertiary analogues, but at the genus and family level it is intermediate between Cretaceous fauna of Europe and the Recent New Caledonia fauna. The Eocene Oamaru fauna from New Zealand also contains undoubted theonellid and pleromid spicules. The Oamaru fauna differs evidently in having important hexactinellid component, including apmhidiscophoran spicules. This sponge fauna is difficult to compare in details, because it consists exclusively of loose spicules.

Evolution of sponge fauna of Lake Baikal from late Miocene to recent times according to micropaleontological results

Elena V. Weinberg{ XE "Weinberg, Elena V." }⁽¹⁾; Carsten Eckert{ XE "Eckert, Carsten" }^(2*)

(1) Wiciefstr. 17, D-10551 Berlin, Germany; (2) Museum für Naturkunde, Zentralinstitut der Humboldt-Universität zu Berlin, Institut für Systematische Zoologie, Invalidenstrasse 43, D-10115 Berlin, Germany, carsten.eckert@museum.hu-berlin.de

Baikalian endemic sponge fauna (family Lubomirskiidae) is very unusual and unique. Long time between scientists there are opinions about its ocean origin. According to Martinson's paleontological research in South-East Siberia and Lake Baikal area was showed that ocean ancestors did not took part in formation of Baikalian fauna. Embryological and ontogenetic investigations of Lubomirskiidae shown that they are very close to the cosmopolitan family Spongillidae. Recent molecular investigations confirmed this relationship between two freshwater families and calculated the age of divergence of Lubomirskiidae from Spongillidae by using molecular clock for 3.5 million years. By our micropaleontological studies of sponge spicules from bottom sediments recovered by drilling in the Central area of Lake Baikal (BDP98, 601m core, 10 million years BP) was shown that in the Late Miocene (10 million years BP) exist spicules of all the recent sponges genera, which inhabit Lake Baikal today (*Baikalospongia*, *Lubomirskia*, *Swartschewskia*, *Rezinkovia*). Furthermore, according to Martinson spicules endemic of Baikalian sponges are documented from Oligocene-Miocene strata of the extinct lake basin, thus the roots of Lubomirskiidae date back at least 24 million years. The spicule analysis in Late Pliocene sediments (BDP96, 200m core, 5 million years BP) shows that the species composition of sponge fauna in the period of 3.2 - 2.8 million years BP was considerably different from the modern one. In this time interval we found spicules of all recent sponges species, which inhabit Lake Baikal today and alongside with them there were found 24 spicule types without morphological analogous to recent species. Spicules of the cosmopolitan family of Spongillidae, as well as spicules of extinct species of the family of Lubomirskiidae, were abundant. Furthermore it has been clearly shown that sponges reacted to the fluctuations of climate and hydrology with variations of their quantitative and taxonomic composition. The most considerable change have been recorded between 2.5 - 2.1 Ma. In this period, the intensification of the Northern Hemisphere Glaciation (NHG) and a change of tectonic stage in Lake Baikal area took place. Spicules of spongillids and the extinct species of Lubomirskiidae disappear in this time. Later, the composition of the spongal fauna became closer to that of present day, but it never reached the abundance like in Late Pliocene. In Holocene sediments appear two new types of spicules which belong to recent subspecies of family Lubomirskiidae. This give us a supposition for the ongoing process of speciation in the endemic family of Baikalian sponges.

**Characterization of homeobox genes related to bilaterian Hox and Para-Hox genes
in the Homoscleromorpha sponges**
(invited lecture)

Carole Borchellini{ XE "Borchellini, Carole" }; Emmanuelle Deniel-Renard{ XE "Deniel-Renard, Emmanuelle" }; Nicole Boury-Esnault{ XE "Boury-Esnault, Nicole" }

UMR 6540 "Dimar", Centre d'Océanologie de Marseille, Rue de la Batterie des Lions, 13007 Marseille, France, borchellini@com.univ-mrs.fr; esnault@com.univ-mrs.fr

The major concept of evolutionary developmental genetics is that the main genetic pathways controlling the development are conserved among the Bilateria. For example, it had been shown that all triploblastic animals share a common set of genes controlling the antero-posterior patterning during the development, called the Hox genes. Because their phylogenetic position at the basis of the metazoan tree, non bilaterian animals are key taxa to understand evolutionary origin of genetic mechanisms known to control the development in Bilateria. We have focused attention on Porifera and particularly on Homoscleromorpha. The phylogenetic status of the sponges has long been a controversial question, but recent works proposed grouping them among the Metazoa as early paraphyletic lineages, leading the hypothesis that the Homoscleromorpha could be sister-group of Eumetazoa. This phylogenetic hypothesis associated with clearly polarized body plan and morphological differentiation between basal and apical region observed in this taxon suggest that homologous genes are involved in the control of polarity between Homoscleromorpha and Eumetazoa. Using degenerated PCR primers for homeobox genes of the antennapedia class, we cloned and sequenced homeoboxes presenting sequence similarity with bilaterian developmental genes involved in regional developmental specification, notably with genes of the bilaterian Hox and ParaHox cluster. These preliminary results, if confirmed, let us expect this taxon to become a leading model for the understanding of the early evolution of developmental mechanisms in Metazoa.

Molecular phylogeny of the Geodiidae Gray, 1867 (Porifera, Demospongiae) with a reassessment of the NE Atlantic species

Paco Cardenas{ XE "Cardenas, Paco" }⁽¹⁾; Hans Tore Rapp{ XE "Rapp, Hans Tore" }^(1*);
Christoffer Schander{ XE "Schander, Christoffer" }⁽¹⁾; Ole Secher Tendal{ XE "Tendal, Ole
Secher" }⁽²⁾

(1) Department of Biology, University of Bergen, Bergen High-Technology Center, PO Box 7800, N-5020 Bergen, Norway, cardenas_paco@yahoo.fr; hans.rapp@bio.uib.no; christoffer.schander@bio.uib.no; (2) Zoological Museum, University of Copenhagen, Universitetsparken 15, DK - 2100 Copenhagen, Denmark, ostendal@snm.ku.dk

The Geodiidae (Porifera: Demospongiae, Astrophorida) encompass around 220 species widely distributed in all oceans, both geographically and bathymetrically. In tropical and parts of temperate waters representatives are common at quite shallow depths, while in boreal/antiboreal and Arctic/Antarctic waters they are normally deep-water species. They often dominate in terms of abundance and biomass in gravely hard-bottom habitats on the outer shelf and upper slope, and over time they may accumulate into mass occurrences over large areas. The evolutionary history of the Geodiidae is not fully resolved and recent molecular phylogenetic studies have suggested their paraphyly or even their polyphyly. The small number of Geodiidae species in the NE Atlantic is an opportunity to test those previous studies. Fourteen species live in the boreal and arctic waters of the Atlantic, they belong to five of the six valid genera: *Geodia*, *Sidonops*, *Isops*, *Pachymatisma* and *Erylus*. Here we present a phylogenetic analysis of the relationships within the Geodiidae based on the mitochondrial cytochrome oxidase subunit I (COI) gene. The main goal of this study is to test the 1) monophyly of the Geodiidae, 2) the Geodinae/Erylinae subdivision (Sollas 1888) and 3) the monophyly of the *Geodia-Isops* complex (since *Isops* and *Sidonops* are considered by some authors as synonyms of *Geodia*). In order to test the monophyly of the Geodiidae, we have broadened our sample with other NE Atlantic Astrophorida belonging to the Ancoronidae and Pachastrellidae. Preliminary results suggest that 1) the Geodiidae is monophyletic, 2) the subfamilies Geodinae/Erylinae do not appear as Sollas (1888) defined them and 3) the *Geodia-Isops* complex is not monophyletic. Furthermore, it also appears that the Ancoronidae and the Pachastrellidae are paraphyletic. A taxonomic revision of some of the NE Atlantic species of the Geodiidae is also proposed based on molecular data.

Phylogeny of Hexactinellida

Martin Dohrmann{ XE "Dohrmann, Martin" }⁽¹⁾; Dorte Janussen{ XE "Janussen, Dorte" }⁽²⁾;
Joachim Reitner{ XE "Reitner, Joachim" }⁽¹⁾; Gert Wörheide{ XE "Wörheide, Gert" }^(1*)

(1) Dept. of Geobiology, Geoscience Centre Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany, mdohrmal@gwdg.de; gert.woerheide@geo.uni-goettingen.de; (2) Marine Evertibraten I, Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany, Dorte.Janussen@senckenberg.de

Hexactinellida are an important component of the sessile benthos and provide a major fraction of the biomass, especially in deeper waters. They are known from the late Precambrian and are therefore amongst the oldest known metazoans. It is nowadays commonly hypothesized that Hexactinellida are the sister-group to Demospongiae sensu stricto, which is primarily supported by the shared intracellular formation of siliceous spicules along a protein filament, rDNA sequence data as well as the shared possession of demosponginic acids, a siliceous sponge-specific biomarker. A strict phylogenetic system of Hexactinellida has only been proposed once (by one of us, D.J., based on spicule morphology) and remains problematic due to homoplastic or uninformative characters. Further cladistic analyses of morphological character data as well as molecular studies to resolve internal relationships of Hexactinellida had not been conducted so far. Here, we present the first estimate of a hexactinellid phylogeny based on Bayesian analysis of full-length 18S rDNA sequences, taking into account rDNA secondary structure models for evolutionary model selection. Our results so far support some of the classically recognized higher hexactinellid subtaxa as monophyletic, but challenge the monophyly of many other. Further sequencing efforts with respect to taxonomic sampling and inclusion of additional, independent phylogenetic markers as well as morphological character analysis and incorporation of data from the fossil record will allow for a much better understanding of the evolution of hexactinellid sponges through the Earth's history.

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Evolutionary patterns of nucleotide composition in demosponge rRNA

Dirk Erpenbeck{ XE "Erpenbeck, Dirk" }^(1*, 3); Scott A. Nichols{ XE "Nichols, Scott A." }⁽²⁾;
John N.A. Hooper{ XE "Hooper, John N.A." }⁽³⁾; Gert Wörheide{ XE "Wörheide, Gert" }⁽¹⁾

(1) Department of Geobiology, Geoscience Centre Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany, derpenb@gwdg.de; gert.woerheide@geo.uni-goettingen.de; (2) Department of Molecular & Cell Biology, University of California, 142 Life Sciences Addition #3200, Berkeley, CA 94720, USA, nichols1@berkeley.edu; (3) Biodiversity Program, Queensland Museum, PO Box 3300, 4101 South Brisbane, Queensland, Australia, john.hooper@qm.qld.gov.au

The ribosome is the location of protein translation and therefore a pivotal molecule complex for all organisms. The rRNA molecules involved in the formation of the ribosome are partially single stranded and partially form helices by binding with complementary regions of either themselves or other subunits. This folding pattern provides the RNA with a secondary structure crucial for its functionality. The stability of those structures is mediated by the base compositions: A helix rich of G-C pairs possesses a higher thermodynamic stability than its A-T rich counterpart. However, the base composition of those structures is neither homogeneous throughout the molecule nor throughout the demosponge taxa. Here, we present patterns of biased nucleotide composition in demosponge 28rDNA. We analyse their correlation in respect to biogeographic zonation and water temperatures. Furthermore, we identify taxonomic patterns of the base composition and discuss their effects on phylogenetic reconstructions.

Demosponge phylogenies analysed under secondary structure-based substitution models

Dirk Erpenbeck{ XE "Erpenbeck, Dirk" }^(1*, 2); Scott A. Nichols{ XE "Nichols, Scott A." }⁽³⁾;
Oliver Voigt{ XE "Voigt, Oliver" }⁽¹⁾; Martin Dohrmann{ XE "Dohrmann, Martin" }⁽¹⁾; John
N.A. Hooper{ XE "Hooper, John N.A." }⁽²⁾; Gert Wörheide{ XE "Wörheide, Gert" }⁽¹⁾

(1) Department of Geobiology, Geoscience Centre Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany, derpenb@gwdg.de; ovoigt@gwdg.de; mdohrma1@gwdg.de; gert.woerheide@geo.uni-goettingen.de; (2) Biodiversity Program, Queensland Museum, PO Box 3300, 4101 South Brisbane, Queensland, Australia, john.hooper@qm.qld.gov.au; (3) Department of Molecular & Cell Biology, University of California, 142 Life Sciences Addition #3200, Berkeley, CA 94720, USA, nichols1@berkeley.edu

The number of sponge molecular phylogenies steadily increases. While more and more DNA fragments different from nuclear ribosomal genes are sequenced for phylogenetic reconstructions, still mostly 18S rDNA and 28S rDNA are employed for basal metazoan phylogeny and demosponge evolution. Nevertheless, many sponge rDNA phylogenies result in trees which are incongruent to either alternative gene trees or morphological expectations. One reason might be the suboptimal application of substitution models in phylogenetic analyses. While standard models describe the evolution of independently evolving characters fairly well, they are not suitable for sites coding for non-independent characters. Such non-independent sites are (among others) rRNA strands that form a RNA-helix in the ribosome by pairing with a second strand. Those sites require the use of models, which take the evolution of the complete nucleotide pair (=doublet) into account. We analysed demosponge 28S rDNA phylogenies under doublet models for pairing sites and compared our findings with the results of traditional approaches. We demonstrate that paired and unpaired sites of the same gene harbour different phylogenetic signals and that the usage of doublet models results in statistically more likely demosponge trees. We show the influence of more sophisticated models on the assumed phylogenetic relationships of demosponge orders.

Molecular phylogeny of the family Clionaidae (Demospongiae: Hadromerida)

Yuji Ise{ XE "Ise, Yuji" }^(1*,2); Toshihiko Fujita{ XE "Fujita, Toshihiko" }^(1,2)

(1) Department of Zoology, National Science Museum, 3-23-1, Hyakunin-cho, Shinjuku-ku, 169-0073, Tokyo, Japan, ug@kahaku.go.jp; (2) Department of Biological Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, 118-8654, Tokyo, Japan

Family Clionaidae d'Orbigny, 1851 (Demospongiae: Hadromerida) is the most diverse taxon both in number of species and ecological characters among limestone excavating sponges. However, systematics of clionaid sponges is still largely confused. To resolve it, we examined the phylogenetic relationships among species of Clionaidae, Spirastrellidae, and species possibly related to these families, by molecular analyses using 28S rDNA for the first time. A ML tree of 43 species of 11 genera and 8 families was obtained by using GTR+I+G model. Family Clionaidae was not reconstructed as monophyletic group because Spirastrellidae and Acanthochaetidae were not clearly separated from the large clade of Clionaidae. Genus *Pione* appeared as a sister clade of a large clade including Clionaidae without *Pione*, Spirastrellidae and Acanthochaetidae, suggesting that presence/absence of oxea was more important character than excavation capability in classification of these groups. Genus *Cliona* was not monophyletic and suggested to be divided into 6 subgroups. These subgroups included species of *Cliothosa* and "*Sphaciospongia*" *vagabunda*, and thorough revision of the genus *Cliona* is required. "*Spirastrella*" *insignis* that has diagnostic characters of both Clionaidae (huge and massive external morphology) and Spirastrellidae (cortex of robust spirasters and no endolithic life habit) made a separate clade not together with any species of Clionaidae and Spirastrellidae. This problematic species should be given a new generic status based on both molecular and morphological characters.

**Phylogenetic relationships between freshwater and marine Haplosclerida
(Porifera) based on the full length 18S rRNA and partial COXI gene sequences**

Valeria Itskovich{ XE "Itskovich, Valeria" }^(1*); Sergey Belikov{ XE "Belikov, Sergey" }⁽¹⁾; Sofia M. Efremova{ XE "Efremova, Sofia M." }⁽²⁾; Yoshiki Masuda{ XE "Masuda, Yoshiki" }⁽³⁾; Thierry Pérez{ XE "Pérez, Thierry" }⁽⁴⁾; Eliane Alivon{ XE "Alivon, Eliane" }⁽⁴⁾; Carole Borchellini{ XE "Borchellini, Carole" }⁽⁴⁾; Nicole Boury-Esnault{ XE "Boury-Esnault, Nicole" }⁽⁴⁾

(1) Limnological Institute of the Siberian Branch of Russian Academy of Sciences, Ulan-Batorskaya 3, RUS-664033 Irkutsk, Russia, itskovich@mail.ru; (2) Laboratory of Ontogenesis, Biological Research Institute, St. Petersburg State University, Oranienbaumskoe sch. 2, Stary Peterhoff, 198 904 St. Peterburg, Russia; (3) Department of Biology, Kawasaki Medical School, Kurashiki, 701-0192, Japan; (4) Centre d'Océanologie de Marseille, Université de la Méditerranée, UMR-CNRS 6540, Station marine d'Endoume, rue de la Batterie des Lions, 13007 Marseille, France

The order Haplosclerida comprises a large number of marine sponge families and all sponge families inhabiting freshwater. Taxonomy within this order remains unclear. To investigate origin of freshwater sponges including endemic Lubomirskiidae from Lake Baikal and to study phylogenetic relationships within order Haplosclerida, sequences from the COXI gene of 8 sponge species and full length 18S rRNA of 5 sponge species were obtained. Structures of the full length 18S rRNA gene of Potamolepidae sp., *Swartschewskia papyracea*, *Baikalospongia bacillifera*, *Baikalospongia* sp. and *Baikalospongia intermedia* were obtained. Results based on 18S rRNA data indicated paraphyletic origin for Haplosclerida. On the obtained phylogenetic tree the marine haplosclerid sponge *Haliclona* is situated at the base of the cluster combining freshwater Haplosclerida and other marine species. All freshwater sponge species (families Lubomirskiidae, Spongillidae and Potamolepidae) cluster on the phylogenetic tree together in one monophyletic group. Also partial sequences of COXI gene of the genera *Niphates*, *Callyspongia*, *Xestospongia* and Haplosclerida sp. were obtained. The obtained phylogenetic trees based on the COXI data also confirmed the paraphyletic origin of the Haplosclerida. The tree topology showed that marine and freshwater Haplosclerida belong to the different clusters and genera *Callyspongia* and *Niphates* are not monophyletic. Our results of study of 18S rRNA and COXI gene are in good congruence with each other and reveal the monophyletic origin of freshwater sponges and paraphyletic origin for the order Haplosclerida.

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Mitochondrial genomics of sponges - implications for sponge phylogeny and animal mtDNA evolution

Dennis Lavrov{ XE "Lavrov, Dennis" }^(*); Natalia Frishman{ XE "Frishman, Natalia" }; Karri Haen{ XE "Haen, Karri" }; Zhiyong Shao{ XE "Shao, Zhiyong" }; Xiujuan Wang{ XE "Wang, Xiujuan" }

EEOB Department, Iowa State University, 253 Bessey Hall, Ames, Iowa 50011, USA, dlavrov@iastate.edu

Mitochondria - the energy producing organelles present in most eukaryotic cells - contain their own genome, separate from that of the nucleus. Mitochondrial DNA (mtDNA) of bilaterian animals is typically a small, circular-mapping molecule that encodes 37 tightly packed genes. MtDNA of choanoflagellate *Monosiga brevicollis*, the closest unicellular out-group of animals, is four times larger and contains 1.5 times as many genes. We are investigating this remarkable transition in mtDNA evolution, by studying mitochondrial genomes from representatives of all three classes of sponges and some other groups of “lower” animals. We also use our data to decipher phylogenetic relationships among these animals. To date we determined complete or nearly complete mitochondrial sequences for ten species of demosponges, two species of glass sponges and one species of calcareous sponges. Our primary approach is to PCR amplify each mtDNA in 2-4 overlapping fragments and to determine the sequences of these fragments by the shotgun method. Although the project is still ongoing, it has advanced our understanding of mtDNA evolution and sponge phylogeny in three ways. First, we were able to show that sponge mitochondrial DNA represents an intermediate stage in the evolution of “typical” animal mtDNA. Poriferan mtDNA resembles those of other animals in its compact organization, lack of introns, and a well-conserved animal-like gene order. Yet, it contains some extra genes, encodes bacterial-like rRNA and tRNAs, and uses a minimally derived genetic code. Second, we found that the tempo and mode of mitochondrial DNA evolution are quite dissimilar among the tree major lineages of sponges. In particular, mitochondrial genomes of calcareous sponges have several unusual features that clearly set this group apart from other sponges. The diverse patterns of mitochondrial evolution in three groups of sponges suggest that different genetic architectures are hidden behind the superficially similar morphology of sponges. Third, we tested some existing hypotheses of demosponge relationships by using several different datasets derived from mitochondrial genomes: supermatrices of concatenated rRNA, tRNA and protein-coding gene sequences and gene arrangements. Our results indicate that both mitochondrial gene sequences and mitochondrial gene arrangements are informative for the study of poriferan relationships and may represent the datasets of choice for phylogenetic studies in sponges. In addition, the large amount of genomic data generated for this project creates an important database for future genetic, phylogenetic, population, and barcoding studies that use mtDNA as a marker.

Are sponges monophyletic or paraphyletic? A phylogenomic approach

Michaël Manuel{ XE "Manuel, Michaël" }^(1*); Romain Derelle{ XE "Derelle, Romain" }⁽¹⁾;
Carole Borchellini{ XE "Borchellini, Carole" }⁽²⁾; Jean Vacelet{ XE "Vacelet, Jean" }⁽²⁾; Nicole
Boury-Esnault{ XE "Boury-Esnault, Nicole" }⁽²⁾

(1) UMR 7138 "Systématique, Adaptation, Evolution", Case 05, Bat A 4eme etage, Université Pierre et Marie Curie, 9 quai St Bernard, 75005 Paris, France, Michael.Manuel@snv.jussieu.fr; Romain.Derelle@snv.jussieu.fr; (2) UMR 6540 "Dimar", Centre d'Océanologie de Marseille, Rue de la Batterie des Lions, 13007 Marseille, France, borchellini@com.univ-mrs.fr; jvacelet@com.univ-mrs.fr; esnault@com.univ-mrs.fr

Recent advances in metazoan phylogeny have let unresolved the phylogenetic status of sponges. Even if most published molecular phylogenies using ribosomal RNA sequences have suggested sponge paraphyly, with the calcareous sponges branching closer to the Eumetazoa (non sponge animals) than to the siliceous sponges, the alternative hypothesis (sponge monophyly) could not be rejected. This is because available molecular data sets convey an insufficient amount of phylogenetic signal. Beyond taxonomic implications, this question is crucial to understand the origin and early evolution of animals, in particular because sponge paraphyly would favour a sponge-like organism as the last common ancestor of Metazoa. We addressed this problem through an innovative phylogenomic approach. To a large published data set of about 150 different aligned genes (from various eukaryotes and mostly bilaterian metazoans), we added sequences from sponges and cnidarians. They were obtained through EST sequencing in 4 sponge species (2000 ESTs per species), each belonging to one of the four main monophyletic sponge lineages: *Oopsacas minuta* (Hexactinellida), *Ephydatia muelleri* (Demospongiae), *Oscarella lobularis* (Homoscleromorpha) and *Sycon raphanus* (Calcispongia). Additional ESTs were sequenced from the cnidarian *Clytia hemisphaerica* (Hydrozoa) (10 000 ESTs). Furthermore, sequences from *Reniera* sp. (Demospongiae) and *Nematostella vectensis* (Anthozoa, Cnidaria), were recovered from publicly available data from the genome sequencing projects. The presentation will highlight some methodological issues for analyzing such large sequence data sets. The main results will be commented, with particular focus on the phylogenetic status of sponges and the position of homoscleromorphs and calcisponges.

Phylogenetic relationships of the genus *Agelas* (Porifera, Demospongiae)

Fernando J. Parra-Velandia{ XE "Parra-Velandia, Fernando J." }^(1*,2); Dirk Erpenbeck{ XE "Erpenbeck, Dirk" }⁽²⁾; Sven Zea{ XE "Zea, Sven" }⁽¹⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽²⁾

(1) Departamento de Biología and Centro de Estudios en Ciencias del Mar -CECIMAR Universidad Nacional de Colombia, INVEMAR, Cerro Punta de Betín, A.A. 10-16, Santa Marta, Colombia, fernando.parra@gmail.com; szea@invemar.org.co; (2) Institute for Biodiversity and Ecosystem Dynamics (IBED) & Zoological Museum, University of Amsterdam, P.O. Box 94766 1090 GT Amsterdam, The Netherlands, derpenb@gwdg.de; soest@science.uva.nl

Agelas is a genus of tropical marine sponges present in the Mediterranean Sea, the Indo-Pacific Ocean, and the Caribbean Sea. In the latter area this genus comprises at least thirteen species, several of them being dominant among coral reef fauna. Species belonging to this genus possess characteristic acanthostyles with regularly spaced verticils of thorns and cored and echinated spongin fibers. Apart from their position as an important ecological component of tropical marine bottoms, the genus *Agelas* has been recently investigated due to the diversity of bioactive metabolites that they produce. Our objective was to construct the phylogeny of this genus testing some hypothesis about the origin of its high diversity in the Caribbean, that contrast with other marine reef groups that usually possess higher diversity in the Indopacific. Complete length ITS1, 5.8S, ITS2, and partial CO1 mtDNA sequences were amplified from specimens collected between 2001 and 2003 from each different species and morphotypes of *Agelas* genus found across Caribbean Sea. Additional specimens came from Australia, Indonesia and Spain; other material came from ZMA specimens. The sorting of between Indopacific and Caribbean species allowed us to reject the hypothesis of a single origin for all Caribbean species, initially established by us. The phylogenetic trees show the presence of four great clades associated to species of wide distribution like *A. dispar*, *A. conifera*, *A. citrina* and *A. clathrodes*; all of them with an origin previous to the formation of the Caribbean Sea, probably since the Tethys Sea. On the other hand, the resolution of the molecular tools was not enough to resolve the ends of two taxonomically challenging clades or geographically variant morphotypes, and did not allow for the testing of events of hybridization during sea level changes associated to Pleistocene glaciations in the northern hemisphere.

Do skeletal architecture, spicule type and spicule arrangement reflect phylogenetic history in the marine Haplosclerida?

Jean Raleigh{ XE "Raleigh, Jean" }⁽¹⁾; Niamh Redmond{ XE "Redmond, Niamh" }⁽¹⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽²⁾; Michelle Kelly{ XE "Kelly, Michelle" }⁽³⁾; Pete Cowman{ XE "Cowman, Pete" }⁽¹⁾; Nathaniel Coffolla{ XE "Coffolla, Nathaniel" }⁽¹⁾; Flint Walter{ XE "Walter, Flint" }⁽¹⁾; Grace McCormack{ XE "McCormack, Grace" }^(1*)

(1) Molecular Evolution & Systematics Laboratory, Zoology Dept., National University of Ireland, Galway, Galway, Ireland, jean.raleigh@nuigalway.ie; n.redmond1@nuigalway.ie; peter.cowman@nuigalway.ie; n.coffolla@nuigalway.ie; flint.walter@nuigalway.ie; grace.mccormack@nuigalway.ie; (2) Curator of Invertebrates, Zoology Museum, University of Amsterdam, Amsterdam, Netherlands, soest@science.uva.nl; (3) National Institute of Water and Atmospheric Research Ltd, Private bag 109-695, Newmarket, Auckland, New Zealand, m.kelly@niwa.co.nz

Despite being well described morphologically and attracting a lot of attention, Haplosclerid classification has proven problematical. Disagreements abound regarding higher-level groupings such as the status of the two marine suborders Haplosclerina and Petrosina. In addition, lower-level groupings, such as some genera, have been described as largely ‘convenient’ although, generally, morphological evidence at the family and genus level would appear to be strong. The order is one of the largest sponge groups both in terms of number of species and number of habitats, and members have few spicule types; factors that may contribute to the taxonomic uncertainty. Molecular phylogenies reconstructed using three different gene regions, both nuclear and mitochondrial, show strong evidence that the two suborders, many of the families and even genera are not monophyletic. There is strong support for a clade containing representatives from both *Haliclona* and *Callyspongia* but also *Oceanapia*, *Petrosia* and *Strongylophora*. Within this clade *Haliclona* and *Callyspongia* do not form monophyletic groups. Another clade contains representatives from *Amphimedon*, *Niphates*, *Xestospongia* and also *Petrosia*. This evidence might suggest that sponge skeletal architecture and spicule type and arrangement do not reflect phylogenetic history.

Molecular phylogeny and morphological evolution of the genus *Clathrina* (Porifera, Calcarea)

André Rossi{ XE "Rossi, André" }⁽¹⁾; Claudia Augusta de Moraes Russo{ XE "Russo, Claudia Augusta de Moraes" }⁽²⁾; Hans Tore Rapp{ XE "Rapp, Hans Tore" }⁽³⁾; Michelle Klautau{ XE "Klautau, Michelle" }^(1*)

(1) Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Pau Brasil 211, CCS, 21941-590, Rio de Janeiro, RJ, Brazil, mklautau@biologia.ufrj.br; (2) Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Pau Brasil 211, CCS, 21941-590, Rio de Janeiro, RJ, Brazil; (3) University of Bergen, Department of Biology, PO Box 7800, N-5020 Bergen, Norway

The genus *Clathrina* Gray, 1867 comprises 43 species widely distributed in all seas. The reduced number of morphological characters may be accounted for the problematic taxonomy of the genus and has limited phylogenetic reconstructions among species. In the present study, molecular data were used to provide, for the first time, a phylogenetic reconstruction of *Clathrina*. The evolution of the morphological characters was discussed based on the molecular trees using 553 bp of the rDNA Internal Transcribed Spacers region (ITS I, 5.8 S and ITS II). Ten species of *Clathrina* were analysed: *C. aspina*, *C. aurea*, *C. cerebrum*, *C. clathrus*, *C. conifera*, *C. contorta*, *C. hispanica*, *C. reticulum*, *C. tetractina* and *Clathrina* sp. *Ascandra* sp. was included as the outgroup. For each species, up to three specimens were included in the analysis. Tree topologies were recovered using Neighbor-Joining (bootstrap with a 1000 replicates) and Maximum Parsimony (bootstrap with 500 replicates) algorithms. In the case of the MP method, a total of 243 parsimonious trees (525 steps) were obtained, the CI was 0.72, the RI was 0.88 and the RCI was 0.63. A consensus tree was reconstructed based on all 243 MP trees. Both methods (neighbor-joining and maximum parsimony consensus) resulted in identical trees with high bootstrap support for almost all nodes. Skeleton characters were largely consistent with the phylogenetic groupings. For instance, *C. aurea*, *C. clathrus*, *Clathrina* sp., *C. hispanica* and *C. conifera* formed a monophyletic group of clathrinas that only present triactines. Also, *C. cerebrum*, *C. aspina* and *C. reticulum* clustered as species with triactines and tetractines in the skeleton. Additionally, species with tetractines as main spicules, *C. contorta* and *C. tetractina*, also grouped together. Finally, the last cluster included *C. aurea*, *C. clathrus*, *Clathrina* sp. and *C. conifera*, species with triactines with cylindrical and undulated actines, with rounded tips. Even though, our results show that skeleton characters appeared only once in the evolutionary history of *Clathrina*, it seems to be clear that the increase in complexity of the cormus and development of water collecting tubes appeared many times in the evolution of the genus.

Cryptic speciation and correspondence between spiculation and molecular markers in *Placospongia*

Cintia Paula Jandre Rua{ XE "Rua, Cintia Paula Jandre" }^(*); Adriana Mattos{ XE "Mattos, Adriana" }; Antônio M. Solé-Cava{ XE "Solé-Cava, Antônio M." }

Laboratório de Biodiversidade Molecular, UFRJ. Prédio do CCS, Bloco A, sala A2-098 - Cidade Universitária - Ilha do Fundão, CEP 21941-590. Rio de Janeiro, RJ, Brasil, cintiarua@yahoo.com.br

In spite of the low dispersal capability and the high levels of cryptic speciation found in sponges and other marine invertebrates *Placospongia* species are reported as cosmopolitan. The species recognized in Systema Porifera as valid for the genus are *Placospongia carinata*, *Placospongia melobesioides* and *Placospongia decorticans*. The diagnostic character that differentiates *P. carinata* from *P. melobesioides* is the presence of spirasters in the former. *P. decorticans* also has spirasters, but is diagnosed by the shape of the selenasters, elongated in *P. decorticans* and bean-shaped in the other two species. In this study, we have compared spiculation patterns with allozymes and nuclear (ribosomal internal transcribed spacers) and mitochondrial (cytochrome oxidase I) sequences of individuals from populations of *Placospongia carinata* and *P. melobesioides* from Brazil and from the Atlantic and Pacific coasts of Panama. Allozyme data showed a differentiation between Atlantic and between Pacific specimens, including the presence of possible cryptic species in each ocean. The other molecular markers were less variable, and, basically, separated the individuals into two groups, which correspond exactly with the presence or absence of spirasters. Thus, the sequence data support the taxonomic value of the presence of spirasters to separate species or species groups in the genus. Phylogenetic analyses generated two clades with high bootstrap support inside the group of *Placospongia melobesioides*. One of those clades includes samples from two locations in Brazil (Bahia and Pernambuco) and from the Pacific coast of Panama, while the other clade is made up of samples from Bahia, Brazil. The same topology was found with either molecular marker. A surprising result was the confirmation, both with allozymes and sequence data, of the presence of what seems to be a trans-Isthmian species of *Placospongia*, as suggested by Nichols and Barnes. This could be the first sponge species to have a distribution in two oceans confirmed by molecular markers. The evolutionary (extremely low rates of genetic divergence) or ecological (some means of transport across the freshwater Panama Canal or through the southern tip of South America) factors responsible for this phenomenon await further studies.

ITS-2 and 18S rRNA gene phylogeny of Aplysinidae (Verongida, Demospongiae)

Susanne Schmitt{ XE "Schmitt, Susanne" }⁽¹⁾; Sven Zea{ XE "Zea, Sven" }⁽²⁾; Thomas Dandekar{ XE "Dandekar, Thomas" }⁽³⁾; Matthias Wolf{ XE "Wolf, Matthias" }⁽³⁾; Ute Hentschel{ XE "Hentschel, Ute" }^(1*)

- (1) Research Center for Infectious Diseases, University of Wuerzburg, Roentgenring 11, D-97070 Wuerzburg, Germany, susanne.schmitt@mail.uni-wuerzburg.de; ute.hentschel@mail.uni-wuerzburg.de; (2) Departamento de Biología and Centro de Estudios en Ciencias del Mar (CECIMAR), Universidad Nacional de Colombia, INVEMAR, Cerro Punta de Betín, AA 10-16, Santa Marta, Colombia, szea@invemar.org.co; (3) Department of Bioinformatics, Biocenter, University of Wuerzburg, Am Hubland, D-97074, Wuerzburg, Germany, dandekar@biozentrum.uni-wuerzburg.de; matthias.wolf@biozentrum.uni-wuerzburg.de

18S ribosomal DNA and internal transcribed spacer 2 (ITS-2) full length sequences, each of which was sequenced three times, were used to construct phylogenetic trees with alignments based on secondary structures, in order to elucidate genealogical relationships within the Aplysinidae (Verongida). The first poriferan ITS-2 secondary structures are reported. Altogether eleven *Aplysina* sponges and three additional sponges (*Verongula gigantea*, *Aiolochoia crassa*, *Smenospongia aurea*) from tropical and subtropical oceans were analyzed. Based on these molecular studies, *S. aurea* that is currently affiliated with the Dictyoceratida should be reclassified to the Verongida. *Aplysina* appears as monophyletic. A soft form of *Aplysina lacunosa* was separated from other *Aplysina* and stands at a basal position in both 18S and ITS-2 trees which suggests a possibly different phylogenetic placement. Based on ITS-2 sequence information, the *Aplysina* sponges could be distinguished into a Mediterranean cluster containing the sponges *A. aerophoba* and *A. cavernicola* and a single Caribbean-Eastern Pacific cluster containing all the remaining *Aplysina* sponges. Inside this cluster no further resolution could be obtained. Based on the above data, the following biogeographic scenario is conceivable: a possibly Tethyan ancestor for all *Aplysina* sponges resulted in a Mediterranean clade and in a more diverse Caribbean-Eastern Pacific clade. The high degree of similarity between the genes analyzed would be explained if the progress of adaptive radiation had occurred only recently.

New insights into the phylogeny and evolution of calcareous sponges

Oliver Voigt{ XE "Voigt, Oliver" }; Martin Dohrmann{ XE "Dohrmann, Martin" }; Dirk Erpenbeck{ XE "Erpenbeck, Dirk" }; Eilika Wülfing{ XE "Wülfing, Eilika" }; Gert Wörheide{ XE "Wörheide, Gert" }^(*)

Dept. of Geobiology, Geoscience Centre Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany,
gert.woerheide@geo.uni-goettingen.de

Calcareous sponges (Porifera: Calcarea) play an important role for our understanding of early metazoan evolution, as a number of molecular studies suggested a closer relationship of Calcarea to Eumetazoa than to Demospongiae and Hexactinellida, the other two main poriferan clades. However, the relationships within Calcarea are far from being solved. While the division into the subtaxa Calcinea and Calcaronea is well established, relationships within both subtaxa remain largely unresolved. From the few molecular studies conducted to date it seems that morphological characters are highly homoplastic. Additionally, previous studies suffered from a relatively limited taxon sampling, especially from the subclass Calcinea. Here we present a study on the phylogenetic relationships within Calcarea as inferred from the analyses of full-length 18S and 28S ribosomal DNA gene sequences. By substantially increasing both, taxonomic sampling and nucleotide positions included in the analyses, we show that several of the traditionally recognized supraspecific taxa within Calcinea and Calcaronea are not monophyletic. The reconstruction of ancestral morphological character states on the basis of our inferred phylogenies sheds new light on their evolutionary history, partially contradicting the results of previous studies.

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Molecular phylogeny, origin and diversification of *Vaceletia* spp. 'living fossil sphinctozoan' sponges

Gert Wörheide{ XE "Wörheide, Gert" }^(1*,3); Joachim Reitner{ XE "Reitner, Joachim" }⁽¹⁾; Jean Vacelet{ XE "Vacelet, Jean" }⁽²⁾; John N.A. Hooper{ XE "Hooper, John N.A." }⁽³⁾

(1) Dept. of Geobiology, Center of Earth Sciences, University of Göttingen, Goldschmidtstr.3, 37077 Göttingen, Germany, gert.woerheide@geo.uni-goettingen.de; (2) Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France; (3) Queensland Museum, PO Box 3300, South Brisbane, Qld., 4101

Coralline sponges (formerly also called 'sclerosponges'), were primary reef-builders for much of Earth's early marine history, but were eventually replaced by a coralline algae-scleractinian coral community during the lower Cretaceous (140MYA). Coralline sponges were thought to have been extinct since the end of the Cretaceous (70MYA) until the rediscovery at the turn of the 19th century and several decades ago of a few 'relict' species living in shallow water reef caves of the Indo-Pacific and Caribbean, and more recently also on the deeper fore-reefs. In this study we focus on a group of 'living relict' coralline sponges, *Vaceletia* spp., previously assigned to a mostly fossil 'Phylum Sphinctozoa' or chambered sponges, and now included in the demosponge Porifera. The taxon under study (*Stylothalamia/Vaceletia*) occurred first in the Middle Triassic (Ladinian, 220MYA) with a continuing fossil record to modern days. A solitary form, *Vaceletia crypta* Vacelet, 1977, lives in shallow water caves throughout the Indo-Pacific, including the Great Barrier Reef, although found only on reefs with a certain geomorphology. A colonial form, which has retained its reef building capacity, was discovered by French scientists on the deep fore-reef of New Caledonia during the 1970s, and more recently we discovered several different colonial morphotypes in shallow water reef caves of Osprey, Shark and Bougainville Reefs in the Coral Sea, as well as the North Astrolabe Reef in Fiji. However, phylogenetic and taxonomic relationships between all those morphotypes remained enigmatic based solely on morphometric data. We have now analysed nuclear ribosomal DNA (full length 18S and partial 28S) and mtDNA (partial Cytochrome Oxidase 1) gene sequences allowing us to explore more accurately relationships among distinct *Vaceletia* morphotypes, and whether those represent different species. We further also determined to which main demosponge lineage *Vaceletia* most likely belongs - with surprising results: taxon *Vaceletia* belongs to the Keratosa, with high statistical support. Our results have important implications for the interpretation of the systematic relationships of *Vaceletia* spp. and the evolution of coloniality of fossil chambered 'sphinctozoan' sponges.

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Agreement between allozymes and cytochrome oxidase I sequences in separating Mediterranean and Atlantic *Chondrosia* species (Chondrosida: Demospongiae)

Carla Zilberberg{ XE "Zilberberg, Carla" }^(*); Antônio M. Solé-Cava{ XE "Solé-Cava, Antônio M." }

Laboratório de Biodiversidade Molecular, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, carlazelber@yahoo.com.br

The application of genetic markers to sponge taxonomy has demonstrated that many sponge species considered cosmopolitan, were actually complexes of cryptic species. A recent allozyme study with Mediterranean and west Atlantic samples of *Chondrosia* aff. *reniformis* has shown that they were genetically very divergent (Genetic Identity, $I = 0.48$), but that the Caribbean and west Atlantic populations were not differentiated ($I = 0.93$). In this study we used mitochondrial cytochrome oxidase I (COI) sequences to compare the same samples. We found a very good agreement between the two markers, with high levels of COI sequence divergence between *Chondrosia reniformis* from the Mediterranean and *Chondrosia* sp. from the west Atlantic (p distance = 0.040) and very little differentiation along the west Atlantic samples (p distance = 0.002). This shows that, unlike what was found in other studies, COI sequences can be used to discriminate between congeneric sponge species of *Chondrosia*. Also, we did not find any COI sequence difference between *Chondrosia* sp. and Caribbean *Chondrosia collectrix* both on COI sequences or cortex thickness (*Chondrosia* sp. = 0.43 ± 0.01 mm; *C. collectrix* = 0.37 ± 0.07 mm; *C. reniformis* = 1.31 ± 0.10 mm). It is, thus, possible, that samples of *Chondrosia* sp. and *C. collectrix* are conspecific.

Mitochondrial and ribosomal markers have less resolution than allozymes to separate cryptic species of *Chondrilla* (Demospongiae: Chondrillidae)

Carla Zilberberg{ XE "Zilberberg, Carla" }(*); Antônio M. Solé-Cava{ XE "Solé-Cava, Antônio M." }

Laboratório de Biodiversidade Molecular, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, carlazilber@yahoo.com.br

The genus *Chondrilla* Schmidt 1862 has a very simple morphology, with the presence of spherasters as the sole spicule type. With such a simple morphology, for a long time the species *Chondrilla nucula* was thought to have a world-wide distribution. However, a molecular study using allozymes found at least four cryptic species of *C. nucula* in the Atlantic Ocean. The purpose of the present study was to compare those findings with analyses of DNA sequences, using a mitochondrial (Cytochrome oxidase I, COI) and the two ribosomal internal spacer regions (ITS 1 & ITS 2). Additionally, we included specimens from new localities such as Florida, Bahamas and Belize, in the northern hemisphere, and Abrolhos, Trindade Island, Pernambuco, Guarajuba and Itapuã, in Brazil. We also analyzed samples of *C. australiensis* and of two cryptic species from Australia. Sequences of COI, ITS 1 and ITS 2 provided less resolution than allozymes to separate cryptic species of *Chondrilla*. Phylogenetic analyses of COI and ITS regions recovered trees with many polytomies, but most species were separated with high bootstrap support in those trees, with the exception of two species in Brazil and two in the Bahamas, which were easily separated using allozymes but not with DNA. Using both DNA markers, we were able to detect two new cryptic species of *Chondrilla* in Florida. One interesting finding was the presence of at least two cryptic species living in sympatry in most locations along the Caribbean and Northeast coast of Brazil (*e.g.*, Bahamas, Florida, Belize, and Guarajuba, Itapuã and Abrolhos in Brazil). The total number of genetically different species of *Chondrilla* in the Atlantic, thus, adds up to eight. Although it is clear that DNA sequence data are invaluable for phylogenetic studies, it seems that, at least until more variable DNA markers are found, allozymes remain the best choice to identify and separate cryptic species in sponges.

Brazilian *Amphimedon compressa* Duchassaing & Michelotti, 1864 (Porifera, Haplosclerida): Phenotypic diversity evaluated by morpho-molecular methodology

Leonardo Luis Marques Assumpção{ XE "Assumpção, Leonardo Luis Marques" }^(*); Meiryelen Vieira da Silva{ XE "Silva, Meiryelen Vieira da" }; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }

Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524 - PHLC, sala 205, Maracanã, 20550-013, Rio de Janeiro, RJ, Brasil, leonardo_assumpcao@hotmail.com; meiryelen_vieira@hotmail.com; lobohajdu@alternex.com.br

Amphimedon Duchassaing & Michelotti, 1864 has a complex taxonomy due to the abundance of morphotypes and wide distribution. The aim of this work is to characterize the sponges registered as *Amphimedon compressa* Duchassaing & Michelotti, 1864 or *A. aff. compressa*, which occur along the Brazilian coast. Morphologic and DNA polymorphism techniques were used to check whether different morphotypes belong to the same species. Samples of *A. compressa* and *A. aff. compressa* were borrowed from MNRJ collection, while the holotype of *A. compressa* came from the BMNH collection. Observations and documentation of details of skeletal architecture, spicule dissociation, tangential and perpendicular thick sections mounts and micrometric measurements were analyzed for morphological data. The skeletal architecture (arrangement of sponging fibers and spicules) is identical between Brazilian samples and holotype from the Caribbean. The thickness and length measurements were variable within and between collection sites, and between Brazil and the Caribbean. Distinct morphotypes might be explained by abiotic factors alteration such as: temperature, depth, salinity, light incidence, substrate type, currents, sedimentation, minerals and organic particle content, solved organic matter and pollution agents. Genomic DNA was extracted and the internal transcribed regions (ITS-1 and ITS-2) from ribosomal RNA genes were amplified by PCR with specific primers. All analyzed samples of *Amphimedon* showed ITS1 and ITS2 with around 300 to 400 base pairs. ITS1 is bigger than ITS2 as observed in other Class Demospongiae sponges. The SSCP method detected a very similar pattern between sponge samples. Results suggest that sponges along the Brazilian coast are co-specific to *Amphimedon compressa* from the Caribbean.

LLM Assumpção - Bolsista IC-FAPERJ; MV Silva - Bolsista PIBIC-UERJ

Population genetic structure of the marine sponge *Scopalina lophyropoda* Schmidt (Halichondrida) assessed by microsatellites markers

Andrea Blanquer{ XE "Blanquer, Andrea" }^(*); Maria-J. Uriz{ XE "Uriz, Maria-J." }

Centre d'Estudis Avançats de Blanes (CEAB) CSIC Accés cala St Francesc, 14 17300 Blanes (Girona)
Spain, andrea@ceab.csic.es

The genetic variation of species, across time and space, is often described as a balance of opposing evolutionary forces leading to genetic differentiation or homogeneity. For most sponges, larvae are considered the main agent for dispersal and thus, for the genetic connectivity among populations, since very little is known about spawning. *Scopalina lophyropoda* is an encrusting Mediterranean sponge that presents a strongly aggregated distribution. Individuals (N=222) from six western Mediterranean populations, belonging to three distinct geographic areas (Cabo de Gata, Costa Brava and Port Cros), were sampled and characterized for seven microsatellite loci previously isolated from the species. Only individuals located further than 0.5 m from each other were sampled, to avoid collecting clone-mates, as revealed in a previous study. The analysis was done at both the ramet and the genet levels. Clonality was found in the Port Cros populations that also presented the lowest number of alleles/locus, whereas it was negligible for the other populations. The Cabo de Gata population presented the highest number of alleles/locus and private alleles, possibly related to water circulation patterns in the area. Private alleles were detected in all populations except the smallest one, revealing genetic structure and isolation by distance, seen as a weak, but significant positive correlation between geographic distance and genetic differentiation. FST values among populations were higher than those observed in *Crambe crambe*. This is congruent with the contrasting larval behaviour and adult distribution of the two species. A lack of inbreeding (negative FIS values) was detected for all the populations surveyed, confirming previous results at a finer scale. Philopatric dispersal may explain the species distribution in aggregates, but would fail to explain the lack of inbreeding. Several non-exclusive hypotheses are suggested as responsible for the gene flow and the avoidance of inbreeding detected: young populations, the simultaneous arrival of several genotypes in one dispersal event (mixing of sexual-asexual propagules) or the existence of a dispersal deep corridor.

Philopatric larval dispersal and fission events contrast with molecular evidences of lack of inbreeding and clonality in *Scopalina lophyopoda* Schmidt (Halichondrida)

Andrea Blanquer{ XE "Blanquer, Andrea" }^(1*); Maria-J. Uriz{ XE "Uriz, Maria-J." }⁽¹⁾; Juli Caujapé-Castells{ XE "Caujapé-Castells, Juli" }⁽²⁾

(1) Centre d'Estudis Avançats de Blanes (CEAB) CSIC Accés cala St Francesc, 14 17300 Blanes (Girona) Spain, andrea@ceab.csic.es; (2) Laboratorio de Biodiversidad Molecular y Banco de DNA, Jardín Botánico Canario "Viera y Clavijo", Ap de correos 14 de Tafiira Alta 35017 Las Palmas de Gran Canaria, Canary Islands. Spain

Sponge populations are considered to consist of a combination of clonal organisms, originated by asexual processes, and genetically distinct individuals resulting from sexual reproduction. Microsatellite markers and spatial autocorrelation analysis were applied to evaluate the influence of sexual versus asexual reproduction on the genetic structure at fine-scale of the Mediterranean encrusting sponge *Scopalina lophyopoda*. We sampled and mapped most individuals (N=70) of a target population (Blanes, NW Mediterranean), which consisted in three sub-samples (*i.e.* parallel gorges) located 25 m from each other. We found a few individuals with the same multilocus genotype, but that could be attributed to random mating, as revealed by the significant probabilities of finding identical multilocus genotypes from distinct sexual events. If occurring, clones were only present in one subpopulation and they were located within 0.5m. The analysis was performed at both, the genet and the ramet level. The genetic structure of *S. lophyopoda* is best explained by short-range sexual reproduction, as revealed by autocorrelation statistics. Philopatric larval dispersal and isolation by distance would explain the aggregated distribution and the significant genetic structure found in the species ($F_{ST}=0.0268$; and private alleles in each subpopulation), specially considering that the two most isolated individuals were located at 59 m. from each other. However, we found a paradoxical lack of inbreeding (negative FIS values) in all subpopulations and in the pooled population, which suggests that *S. lophyopoda* presents some mechanism to avoid inbreeding. In the absence of evidence for selection, several hypotheses are discussed that may explain the outcrossing detected in the species.

Estimation of gene flow using microsatellite markers on *Hymeniacidon sinapium*

Sayumi Hoshino{ XE "Hoshino, Sayumi" }^(1*,2), Daichi S. Saito{ XE "Saito, Daichi S." }⁽¹⁾;
Toshihiko Fujita{ XE "Fujita, Toshihiko" }^(1,2)

(1) Department of Zoology, National Science Museum, Tokyo, 3-23-1 Hyakunin-cho, Shinjuku-ku, 169-0073 Tokyo, Japan; (2) The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033 Tokyo, Japan

Recent phylogeographical studies on sponges using nDNA ITS region have revealed genetic structure within species, indicating restricted gene flow between populations. Our study on *Hymeniacidon flavia* has also revealed genetic differentiation between populations using the same marker. However, closely related *H. sinapium* showed no polymorphism in ITS sequence, despite they were collected in wider geographical range than *H. flavia*. It is highly likely that *H. sinapium* has high gene flow, but level of gene flow cannot be estimated by genetic marker without any polymorphism. To address this problem, we developed microsatellite markers and investigated geographical genetic structure and gene flow in *H. sinapium*. We discuss orientation of gene flow and their dispersal pattern.

Intraspecific variation in *Aplysina lacunosa* (Pallas, 1766) (Aplysinidae, Verongida, Demospongiae) by analysis of ITS1 and ITS2 fragments

Estéfane Cardinot Reis{ XE "Reis, Estéfane Cardinot" }^(*); Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }

Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524 - PHLC, sala 205, Maracanã, 20550-013, Rio de Janeiro, RJ, Brasil, estcr@brasilvision.com.br; lobohajdu@alternex.com.br

The taxonomy of *Aplysina* Nardo, 1834 is difficult, mainly because of the scarcity of morphological characters, which are all related to color and plasticity of specimens, as well as to their anatomy, which is primarily composed of spongin fibers (without spicules). This genus stands out for its diversified chemistry with potential pharmacological uses, and for its great diversity in the Atlantic Ocean, which suggests the use of molecular methods in the search for more sound species limits. The aim of the present study is to compare individuals of the species *Aplysina lacunosa* (Pallas, 1766) from the Brazilian coast with others from the Caribbean region, by the analysis of molecular markers to test the co-specificity of the sampled populations and the possible occurrence of intra and inter-specific variation. Samples conserved in alcohol were obtained from the collection of the Museu Nacional of Universidade Federal do Rio de Janeiro. The specimens analyzed were: 26 individuals of *A. lacunosa* (21 from the Brazilian coast and five from the Caribbean region), two of *A. fulva* Pallas, 1766 (Brazilian coast and the Caribbean region), four of *A. fistularis* Lendenfeld, 1889 and five of *A. cauliformis* Carter, 1881 (Brazilian coast). They were macerated in guanidine hydrochloride lyses solution and, after removing proteins and lipids by extraction with phenol: chloroform, the total genomic DNA was precipitated with ethanol. Soon after, the ITS1 and ITS2 (transcribed internal spacers) regions of the nuclear ribosomal RNA were amplified by the Polymerase Chain Reaction (PCR) method, using specific primers. After the amplification, the samples were submitted to the Single Strand Conformation Polymorphism (SSCP) technique, to analyze the different migration patterns obtained in 8.0% polyacrilamide gel visualized with silver nitrate. The size in base pairs expected for the ITS fragments is different for each species and sponge genus. Moreover, the differences in the migration patterns of the ITS fragments in the SSCP gel reflect mutations in the DNA that can be used to infer phylogeny among close taxa. Four possible alleles for both the ITS1 (classified as A, B, C and D) and ITS2 (classified as A*, B*, C* and D*) regions were obtained. These different alleles were correlated for 91.6% of the samples, resulting in A/A*, A/B*, B/B*, B/C*, C/C* and D/D* haplotypes for *A. lacunosa*. The haplotype C/C* was the most frequent. The ITS2 was more variable than the ITS1 and the ITS fragments, in general, showed more occurrence of intra-specific variation than inter-specific one. Caribbean specimens presented alleles in common with samples of the Brazilian coast, but results are considered still preliminary, thus recommending the analysis of a larger number of individuals.

Population genetics study of five broad range sponge taxa associated with cold-water coral reefs along the European margins

Julie Reveillaud{ XE "Reveillaud, Julie" }⁽¹⁾; Thomas Remerie{ XE "Remerie, Thomas" }⁽¹⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽²⁾; Ann Vanreusel{ XE "Vanreusel, Ann" }⁽¹⁾; Jean-Pierre Henriët{ XE "Henriët, Jean-Pierre" }⁽³⁾

(1) Marine Biology Section, Ghent University, Krijgslaan 281-S8, B-9000 Gent, Belgium / CeMoFE, Center for Molecular Phylogeny and Evolution, Ghent University, Belgium, julie.reveillaud@ugent.be; thomas.remerie@ugent.be; ann.vanreusel@ugent.be; (2) Institute for Biodiversity and Ecosystem Dynamics, Zoologisch Museum, University of Amsterdam, P.O.Box 94 766, 1090 GT Amsterdam, Netherlands, soest@science.uva.nl; (3) Renard Centre of Marine Geology, Department of Geology and Soil Science, Geological Institute, Ghent University, Krijgslaan 281 s.8, B-9000 Gent, Belgium, jeanpierre.henriet@ugent.be

Deep-water corals (most commonly *Lophelia pertusa* and *Madrepora oculata*), banks or patches occur all along the European shelf margin, in the Mediterranean Sea and from Gibraltar up to Norway. Hosting nurseries for fishes and a multitude of invertebrates - some new to science and potential coral reefs endemics- they are Hotspots of Biodiversity. However, with the depletion of shallow-water fish stocks and the increasing commercial exploitation of the deep-sea environment, there is actually a high concern of Europe to better understand these ecosystems in order to achieve a sustainable management of these complexes but fragile ecosystems. Particularly, there is an urgent need to understand the connectivity of these ecosystems along the European margin. Sponges, because they dominate these aphotic ecosystems, where nutrients and food is generated through pelagic production are of great potential. Besides, because their short pelagic larvae life and their asexual reproduction mode don't allow them to go away from the seafloor, sponges are totally dependent from the currents and are perfect organisms to study the relationship between their distribution and the water masses flux that occur along the European margins. From preliminary comparison of datasets on bathyal (>200m) sponges biodiversity survey and robust assessment of species distributional range, we highlighted actually several broad ranges sponges species associated with different deep-sea coral reefs populations along the European margin. Five broad range taxa; *Hexadella* sp, *Desmacella* sp, *Mycale* sp, *Plocamionida* sp, *Pheronema* sp were selected as model species. The chosen species are (1) representative for a limited series of generalized habitus (thin/hairy, thin/soft, hollow/bladder, massive/soft, megabenthic/siliceous), and (2) appear to be abundant in these coral ecosystems. These broad range taxa will be subject to a molecular phylogenetic study, in order to address the identification of species and/or cryptic species. Then, an intra-specific (genetic) diversity and population structure/ phylogeographic study will be aimed to assess the connectivity of deep-sea reefs from the Mediterranean Sea up to Norway. We expect, from data about the genetic structure of species in several localities, to determine whether the populations are made up of one or a number of smaller local populations, and to estimate how isolated these local populations are from each other through calculation of the degree of gene flow occurring. These data have a direct application for management; if gene exchange exists between populations then the loss of areas of reef will not be damaging to the overall genetic diversity of the species.

Morphological and molecular variation in *Drarmacidon reticulatus* (Porifera, Demospongiae) from the Brazilian coast

Adriana Salgado{ XE "Salgado, Adriana" }^(1, 2*); Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }⁽²⁾

(1) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, São Cristóvão. 20940-040 Rio de Janeiro, RJ, Brazil; (2) Departamento de Biologia Celular e Genética, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524 - PHLC, sala 205, 20550-013, Rio de Janeiro, RJ, Brazil

Drarmacidon reticulatus (Ridley & Dendy, 1886) is widely distributed in the Tropical Western Atlantic Ocean from North Carolina (USA) to Southern Brazil (Santa Catarina state). The species shows high morphological plasticity in Southeastern Brazil, being massive or encrusting, with flush or elevated oscula, with smooth or reticulated surface, and soft or tough in consistency, although characterized by a bright red to orange color. The spicules are oxeas and styles in only one size category, and microscleres are absent. The purpose of this work was to test if Brazilian populations of *Drarmacidon reticulatus* belong to a single species or if they form a species complex. Combined morphological and molecular data were used to evaluate the specific status of Brazilian populations of *Drarmacidon reticulatus*. The genic regions used in this study were the two internal transcribed spacers (ITS 1 and ITS 2) of the ribosomal RNA (rRNA). The products of the PCR were submitted to the technique of single strand conformation polymorphism (SSCP). Four populations were studied: Caraguatatuba-SP; Búzios-RJ; Angra dos Reis-RJ and Bombinhas-SC. The skeleton was not informative to distinguish morphotypes, but the external morphology allowed distinction of eight morphotypes. DNA sequencing confirmed that the products of PCR corresponded indeed to the ITS region. Preliminary SSCP results showed that the banding patterns of ITS 1 and ITS 2 of both sympatric and allopatric specimens were similar, with low genetic variation. This suggests either that the different populations and morphotypes belong to the same biological species, or that the ITS region is not a good marker to distinguish intraespecific variation in this species. These results corroborated previous data on *Aplysina* and *Hymeniacidon* species of low ITS variability at the species level in sponges.

Morphologic and genetic variation in Brazilian *Amphimedon viridis*

Meiryelen Vieira da Silva{ XE "Silva, Meiryelen Vieira da" }^(*); Leonardo Luis Marques Assumpção{ XE "Assumpção, Leonardo Luis Marques" }; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }

Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524 - PHLC, sala 205, Maracanã, 20550-013, Rio de Janeiro, RJ, Brasil, meiryelen_vieira@hotmail.com; leonardo_assumpcao@hotmail.com; lobohajdu@alternex.com.br

Amphimedon viridis Duchassaing & Michelotti, 1864 (Niphatidae, Haplosclerida) populations from the Brazilian Northeast and Southeast coasts present two distinct morphotypes according to color trait: a common green and a rare reddish-brown morphotype. The holotype of *A. viridis* from the BMNH collection was used for comparison with Brazilian samples on loan from the MNRJ collection, originated from 3 - 230S. Morphological data were obtained from observations and documentation of details of skeletal architecture, spicule dissociation, tangential and perpendicular thick sections mounts and micrometric measurements. The internal transcribed regions (ITS-1 and ITS-2) from ribosomal RNA genes were amplified by PCR with specific primers and analyzed through the SSCP method. The arrangement of spongin fibers and spicules is identical between Brazilian samples and the holotype from Saint Thomas, Caribbean Sea. The thickness and length measurements were variable within and between collection sites among Brazilian populations. When samples were sorted by depth or light incidence, groups could be joined by similar spicule categories. Samples of *A. viridis* showed ITS1 and ITS2 with around 300 to 400 base pairs, ITS2 being smaller than ITS1. Results support the hypothesis that the two color morphotypes occurring along the Brazilian coast belong to the same species.

MV Silva - Bolsista PIBIC-UERJ; LLM Assumpção - Bolsista IC-FAPERJ

Finding the relevant scale: Clonality and small-scale genetic structure in *Crambe crambe*

Xavier Turon{ XE "Turon, Xavier" }^(*); Isabel Calderón{ XE "Calderón, Isabel" }⁽¹⁾; Sandra Duran{ XE "Duran, Sandra" }⁽¹⁾; Natalia Ortega{ XE "Ortega, Natalia" }⁽¹⁾; Mikel A. Becerro{ XE "Becerro, Mikel A." }⁽²⁾

(1) Dept. of Animal Biology (Invertebrates). Fac. of Biology. Univ. of Barcelona. 645, Diagonal Ave. 08028 Barcelona. Spain, xturon@ub.edu; (2) Centre d'Estudis Avançats de Blanes (CEAB-CSIC). Accés Cala S. Francesc, 14, 17300 Blanes (Girona). Spain

Sponges, having short-lived larvae and being able of asexual reproduction, are ideal models for the study of the small-scale distribution of genetic variability. Finding the relevant scale at which this variability is the highest is crucial for studies of population dynamics, reproduction, and conservation of sponges, yet this is a largely neglected field. We have performed a small-scale study of the population structure of the poecilosclerid sponge *Crambe crambe*. We genotyped 177 individuals found on the same rocky wall (interindividual distances ranging from 0 to 7 m) in a locality of the NW Mediterranean, using the microsatellite markers available for this species. The results were analysed by means of autocorrelation analyses. Genetic relatedness within the wall was then compared with the one obtained with sponges from the same locality (separated by tenths of meters) and from other localities (separated from tenths to hundreds of kilometres), genotyped in a previous study. Out of 177 individuals, 101 were unique genotypes and the remaining 76 formed 24 clones of 2 to 8 individuals each. The clones showed a strongly clumped distribution (intraclone distance: 19.97 ± 1.6 cm, mean \pm SE). The autocorrelation analyses using different indices of similarity and distance showed a drastic decrease in genetic relatedness over the first 2 metres of distance. If the contribution of clonality to this pattern was eliminated (by treating clonemates as one single sponge and recalculating physical distances), the trend was reduced but was still marked and significant. Interestingly, indices based on allele frequencies and an index based on allele sizes gave the same pattern, indicating that in this sponge the stepwise mutation model for microsatellites may apply. Similarities (or distances) with sponges of the same locality, or from other Mediterranean localities, were within the same range as those found in sponges 2-7 m apart. Only with samples from the Atlantic did the indices based on allele frequencies decrease (similarity) or increase (distance) significantly. It is concluded that asexual reproduction plays an important role in structuring populations in this species, despite low rates of fission reported in previous works. However, over and above the effects of clonality, a strong fine-scale genetic structure was present at distance ranges of less than 2 m, indicating extremely short dispersal of larvae, again in contrast with predictions of previous studies in this species. Still more surprisingly, our indices showed that genetic structure found at distances of several meters is of the same order as that corresponding to tenths of kilometres. In other words, relevant changes in genetic relatedness occur at extremely small scales in this species. If this case proves to be general, the sampling schemes usually used in population genetic studies are prone to miss key information about structuring processes in sponge populations.

**Larval dispersal behaviours and reproductive strategies determining the
population genetic structure of *Rhopaloeides odorabile***

Stephen Whalan{ XE "Whalan, Stephen" }^(1*); Dean Jerry{ XE "Jerry, Dean" }⁽¹⁾; Christopher
Battershill{ XE "Battershill, Christopher" }⁽²⁾; Rocky de Nys{ XE "Nys, Rocky de" }⁽¹⁾

(1) School of Marine Biology & Aquaculture, James Cook University, Townsville, QLD, Australia,
stephen.whalan@jcu.edu.au; (2) Australian Institute of Marine Science, Townsville, QLD, Australia

Elucidating patterns of larval dispersal and recruitment of marine organisms is fundamental in guiding our understanding of adult distributions in both time and space. This is particularly relevant for sponges that exhibit a mobile larval phase followed by a sedentary adult life. Indirect estimates of larval dispersal using molecular markers have not routinely been applied to sponges, but for those few species where such data exists larval dispersal has been inferred to be restricted and to contribute to population subdivision for these taxa. There is a conspicuous absence of data, however, that links quantitative assessments of larval behaviours and reproductive strategies to their corresponding population genetic structures. This study collected data on the larval dispersal behaviours, reproductive strategies and population genetic structure for the tropical Australian sponge *Rhopaloeides odorabile*. To develop an understanding of reproductive strategies samples were collected each month from 50 individuals, including 15 tagged sponges, which were repetitively sampled. Sample collections were conducted over a two year period and processed using standard histological methods to reveal the reproductive biology of this species. Larval dispersal behaviours were assessed by experiments on swimming speeds, larval longevity, and vertical orientation within the water column over time. Settlement preferences were also determined. Population genetic structure was elucidated using allozyme and mtDNA markers on samples collected using a hierarchical sampling design to assess genetic structure at spatial scales ranging from metres to hundreds of kilometres. Sample collections showed that *Rhopaloeides odorabile* broods larvae, which are 'dribble spawned' over several weeks in late January to late February. Larvae are released in the afternoon and are positively phototactic. They have the ability to control their vertical position within the water column and prefer to occupy surface waters before returning to the benthos approximately 24-36 hours post-release. Low levels of genetic differentiation amongst samples indicate that minimal population subdivision exists and suggests sufficient genetic exchange is occurring to prevent genetic divergence. This contrasts with population genetic structure for other viviparous sponge species. The ability of larvae to utilise wind driven surface currents for approximately 24-36 hours contributes to their ability to disperse over sufficient distances to promote genetic connectivity among reefs separated by hundreds of kilometres. This is the first study on sponges, within the Great Barrier Reef, that clearly demonstrates the influence of larval behaviours and reproductive strategies on population genetic structure.

**Phylogeography of tropical Indo-Pacific coral reef sponges: Insights into
population history and speciation**
(invited lecture)

Gert Wörheide{ XE "Wörheide, Gert" }

Geoscience Centre Göttingen, Dept. of Geobiology, Göttingen, Germany; and Queensland Centre for
Biodiversity, Queensland Museum, Brisbane, Australia, gert.woerheide@geo.uni-goettingen.de

The biogeographic history of marine invertebrates still remains one of the greatest mysteries in natural history. Surprisingly little is known about processes that lead to the divergence of marine invertebrate populations, including sponges, and ultimately speciation. However, recent advances in analytical methods, such as coalescent theory, now provide powerful tools that aid in unraveling those mysteries. Here, I will provide some background information on phylogeography and the Coalescent, and then focus on new results of such ongoing research on Indo-Pacific sponges. Previous biodiversity analyses of australasian tropical sponges at several spatial scales showed that latitudinal gradients in sponge diversity were not evident, whereas various environmental factors were prominent at small spatial (a) scales and biogeographic factors were prominent at larger spatial (b and g) scales of diversity. However, investigations based on morphometric data alone could not elucidate which biogeographic factors were responsible. Genealogical relationships, diversification patterns as well as migration rates between populations remained enigmatic. Here, the widely distributed Indo-Pacific coral reef sponge taxon *Leucetta 'chagosensis'* (Calcarea) was used as a model species to investigate phylogeographic and population genetic relationships of populations ranging from the northern Gulf of Aqaba (Red Sea, Egypt) to the Tuamotu Archipelago (Eastern Central Pacific), from Okinawa (Japan) to Brisbane (Australia), covering all of its known geographic range. Phylogeographic analyses of ribosomal DNA (ITS and partial 28S rDNA sequence types) showed that populations throughout the Indo-Pacific were phylogeographically structured into several clades, with two distinct ones discovered on the Great Barrier Reef and Queensland Plateau (Coral Sea) that appear to be more closely related to the remaining clades than to each other. Analyses of a newly developed single-copy nuclear intron-marker are congruent with rDNA results and provide enhanced resolution and robust support for the major clades detected, some of which were deeply divergent and reciprocally monophyletic. Using coalescent methods, demographic parameters, such as migration rates and directions, population sizes and population growth rates were estimated. Results were accomplished and compared with data from *Pericharax heteroraphis* (Calcarea), focussing on the Great Barrier Reef, and now allow further exploration of patterns and processes of diversification of marine coral reef sponges in the Indo-Pacific.

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Phylogeography and taxonomy of sponges from the Azores Archipelago and North Atlantic seamounts

Joana Xavier{ XE "Xavier, Joana" }^(1*); Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽²⁾;
Johannes Breeuwer{ XE "Breeuwer, Johannes" }⁽¹⁾; Antonio Frias Martins{ XE "Martins,
Antonio Frias" }⁽³⁾

(1) Institute for Biodiversity and Ecosystem Dynamics (IBED). University of Amsterdam. P.O.Box 94766, 1090 GT. Amsterdam, The Netherlands, xavier@science.uva.nl; breeuwer@science.uva.nl; (2) Zoologisch Museum. University of Amsterdam. P.O.Box 94766, 1090 GT. Amsterdam, The Netherlands, soest@science.uva.nl; (3) Seccao de Anatomia e Taxonomia Zoologicas. Departamento de Biologia, Universidade dos Acores. Apartado 1422 9501-855. Ponta Delgada. Portugal, frias@notes.uac.pt

The way that marine species with low dispersal capabilities colonize remote habitats such as oceanic islands and the effect of macro- and microevolutionary processes in the genetic structure of such populations remain poorly studied. Here we present a study of faunal evolution in oceanic island marine biota using sponges as model taxa. At the spatial scale two levels are taken into account: a large spatial scale (Northeast Atlantic and Mediterranean); and a small spatial scale at an intra-archipelagic level. Specimens from some North Atlantic shallow seamounts (Gettysburg and Ormonde peaks, Ampere seamount, D. Joao de Castro and Dollabarat Banks) occurring in the intervening areas are also included in order to test for colonization through a stepping stone model. This project's aims are to: (1) check the biogeographic affinities of the littoral sponge fauna from the Azores; (2) test for population differentiation in sponge species at different spatial scales and try to determine probable directions of distribution and common ancestry; (3) assess the genetic structure of island populations (within population, between populations within the island, between populations from different islands and different island groups) and compare it with continental populations; (4) check for congruence of morphological and molecular data in the phylogenetic relationships of these populations; (5) check the role played by the shallow seamounts in the intervening areas in long distance dispersal of these species (stepping-stones?). A total of 6 species with a wide distributional range and different reproductive strategies were selected to perform this study: *Erylus discophorus*, *Cliona celata*, *Cliona viridis*, *Phorbast fictitius*, *Tedania anhelans* and *Petrosia ficiformis*. Preliminary results are presented about the phylogeography and genetic structure of populations of the poecilosclerid sponge *Phorbast fictitius*. A total of 86 specimens from 8 locations separated between 10 and 1500 km, were subjected to taxonomic and molecular analysis. For the molecular work the nuclear ribosomal internal transcribed spacers (ITS-1 and ITS-2) and the mitochondrial DNA gene cytochrome oxidase subunit I (COI) were sequenced. Sequence data was transformed in haplotypes and used to perform phylogenetic and nested clade analysis in order to infer the patterns and processes involved in the geographical distribution of genetic variation of these populations and therefore contribute for a better understanding of evolution at oceanic islands.

Growth and morphology of a reef-forming glass sponge, and implications for recovery from widespread trawl damage

William C. Austin{ XE "Austin, William C." }⁽¹⁾; Kim W. Conway{ XE "Conway, Kim W." }⁽²⁾,
J. Vaughn Barrie{ XE "Barrie, J. Vaughn" }⁽²⁾; Manfred Krautter{ XE "Krautter, Manfred" }⁽³⁾

(1) Marine Ecology Centre & Khoyatan Marine Laboratory, 9835 Seaport Place, Sidney, B.C., Canada, baustin@mareco.org; (2) Pacific Geoscience Centre, Geology Survey of Canada, P.O. Box 6000, Sidney, B.C., Canada, conway@pgc-gsc.nrcan.gc.ca; (3) Institut für Geologie und Paläontologie der Universität Stuttgart, Herdweg 51, D-70174, Stuttgart, Germany, manfred.krautter@geologie.uni-stuttgart.de.

Hexactinellid sponge reefs are found widely distributed on the western Canadian shelf in both Georgia and Queen Charlotte Basins. Multibeam surveying has provided accurate maps of sponge reef distribution in water depths of 100 to 240 m. The largest reef complex is roughly 40 kilometres long and has been growing for about 9,000 years with bioherms commonly up to 15 m in height. These reefs form a stable and complex habitat for many species of invertebrates and fish, though trawling or bottom dragging has damaged reefs in most areas. Recovery of these damaged or destroyed reefs depends on many interrelated factors including sponge larval settling and survival, sponge growth rates and the balance between suspended sediment trapping by the sponges and smothering by sedimentation. Shallow and accessible populations of *Aphrocallistes vastus*, an important reef former at all known reef sites, are present in southern inshore waters of BC allowing these sponges to be studied as proxies for the sponges of the vast, but remote, northern reefs. Saanich Inlet, a fjord near Victoria, BC is one such site where these sponges are found in 25-35 m depths and where dive surveys at two study areas have been undertaken. Here divers could directly measure growth rates in surface area over time. Growth only occurred in regions where the spicules were not fused together. However, when a fused portion of a sponge was artificially removed, regeneration sealed the opening. The largest sponge measured was 3.4 m long by 0.5 m wide by 1.1 m high. Without measurements of growth rates over a large size range, we can only surmise that such a large sponge is, at least, many decades old. What is the impact of sedimentation on the sponges? *Aphrocallistes vastus* characteristically has mitten like out-pouchings with the long axis oriented vertically. This area contains fused skeleton. If there is an un-fused portion, it is at the upper and/or outer margin. Sediment was artificially added to the water where a sponge was maintained in the laboratory. Sediment only collected on the horizontal surfaces which had a considerably smaller area than the vertical surfaces. In effect the ostia over most of the surface area would not be clogged by sediment. In one case the un-fused tissue lying under sediment opened up and the sediment fell into the sponge. The opening subsequently closed. Otherwise, no sediment or carmine particles were observed to move through the tissue into the sponge which was actively pumping water at this time. At both sites divers observed that the basal area of approximately 50% of living sponges was mainframe skeleton only. Dissolution of such skeletons is evidently slow. The living syncytial tissue may die back, or alternatively, may move away from the base as the sponge grows. Such a mechanism could explain how sponges in reefs continue to grow without being smothered by sediments.

**The marine lakes enclosed in Karsts Islands of the Ha Long Bay (North Vietnam):
A unique environment for sponges**

Francesca Azzini{ XE "Azzini, Francesca" }⁽¹⁾; Barbara Calcinai{ XE "Calcinai, Barbara" }⁽¹⁾;
Carlo Cerrano{ XE "Cerrano, Carlo" }⁽²⁾; Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽¹⁾;
Maurizio Pansini{ XE "Pansini, Maurizio" }^(2*)

(1) Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, 60131
Ancona, Italy; (2) Dip. Te. Ris. Università di Genova, Corso Europa 26, 16132 Genova, Italy,
mpansini@dipteris.unige.it

Salt water lakes enclosed within karsts islands of the Ha Long Bay in the northern part of the Tonkin Gulf are a peculiar environment for marine life. Some lakes show a communication with the neighbouring sea, evidenced by the flow of tidal streams. Others, the most interesting ones, are apparently closed but connected to the sea through the cavities of the karsts system. A sponge assemblage of at least 26 species, 11 of which were never found outside, was recorded in the marine lakes. Some of the recorded species occur also on the coasts of the Ha Long Bay islands, characterized by shallow depth (less than 7 m), murky waters with abundant suspended sediment, patchy and impoverished reefs, very low water movement. Forty-five demosponge species were identified in total in the area. Two species, belonging to the genera *Plicatellopsis* and *Protosuberites*, were present only in Hang Du I and Bui Xam lakes which are devoid of any apparent connection with the sea. A coastal, visual transect was performed in the Hang Du I lake for a first assessment of sponge quantity and distribution. The Ha Long Bay marine lakes are very shallow basins, less than 8 m deep, with a bottom covered by mud and vegetable debris. Therefore sponges can live at the base of the coastal rocky cliffs and on a coarse detritus belt down to 3-4 m depth. Clionaid sponges are abundant and diverse, due to the carbonate substrate and to the presence of large sessile bivalve mollusc communities. Patches of mangroves were seldom observed, while isolated colonies of massive corals are frequently found. No lake is in pristine condition, because oyster harvesting and aquaculture assays are practised by local people, which dug artificial canals to enhance sea water exchange. Extreme variations of the environmental conditions yearly occur, due to the tropical climate with heavy monsoon rains. A thermal crisis was recorded at the end of the summer in the Hang Du I lake, with bottom temperatures as high as 36°C and a 1.5 m thick rainwater layer at the surface. Sponges can stand these conditions but undergo important rearrangements with a late summer degeneration followed by a very quick fall and winter growth. The results of two spring (April 2003 and 2004) and one summer (September 2004) campaigns are here reported. A two year study with seasonal monitoring is expected to be founded.

Biodiversity of sponges in marine caves on Dalmatian islands (Croatian coast of the Adriatic Sea)

Tatjana Bakran-Petricioli{ XE "Bakran-Petricioli, Tatjana" }^(1*); Donat Petricioli{ XE "Petricioli, Donat" }⁽²⁾; Irena Bogdanovic{ XE "Bogdanovic, Irena" }⁽¹⁾; Petra Žvorc{ XE "Žvorc, Petra" }⁽¹⁾; Sandro Dujmovic{ XE "Dujmovic, Sandro" }⁽¹⁾; Veljko Vorkapic{ XE "Vorkapic, Veljko" }⁽¹⁾; Jean Vacelet{ XE "Vacelet, Jean" }⁽³⁾

(1) Division of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia, tatjana.bakran-petricioli@zg.htnet.hr; (2) Oikon Ltd. - Institute for Applied Ecology, Prekratova 20, HR-10000 Zagreb, Croatia, dpetricioli@oikon.hr; (3) DIMAR, Centre d'Océanologie de Marseille, Station Marine d'Endoume, Rue de la Batterie des Lions, F-13007 Marseille, France, jvacelet@com.univ-mrs.fr

Marine caves, half-caves and submarine passages are numerous along the Croatian coast due to geomorphologic characteristics of the eastern coast of the Adriatic Sea and the Holocene sea transgression. Sponges are an important group of organisms, which inhabits these submarine habitats. In this work five caves on the southwestern coasts of Premuda, Dugi otok and Garmenjak Veli islands were prospected. Sponge samples had been collected for five years (2000-2005) during numerous SCUBA dives. Altogether more than 80 sponge species were determined on the basis of microscopic slides of skeletal structures. Although all the species were not recorded in all the caves, a high level of similarity was noted among the caves. Some of the species, such as *Thymosiopsis conglomerans* Vacelet *et al.* and *T. cuticulatus* Vacelet & Perez were recorded for the first time in the Adriatic Sea. Up to now the biodiversity of sponge species in the caves along the Dalmatian coast was not well studied and the results of our research show that it deserves further attention.

Freshwater habitats indicated by sponge assemblages at the Amazonian protected area of "Meandros do Rio Araguaia", Brazil

Twiggy Cristina A. Batista{ XE "Batista, Twiggy Cristina A." }^(1*); Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }⁽²⁾; Maria da Graça G. Melão{ XE "Melão, Maria da Graça G." }⁽³⁾

(1) Programa de Pós-Graduação em Ecologia e Recursos Naturais, UFSCar, Rodovia Washington Luis, Km 235 CP 676, 13565-905 São Carlos, SP, Brasil, twiggybat@hotmail.com; (2) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, R. Dr. Salvador França, 1427, Jardim Botânico. Porto Alegre, RS, 90690000, cvolkmer@fzb.rs.gov.br; (3) Depto de Hidrobiologia/CCBS, UFSCar, Rodovia Washington Luis, Km 235 CP 676, 13565-905. São Carlos (SP) Brasil, dmgm@power.ufscar.br

A previous survey for freshwater sponges at the lower reach of river Araguaia evinced two assemblages of sponges, one encrusting the river rocky bottom and the other the seasonally flood forest. A second step has now been taken towards an extension of the survey into a protected area that encompasses a stretch of the river with its complex network of meanders and tributaries. The research aims to explore the correlation of the sponge assemblages with some of the outstanding aquatic habitats and their physical, chemical and productivity characteristics as well as the spicule contents in the sediments. We report now the first results bearing on the correlation of the sponge assemblages with the different tributaries and habitats sampled. The sponge sampling was undertaken at low (2002-2004) and high (2004-2005) water periods at 27 GPS referred stations encompassing several exposed rocky bottoms or rocky river banks, ponds left behind the river banks by the flood, and the riparian flooded forest. The sponges were collected by hand, preserved dried, deposited and catalogued in the Porifera Collection of Museu de Ciências Naturais. Taxonomic analysis of the specimens was carried out after microscopic examination following preparation of the skeletal spicules and gemmules after dissociation with Nitric Acid. Twenty-one species of freshwater sponges were detected. The maximum specific richness reached from 01 to 11 species per station (medium \pm DP = 5.3 ± 2.6). Two assemblages of sponges were detected. To the one belong the sponges which adhere to the exposed surfaces as well as to the crevices in rocky or woody hard substrates subjected to the rivers strong currents at high waters and which form grayish to whitish hard, shallow crusts: *Oncosclera spinifera*, *Oncosclera navicella*, *Spongilla spoliata*, *Corvospongilla seckti*, *Drulia ctenosclera*, *Drulia uruguayensis* and *Metania reticulata*. To the second group belong sponges with spheric to sub-spheric, brown or yellowish, extremely fragile skeletons adhering to the flooded vegetation: *Saturnospongilla carvalhoi*, *Trochospongilla paulula*, *T. delicata*, *T. gregaria*, *T. pennsylvanica*, *T. variabilis*, *T. lanzamirandai*, *Acalle recurvata*, *Heteromeyenia* sp and *Radiospongilla amazonensis*. The Principal Component Analysis evinced that the sponge assemblages were in fact related to the sampling stations with 41% of the variations explained by the two first axes. The first one put apart the stations at the rivers bottom (rivers Cristalino and Araguaia), represented by *Oncosclera spinifera* and *Corvospongilla seckti*, from those of the lakes at the flooded banks represented by particularly *Trochospongilla gregaria*, *Trochospongilla paulula* and *Saturnospongilla carvalhoi*. The second axis recognized the species accordingly to the river considered, setting apart the assemblages of river Cristalino (with the remarkable presence of *Acalle recurvata*) from those of river Araguaia (with *Trochospongilla delicata*).

C. Volkmer-Ribeiro: Fellow researcher of CNPq

Sponges from a submarine canyon in the Argentinian Sea

Marco Bertolino{ XE "Bertolino, Marco" }⁽¹⁾, Laura Schejter{ XE "Schejter, Laura" }⁽²⁾, Barbara Calcinai{ XE "Calcinai, Barbara" }⁽³⁾, Carlo Cerrano{ XE "Cerrano, Carlo" }⁽¹⁾, Maurizio Pansini{ XE "Pansini, Maurizio" }^(1*), Claudia Bremec{ XE "Bremec, Claudia" }⁽⁴⁾

(1) Dipartimento per lo studio del Territorio e delle sue Risorse, Corso Europa, 26, 16132, Genova, Italy, marco.bertolino75@libero.it; cerrano@dipteris.unige.it; mpansini@dipteris.unige.it; (2) Laboratorio de Bentos, Instituto Nacional de Investigación y Desarrollo Pesquero, Paseo Victoria Ocampo 1, (B7602HSA) Mar del Plata, Argentina, schejter@inidep.edu.ar; (3) Dipartimento di Scienze del Mare, Via Brece Bianche, 60131, Ancona, Italy, b.calcinai@univpm.it; (4) Laboratorio de Bentos, Instituto Nacional de Investigación y Desarrollo Pesquero, Paseo Victoria Ocampo 1, (B7602HSA) Mar del Plata, Argentina, cbremec@inidep.edu.ar

During an assessment research cruise (R/V "Oca Balda", INIDEP, April 2005) of the Patagonian scallop (*Zygochlamys patagonica*) a submarine canyon was discovered using a multibeam SIMRAD EM1002 sonar. The canyon is positioned at 43°35'S and 59°33'W, close to the southern commercial scallop beds, at 360 m depth, in the Argentine Sea. The existence of submarine canyons on the continental shelf of Argentina was already known, but their exact location, their number, their edaphic and biotic conditions are still unstudied. A sample of the benthic community was collected at the "head" of the canyon, using a non-selective dredge 2.5 m wide. The sample was frozen on board and was sorted and studied at the INIDEP laboratory. Together with Patagonian scallops, gastropods, asteroids, sea urchins, ophiuroids, crustaceans, and polychaetes, many sponges were found. Sponges represented 24% of the total biomass. They were fixed in formaldehyde and then preserved in alcohol. Species identification was made on preserved material by classical procedures considering spicules and skeletal characteristics. A total of 9 species of demosponges were identified: *Tedania* (*Trachytodania*) *mucosa*, *Tedania* (*Tedaniopsis*) *massa*, *Tedania* (*Tedaniopsis*) *charcoti*, *Myxilla mollis*, *Guitarra dendy*, and *Tetilla leptoderma*. Some of them represent new records for the Argentine Sea and in particular *Guitarra dendy* was previously known only from the Antarctic. Three other species: *Tedania* (*Tedaniopsis*) sp., *Myxilla* sp., and *Pseudosuberites* sp. are probably new for science.

Silica banding in the deep-sea lithistid sponge *Corallistes undulatus*: Investigating the influence of diet and environmental conditions on growth

Michael J. Ellwood{ XE "Ellwood, Michael J." }^(1*); Michelle Kelly{ XE "Kelly, Michelle" }⁽²⁾;
Bertrand Richer de Forges{ XE "Forges, Bertrand Richer de" }⁽³⁾

(1) National Institute of Atmospheric and Water Research (NIWA) Ltd, P.O. Box 11 115, Gate 10 Silverdale Road, Hillcrest, Hamilton, New Zealand, m.ellwood@niwa.co.nz; (2) National Centre for Aquatic Biodiversity and Biosecurity, National Institute of Atmospheric and Water Research (NIWA) Ltd, Private Bag 109695, 269 Khyber Pass Road, Newmarket, Auckland, New Zealand, m.kelly@niwa.co.nz; (3) Connaissance des Faunes et Flores Marines, Institut de Recherche pour le Développement, B.P. A5 98848 Nouméa Cedex, Nouvelle-Calédonie, richer@noumea.ird.nc

Unraveling the life histories of deep-ocean organisms has recently shown that the deep-ocean environment is not as benign or as constant as was previously thought; it varies considerably, both chemically and physically, and greatly influences the ecology of organisms therein. Food supply strongly influences the growth of deep-sea organisms, and we know that the 'rain' of particulate organic matter (POM) to the ocean floor is strongly coupled with climate. How do we gauge the influence of current climate on such 'food-rain' events, and more importantly, can we look at these events in the organism's life history? The major hurdle is finding a sessile organism with a long life span and a skeleton that archives the history of the animal's diet and growth. A large foliose vase-shaped specimen of the lithistid sponge *Corallistes undulatus* Lévi & Lévi, collected from a seamount located south of New Caledonia, was X-rayed and found to have 144 light and dark density band pairs within the siliceous skeleton. An immediate question that arose from this exciting discovery was: Are these bands annual in nature? Silicon-32 dating of the sponge has shown that band pairs represent roughly annual depositions of silica, confirming the seasonal nature of silica deposition and the organism's growth. A further critical question that arose from this discovery of density banding along the growing axis of the sponge was: What are the environmental mechanisms and sources of variability that facilitate the formation of silica bands in a supposedly constant environment? We have shown recently that the zinc:silicon ratio in a sponge can be used as a proxy for the rain of POM to the deep ocean. High-resolution zinc records from silica sections removed along the axis of growth suggest that the rain of particulate organic matter (POM) on the sponge as a food source has been low over the life of the sponge. Isotopic analysis of the carbon ($\delta^{14}\text{C}$ and $\delta^{13}\text{C}$) trapped within the silica matrix was used to help elucidate the source of carbon acquired by the sponge. Surprisingly, the $\delta^{14}\text{C}$ results suggest that surface derived POM is not the only source of carbon to our sponge. A high-resolution manganese record extracted from silica sections removed along the axis of growth showed subtle decadal variations that correlate well to other environmental records and indices. This record suggests that short and longer-term climatic events such as the Interdecadal Pacific Oscillation (IPO) and the El Niño-Southern Oscillation (ENSO), are a source of environmental variation that is likely to effect sponge food supply and growth. Using a multifaceted approach, we will show that the seasonal growth bands seen within *Corallistes undulatus* require a good understanding of the links between food supply, and silica availability; two parameters that underpin the ecology of these extraordinary organisms.

Deep-water sponges and their associated fauna collected off the Southeastern United States

Cara Fiore{ XE "Fiore, Cara" }; Pamela Cox Jutte{ XE "Jutte, Pamela Cox" }

Department of Biology, Grice Marine Lab College of Charleston, 205 Fort Johnson Rd, Charleston, SC 29412, USA, cfiore1@edisto.cofc.edu; juttep@yahoo.com

Sponges are known to serve as hosts to many species that live and reproduce within them and may therefore be considered ecological communities. Sponges also constitute a major part of the benthic epifaunal community on the continental shelf off the southeastern United States; however, little is known about these sponges and the communities they harbor. In this study, the associated fauna from a variety of sponges collected from the continental shelf and slope of the southeastern U.S. at depths ranging from 58 to 800 m were examined. Sponges were collected by the submersible the Johnson Sea Link II. Seventeen sponges comprising eight species (*Ircinia campana*, *Topsentia* sp., *Geodia* sp., *Characella* sp., *Erylus* sp., *Apylsina archeri*, *Cliona* sp., and *Schulzeviella* sp.) have been fully dissected and all organisms identified and counted. Another sponge, *Pheronema* sp. was collected and identified although the associated faunal community was not examined. Each sponge was identified to the lowest possible taxonomic level using spicule preparations and sectioning. Diversity (H') and abundance of sponge associates varied greatly among hosts; densities of all associates ranged from 0.2 to 5842 per 500 ml. Polychaete worms were the most common organisms found in these hosts, with one species, *Haplosyllis spongicola*, being especially abundant in *Ircinia campana*, *Topsentia* sp., and *Cliona* sp. The amphipods *Erichthonius brasiliensis* and *Leucothoe spinicarpa* as well as decapods such as snapping shrimp (*Synalpheus* sp.) and crabs were also common. The number of inquiline taxa increased as sponge size increased. Weak positive trends were also found between the diversity and abundance of associates and increasing canal diameter and sponge wall thickness. Results of this study illustrate the important contribution that sponges make to the biodiversity of many benthic habitats. It has also added to the sparse previously documentation of sponge taxonomy in this area and will hopefully initiate future in-depth studies of sponges inhabiting the continental shelf and slope off of the southeastern United States.

Deep-sea sponges from Southeastern Brazil: Campos Basin Deep-Sea Environmental Project (CENPES/PETROBRAS)

Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(1*); Daniela A. Lopes{ XE "Lopes, Daniela A." }⁽¹⁾; Mariana S. de Carvalho{ XE "Carvalho, Mariana S. de" }⁽¹⁾; Ana Paula Falcão{ XE "Falcão, Ana Paula" }⁽²⁾; Helena P. Lavrado{ XE "Lavrado, Helena P." }⁽³⁾; Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾; Henry M. Reiswig{ XE "Reiswig, Henry M." }⁽⁴⁾; Konstantin Tabachnick{ XE "Tabachnick, Konstantin" }⁽⁵⁾;

(1) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil, hajdu@acd.ufrj.br; (2) CENPES - Petrobras, Avaliação e Monitoramento Ambiental, Ilha do Fundão, Cidade Universitária, 21949-900, Rio de Janeiro, RJ, Brazil; (3) Instituto de Biologia, Departamento de Biologia Marinha, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Cidade Universitária, CCS, 21941-590, Rio de Janeiro, RJ, Brazil; (4) Department of Biology, University of Victoria, and Natural History Section, Royal British Columbia Museum, P.O. Box 3020 Stn CSC, Victoria, B.C. V8W 3N5, Canada, hmreiswig@shaw.ca; (5) Institute of Oceanology of Academy of Sciences of Russia, Nahimovky 36, RU-117218 Moscow, Russia, tabachnick@mail.ru

This study reports the sponges collected during Petrobras deep-sea environmental assessment of Campos Basin, SE Brazil. The collections were made between 02/2002 and 08/2003 in the oceanic region of Campos Basin, between 700 and 2000m depth. The sampling design included 18 stations, distributed around several oil fields under commercial exploitation. A total of 110 specimens were collected with an otter-trawl semi-ballon (OTSB) between 1130 and 1630m depth, 58 belonging to the Class Demospongiae (16 species) and 52 to the Class Hexactinellida (13 species). Sponges were found in nine stations, comprising 6% of the total megafauna biomass trawled and ca. 40% of specimens were associated to deep-sea corals (specimens are attached to pieces of coral). The demosponges collected were: *Acanthella* aff. *flageliformis*, *Atergia* sp., *Bubaris* aff. *vermiculata*, *Clathria* sp., *Desmacella* aff. *informis*, *Echinostylinos* aff. *mycaloides*, *Geodia* aff. *australis*, *Haliclona* sp., cf. *Halicometes* sp., *Iophon* sp., *Lyssodendoryx* sp., *Penares* sp. nov., *Radiella* sol, *Stelletta* sp., *Tetilla* sp. and *Vulcanella cribrifera*. The hexactinellids were: cf. *Bathydorus* sp., *Chonelasma choanoides*, cf. *Conorete* sp., *Euplectella suberea*, *Eurete* sp. nov., *Euretidae* sp., *Farrea occa*, *Farrea* sp. nov., *Hyalonema* sp., *Rossellidae* sp. 1 and sp. 2, *Saccocalyx pedunculatus* and *Sympagella* sp. nov. Six demosponges and eight hexactinellids were new records for the SW Atlantic, some of which at family level (*Bubaridae*, *Euretidae*, *Farreidae* and *Phellodermidae*). Most sponges were concentrated in a few stations, where deep-sea corals occurred. This preliminary list reveals the outstandingly poor knowledge of the deep-sea sponge fauna at Campos Basin in particular, and the entire SW Atlantic in general, suggesting the area to be a highly rewarding front for new deep-sea research.

What is characteristic for the deep-sea sponge fauna in the Weddell Sea? First results from the ANDEEP I, II and III expeditions, Antarctica

Dorte Janussen{ XE "Janussen, Dorte" }^(1*); Ole Secher Tendal{ XE "Tendal, Ole Secher" }⁽²⁾

(1) Marine Evertebraten I, Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany, Dorte.Janussen@senckenberg.de; (2) Zoological Museum, Universit tsparken 15, DK-2100 Copenhagen, Denmark, ostendal@snm.ku.dk

The bathymetric distribution of Antarctic sponges is markedly different from that along other continents, probably because of the combination of special topographic and hydrographic conditions. Many otherwise shallow-shelf taxa extend to larger depths than expected, and part of the deep-sea fauna ascends onto the slope and outer shelf. During the ANDEEP I and II expeditions (26.01-4.04.2002), 49 sponge species were taken at depths between 800 and 5000 m: 29 Demospongiae (1 new sp., 11 new for Antarctica), 4 Calcarea (3 new spp.) and 16 Hexactinellida (5 new spp., 6 new for Antarctica). Several hexactinellid species, originally described only on the basis of a fragment, were re-described and partly revised on the basis of the new and better material. Most surprising were the deep-sea Calcarea, reported for the first time in Antarctica, collected during ANDEEP II and III at depths down to 4400 m. The eurybathic shelf sponge fauna, mainly Demospongiae, extends down to about 3000 m, and a few deep-sea species (*Chondrocladia* spp.) were found at shallower depths (800 - 1000 m). Thus, the bathyal sponge fauna in Antarctica is characterized by a mixture of shallow and deep-sea fauna elements. The sponge community of the abyssal plain (4000-5000 m) is fundamentally different from that of the slope: whereas sponges in the bathyal are mostly shelf species, many endemic to Antarctica, the abyssal sponge community comprises, at least in certain areas, mainly predatory demosponges (Cladorhizidae) and shows affinity to the sponge faunas of other deep-sea areas. During the recent ANDEEP III expedition (21.01. - 06.04.2005), large scale transects within the following areas were sampled: Cape Basin - Atka Bay, Kapp Norwegia, central Weddell Sea, Powell Basin and Drake Passage. The first evaluation of the sponges points towards some new conclusions compared with the results of sampling during ANDEEP I and II: the deep-sea sponge fauna off Kapp Norwegia and in the Weddell Sea differs substantially from that of the adjacent areas (Powell Basin, Drake Passage, Scotia Sea and Cape Basin): The first is highly diverse, rich in Hexactinellida and large eurybathic Demospongiae and yields some Calcarea as well, whereas the latter comprises almost exclusively very small predatory sponges (Cladorhizidae). Although calcareous sponges are rare in the Antarctic, they seem to constitute a constant component of the Antarctic deep-sea fauna. Large size of Antarctic sponges is not restricted to the shelf areas. The deep-sea in the Antarctic is heavily undersampled. Therefore, it is currently not possible to decide to which degree we are dealing with local phenomena of the Weddell Sea or with a general feature of the entire Antarctic Ocean.

Freshwater sponges in Estonia: Distribution, morphological and genetic identification

Annika Lopp{ XE "Lopp, Annika" }^(1*); Tõnu Reintamm{ XE "Reintamm, Tõnu" }⁽¹⁾; Kerli Vallmann{ XE "Vallmann, Kerli" }⁽²⁾; Mailis Päril{ XE "Päril, Mailis" }⁽²⁾; Valdek Mikli{ XE "Mikli, Valdek" }⁽³⁾; Evelyn Richelle-Maurer{ XE "Richelle-Maurer, Evelyn" }⁽⁴⁾; Merike Kelve{ XE "Kelve, Merike" }^(1,2)

(1) Department of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia, annika@kbfi.ee; tonu@kbfi.ee; merike@kbfi.ee; (2) Institute of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 19086 Tallinn, Estonia, kerli.vallmann@mail.ee; mailis@kbfi.ee; (3) Faculty of Chemical and Materials Technology, Tallinn University of Technology, Ehitajate tee 5, 19806 Tallinn, Estonia, miku@staff.ttu.ee; (4) Laboratoire de Physiologie Cellulaire, IBMM-CP 300, Université Libre de Bruxelles, Rue des Professeurs Jeener et Brachet 12, B 6041 Charleroi, Belgium, emaurer@telenet.be

Up to now, only a few studies have been carried out on the occurrence of freshwater sponges in lakes and rivers of Estonia. Three species, *Spongilla lacustris*, *Ephydatia fluviatilis* and *Ephydatia muelleri*, described already by Dybowski had been found from a few localities. This study is a first comprehensive survey on the presence of sponges in different rivers throughout Estonia. In addition to morphological identification, genetic identification based on sequencing of the extended D3 domain of the 28S rDNA was used for the confirmation of the identity of sponge species. The sponges were collected from 31 rivers at 56 sites throughout Estonia during the summers/autumns of 2004 and 2005. The number of sponge samples taken from one locality varied from 1 to 15. Some sponge material from each sample was dissolved in concentrated HNO₃ to remove organic matter. The resulting spicule preparations were studied by using light microscopy and scanning electron microscopy. The material for DNA analysis was frozen in liquid nitrogen and stored at -20°C. Genomic DNA was extracted and the D3 domain of the 28S rDNA together with approximately 150 bp of 3' core sequence was amplified. The amplification products were purified and subjected to direct sequencing. The most frequently found sponge in the rivers was *E. fluviatilis*, collected from 39 sites in all regions throughout Estonia. *S. lacustris* and *E. muelleri* were present at 20 and 17 collecting sites, respectively. These three species were represented at each locality either as a single species or occurring together with other species. Co-occurrence of *E. fluviatilis* and *S. lacustris* was observed at 11 sites; *E. fluviatilis* and *E. muelleri* were found together at 9 sites and *S.l.* and *E.m.* at 7 collecting sites. In addition, we found the fourth species, *Eunapius fragilis*, from several localities in Central and Southern Estonia. This is a first record of *E. fragilis* in Estonia. We studied if a molecular approach based on sequencing of the extended D3 domain of the 28S rDNA could be used for the confirmation of the identity of the freshwater sponge species. As the particular data for Spongillidae were missing in 28S Genbank, we first obtained the reference sequences, using samples from the sponge type species, collected in Belgium. The sequenced DNA fragments of the samples, collected in Estonia, showed an identity with those from the corresponding spongillid type species. The present study demonstrates the presence of four freshwater sponge species, *E. fluviatilis*, *E. muelleri*, *S. lacustris* and *E. fragilis*, in the rivers of Estonia. While the three previously reported species are widely distributed throughout Estonia, *E. fragilis* was found to be a less frequent species. The results of the DNA analysis confirmed the adequacy of the chosen DNA fragment for the discrimination between the studied freshwater sponge species.

A new morphological trait for Northern Australian Pectispongillas

Renata Manconi { XE "Manconi, Renata" }^(1*); Tiziana Cubeddu { XE "Cubeddu, Tiziana" }⁽¹⁾;
Cristina Ferretti { XE "Ferretti, Cristina" }⁽²⁾; Roberto Pronzato { XE "Pronzato, Roberto" }⁽²⁾

(1) Dipartimento di Zoologia e Antropologia Biologica, University of Sassari, Via Muroni, 25, I-07100 Sassari, Italy, r.manconi@uniss.it; (2) DIPTERIS, Dipartimento per lo Studio del Territorio e delle sue Risorse, University of Genoa, Corso Europa, 26, I-16132 Genoa, Italy

A population of freshwater sponges discovered in a dry creek of the Kakadu Park displays a peculiar morphological trait. Sponges in the dry season were represented exclusively by hard white bodies, scattered and strictly adhering to the rocky bed. They are shaped as hemispherical inverted cups with a single conspicuous circular aperture at the apex. The cup's wall appears by SEM as a compact and dense arrangement of megascleres (acanthoxeas and acanthostrongyles) and rare microscleres (acanthoxeas) embedded more or less tangentially in a quite abundant spongin layer. The inverted cup is empty and the cavity bears some free gemmules with traits typical for the genus. A comparative analysis of these sponges vs. the other species of pectispongillas, from the Indo-Australian geographic range, showed that they share all gemmular traits, while the reversed cup is absent in all the previously described species. If the new trait could be considered diagnostic at the genus level, the sponges do not match the definition of the genus *Pectispongilla*. The morpho-functional role of the reversed cup could be comparable to that performed by gemmular cages of megascleres found in other genera. It seems however a new trait never described before for the suborder Spongillina.

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New records of *Petrobiona massiliana* (Porifera, Lithonida) in the W-Mediterranean Sea

Renata Manconi { XE "Manconi, Renata" }^(*); Fabio Ledda { XE "Ledda, Fabio" }; Maria Francesca Ortu { XE "Ortu, Maria Francesca" }; Annalisa Serusi { XE "Serusi, Annalisa" }; Giacinta Stocchino { XE "Stocchino, Giacinta" }

Dipartimento di Zoologia e Antropologia Biologica, University of Sassari, Via Muroni, 25, I-07100 Sassari, Italy, r.manconi@uniss.it

We report new findings of *Petrobiona massiliana* Vacelet & Lévi, 1958 from a NW Sardinian Marine Protected Area extremely rich in karstic submerged caves. *Petrobiona massiliana* is a rare troglobious calcareous sponge endemic to the Mediterranean Sea. Dark zones of four almost horizontal caves, out of eight surveyed ones, are characterized by the presence of conspicuous populations of *P. massiliana*, also in large facies, associated to serpulids, bryozoans and lithistid sponges. At a phenotypic level *P. massiliana*, typically of small size, displays a notable morphological plasticity with a growth form ranging from encrusting to subspherical or multilobed according to the cave typology. A comparative analysis vs. morphological and ecological data of previous records tests the presence of *P. massiliana* in a more wide environmental range. The new records enlarge the geographic range of *P. massiliana* to the Sardinian Sea and suggest the need of more investigations on diversity of the relic spongefauna hosted in dark caves.

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Life styles and strategies of freshwater sponges: A morpho-functional and biogeographic study
(invited lecture)

Renata Manconi { XE "Manconi, Renata" }^(1*); Roberto Pronzato { XE "Pronzato, Roberto" }⁽²⁾

(1) Dipartimento di Zoologia e Antropologia Biologica, University of Sassari, Via Muroni, 25, I-07100 Sassari, Italy, r.manconi@uniss.it; (2) DIPTERIS, Dipartimento per lo Studio del Territorio e delle sue Risorse, University of Genoa, Corso Europa, 26, I-16132 Genoa, Italy

Taxa richness of the six families belonging to the suborder Spongillina (Demospongiae, Haplosclerida) is notably high if compared to the other sessile filter feeder benthic taxa living in freshwaters such as Cnidaria and Bryozoa. Although extremely conservative for some characters the plastic bauplan of Porifera favoured the adaptive radiation of the taxon Spongillina (Demospongiae, Haplosclerida) in inland waters worldwide and produced both structural and functional evolutionary novelties mainly at the level of resting bodies. Clonation, modular architecture and cryptobiosis represent successful adaptive strategies to support survival and dispersal of freshwater sponges at all latitudes from the Arctic Circle to Patagonia. Sponges colonised a wide variety of extremely discontinuous freshwater habitats such as alpine lakes, caldera lakes, Saharan ouadi, springs, thermal springs, terrestrial caves, oceanic islands, ancient lakes, and man-made water bodies. A comparative analysis on morpho-functional diversity of asexual resting bodies based on literature data vs. new investigations by scanning electron microscopy highlighted some trends in the evolution of the genera belonging to the families Metaniidae, Potamolepidae and Spongillidae that display typical gemmular architectures. Other freshwater sponges endemic from ancient lakes that are exclusively sexual, namely Lubomirskiidae, Malawispongiidae and Metschnikowiidae, share this reproductive strategy by swimming larvae (parenchymellas) with the gemmule-producing families. The evolutionary success of the taxon Spongillina is not easy to be interpreted. In some cases a dispersalist model seems to fit the natural history of the species till now considered cosmopolitan, as in the case of *Ephydatia fluviatilis*. In other cases, e.g. the genus *Corvospongilla*, the vicariance model explains better its speciosity and endemism. The distribution pattern of genera/families from ancient lakes represents a peculiar case; these taxa share both morphological (spiculation, skeletal architecture) and biological (absence of gemmules) traits in spite of their extremely disjunct geographic distribution and high levels of endemism.

A study of the distribution of freshwater sponges in Lake Baikal

Yoshiki Masuda{ XE "Masuda, Yoshiki" }^(1*); Valeria Itskovich{ XE "Itskovich, Valeria" }⁽²⁾.
Elena Veinberg{ XE "Veinberg, Elena" }⁽²⁾; Sofia M. Efremova{ XE "Efremova, Sofia M." }⁽³⁾

(1) Dept. of Biology, Kawasaki Medical School, Matsushima, Kurashiki City, Okayama, 701-0192, Japan, masuda@med.kawasaki-m.ac.jp; (2) Limnological Institute of the Siberian Division of the Russian Academy of Science, Ulan-Batroskaya str.3, 664033, Irkutsk, Russia; (3) Biological Institute, St. Petersburg State University, Organienbaumschoye sch. 2, Stary Peterhof, St. Petersburg 198904, Russia

During the summers of 1993, 1994, 1995 and 1996, expeditions were carried out to collect freshwater sponges to study their distributions in Lake Baikal. The sponges were classified into 2 families, 6 genera, and 14 species. Most belonged to the Lubomirskiidae, and were widely distributed in Lake Baikal except on inappropriate substrata. A few sponges belonged to the Spongillidae and were confined to the Little Sea near Olkhon island and an estuary in the North Basin. During the summers of 1997 and 1998, other expeditions to survey the vertical distributions of sponges in Lake Baikal from the surface to a depth of 45m were carried out by shore survey and diving surveys. Lubomirskiidae were distributed from a depth of 1m to 40m. On the other hand, Spongillidae were confined to a depth of 0.5m. *Baikalospongia intermedia* had the highest frequency among all species and also showed higher frequency in the shallow zone. *Lubomirskia baicalensis* had the second highest frequency and showed higher frequency in the shallow zone. Other *Baikalospongia* species showed higher frequency in the deeper zone. Until now, it had been believed that *Swartchewskia papyracea* inhabits only the deeper zone, but this species was also found in the shallow zone.

Remarks on spongillid sponge species in Lake Constance, Germany

Martin Pfannkuchen{ XE "Pfannkuchen, Martin" }^(1*); Franz Brümmer{ XE "Brümmer, Franz" }⁽¹⁾; Sabine Schlesinger{ XE "Schlesinger, Sabine" }⁽¹⁾; Gisela Fritz{ XE "Fritz, Gisela" }⁽¹⁾; Martin Meixner{ XE "Meixner, Martin" }⁽²⁾

(1) Biological Institute, Department of Zoology, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany, martin.pfannkuchen@bio.uni-stuttgart.de; franz.bruemmer@bio.uni-stuttgart.de; sabine.schlesinger@bio.uni-stuttgart.de; gisela.fritz@bio.uni-stuttgart.de; (2) SMB, R.-Breitscheid Str. 70, D-15562 Rüdersdorf, Germany, ma_meixner@yahoo.com

Sponges of the family Spongillidae are found around the world in the most different habitats and thus are said to be cosmopolitan species. We investigate two populations of freshwater sponges that at first glance inhabit the very same habitat, a drop off in Lake Constance, Germany. By this example we will demonstrate today's difficulties in taxonomic work on spongillid sponges and possible outcomes. The upper population built of *Spongilla lacustris* covers the sandstone drop off from 7 to 14 m water depth; this species is easy to determine by established taxonomic characters. Beneath 14 m water depth we find a dense population of *Ephydatia* sp. growing on a dense cover of the so called zebra mussel *Dreissena polymorpha* (Bivalvia). The most intriguing feature of this observation is that both populations are directly adjacent to each other but not overlapping. A very strict borderline is formed at 14 m water depth. There is a strict correlation for the lower population to the presence of *Dreissena polymorpha*, which acts as edificator organism in the observed consortium. As the lower population is built up by a non-gemmulating *Ephydatia* species, macroscopic morphological features as well as molecular data have to be taken into account for the taxonomic description. We therefore investigated 28S, 18S, ITS and COI sequences as well as additional morphological and histological characters like canal system and growth form. The combination of the markers we investigated gives a clear hint for the classification as *Ephydatia fluviatilis*. Our approach is using ecological, morphological and molecular data sets as a synthesis of all available data on the species. Only this synthesis enables us to uncover the taxonomic relationship of the observed non-gemmulating *Ephydatia* species.

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Morphologic variability of *Spongilla alba* Carter, 1849 on distinct geographic scales (Spongillidae, Haplosclerida, Demospongiae)

Ulisses S. Pinheiro{ XE "Pinheiro, Ulisses S." }^(1*, 2); Loyana Docio Santos{ XE "Santos, Loyana Docio" }⁽¹⁾; Yoshiki Masuda{ XE "Masuda, Yoshiki" }⁽³⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽²⁾

(1) Universidade Estadual do Sudoeste da Bahia - Campus Jequié, Departamento de Ciências Biológicas, Rua José Moreira Sobrinho s/n. Jequiezinho, 45200-000, Jequié, BA, Brazil, ulisses@uesb.br; (2) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil; (3) Dept. of Biology, Kawasaki Medical School, Matsushima, Kurashiki City, Okayama, 701-0192, Japan

Spongilla alba Carter, 1849 was originally described from Bombay (India), but had its range subsequently extended to North, Central and South America, Africa and Oceania, making up a highly discontinuous distribution. A large morphologic variability has been reported for the species, partly explained as ecophenotypic. The maintenance of populations on these widely separated landmasses is defended on the basis of alleged capacity for long distance dispersal through gemmules. No consensus exists on this matter though. In an alternative scenario, *S. alba* would comprise a complex of sibling species instead. In this work we aim to describe the morphologic variability in *S. alba* at different geographic scales. The studied material (number of specimens), deposited in the MNRJ collection, or borrowed from the BMNH and ZMA collections, originated from four coastal lagoons on northern Rio de Janeiro state (28), an altitude mountainous habitat at Bahia state (3) and the Amazon (1), in Brazil, as well as from Japan (1), Australia (1), India (2), Africa (1) and Curaçao (2). Several of these specimens, including those from India possess smooth fusiform oxeads, micracanthoxeote microscleres with simple and compound spines, and gemoscleres which are acanthostrongyles with spines which are bent and distributed all over, or more concentrated on both terminations. The Japanese specimen studied possesses small conical spines in their oxeads. This same trend was reported in the literature for Australian and Indonesian specimens, and seems to corroborate the idea that eastern populations of alleged *S. alba* may indeed belong to another species. The specimen from the African lakes has small spines in its oxeads, and no compound spines on the microscleres, and is probably incorrectly assigned to *S. alba*. The Curaçao specimens studied possess much stouter megascleres and microscleres, so that an alternative hypothesis, viz. of their insular endemism, cannot be totally discarded yet. The specimens collected on the coastal lagoons of Rio de Janeiro state, in spite of distinct abiotic parameters (e.g. salinity), do not show significant morphologic differences from each other, thus contradicting earlier results by Poirrier. Our results suggest that the alleged cosmopolitanism of *S. alba* might be a consequence of conservative taxonomy. Molecular taxonomy and larger series of specimens may help disentangle this Gordian knot.

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New lithistid sponges from a submarine cave in Philippines: Affinities and evolutionary significance

Andrzej Pisera{ XE "Pisera, Andrzej" }^(1*); Jean Vacelet{ XE "Vacelet, Jean" }⁽²⁾

(1) Polish Acad Sci, Inst Paleobiol, Ul Twarda 51-55, Warsaw, PL-00818 Poland, apis@twarda.pan.pl; (2) Centre d'Océanologie de Marseille (CNRS-Université de la Méditerranée, UMR 6540), Station Marine d'Endoume, 13007 Marseille, France, jvacelet@com.univ-mrs.fr

Three species of lithistid sponges have been found in a large submarine cave on Philippines. It is the first report of such fauna in the region. Two species represent rhizomorine (choanosomal desmas as rhizoclones) lithistid sponges, which belong to genera *Microscleroderma* and *Gastrophanella*. The third species belongs to sphaerocladine lithistids (desmas as sphaeroclones and only known living representative *Vetulina stalactites*, family Vetulinidae), and represents a new genus and probably a new family as well. Its desmas are very similar to typical sphaeroclones known in sphaerocladine lithistids from the Mesozoic of Europe. It bears ectosomal spicules of dichotriaenes with spinose cladome and acanthorhabd microscleres. It is the first sphaerocladine with ectosomal spicules and microscleres (*Vetulina* has none) known, allowing for better understanding of the evolutionary relationship of this lithistid group. Highly interesting are also specific affinities of the discussed rhizomorine lithistids: they are extremely similar, if not identical, with recently described forms from the shallow submarine cave in the eastern Mediterranean in Lebanon. The investigated here *Gastrophanella*, on the other hand, displays close affinity to some late Cretaceous rhizomorine from Germany. Taking into account of all above discussed features, like affinities between species from the Mediterranean and Philippines, as well as close affinities with some Cretaceous lithistids, the fauna from the Philippine cave may be considered as a relict of Mesozoic Tethyan fauna.

Lithistid sponges from submarine caves in the Mediterranean: Taxonomy and affinities

Andrzej Pisera{ XE "Pisera, Andrzej" }^(1*); Jean Vacelet{ XE "Vacelet, Jean" }⁽²⁾

(1) Polish Acad Sci, Inst Paleobiol, Ul Twarda 51-55, Warsaw, PL-00818 Poland, apis@twarda.pan.pl; (2) Centre d'Océanologie de Marseille (CNRS-Université de la Méditerranée, UMR 6540), Station Marine d'Endoume, 13007 Marseille, France, jvacelet@com.univ-mrs.fr

Lithistid sponges are rare in the Mediterranean Sea and only 8 species of 8 genera have been reported. Lithistid sponges from the submarine caves are even less common and poorly studied. Only 5 lithistid species of 5 genera were reported from the submarine caves in several papers. The most commonly cited is the theonellid *Discodermia polydiscus* that is common in numerous caves (sometimes in large populations) along the Spanish and French coast, as well as in Sardinia. Populations of this sponge found in different submarine caves differ, however, considerably in the desma and microsclere morphology. We have found that they represent (a) new species, which is either highly polymorphic in respect to skeletal characters, or that it is a complex of very similar and closely related 2-3 species. Among corallistids, *Corallistes masoni* has been cited most frequently, but we have found that it is, in fact, *C. bowerbanki*. The other corallistids (at least 2 different species), first time found in the Mediterranean, belongs to the genus *Neophrissospongia*, characterised by spinose cladome of the ectosomal dichotriaenes. This genus has representatives in caves near Marseille as well as in the Croatia coast. Two or even three species of lithistids may occur in one cave, but usually only one species is observed. The discussed here lithistid fauna has clear Atlantic affinities, but some species are known only from the Mediterranean Sea. Earlier described lithistids from the Lebanon have affinities with those occurring in the submarine cave on Philippines, suggesting that they may be relicts of ancient Tethys.

Calcareous sponges (Calcarea: Porifera) from the abyssal Norwegian, Greenland and Iceland seas

Hans Tore Rapp{ XE "Rapp, Hans Tore" }^(1*); Ole Secher Tendal{ XE "Tendal, Ole Secher" }⁽²⁾

(1) Department of Biology, University of Bergen, Bergen High-Technology Center, PO Box 7800, N-5020 Bergen, Norway, hans.rapp@bio.uib.no; (2) Zoological Museum, University of Copenhagen, Universitetsparken 15, DK - 2100 Copenhagen, Denmark, ostendal@snm.ku.dk

The abyssal (>2000 m depth) zones of the GIN Seas (Greenland-, Iceland- and Norwegian 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 fauna are a high number of endemic species, the generally low diversity as compared to the Atlantic abyssal fauna, and that some species are very abundant. 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. It is therefore a striking and peculiar situation that quite a number of species live in the abyssal areas of the GIN Seas at negative temperatures, and some occur in high abundance. Here we report on material of calcareous sponges collected by expeditions during the last 130 years, especially since 1970. More than 4000 specimens are examined, and we recognize 7 species representing 6 different genera, and describe 5 species and 1 genus new to science. For several reasons the occurrence of seven species of calcareous sponges in the abyssal of the GIN Seas is remarkable. They constitute a high proportion of the sponge fauna in the abyssal parts of the GIN Seas (7 out of 25 species). They are also a defined and regularly occurring element in a well-established fauna inhabiting an extensive geographical area. Both these features are globally unique. Abyssal Calcarea are known from other major oceans, viz. in the Weddell Sea, 4065 m, and from the North Pacific both from the Kurilo-Kamchatka trench, 5045 m and from off Northern California, 3500 m, but not in the numbers and abundance as reported here. 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 thriving so well under these circumstances probably have to do with the hydrography and the feeding conditions of the abyssal GIN Seas. In the North Atlantic the calcite compensation depth (CCD) is deeper than in the other oceans. The deep-sea water masses of the GIN Seas are recently formed from surface water masses and are calcium-saturated in contrast to the mostly undersaturated deep-water of the Pacific; they are therefore less corrosive to CaCO₂. CO₂ accumulates as a product from organism metabolism and the decay of organic materials. Accordingly, the amount is dependent on the supply of organic matter from the surface layers and on the benthic biomass. Generally speaking, the calcium saturation, the limitation to low biomasses and the strong seasonality in surface production can be supposed to be the key factors behind the subsistence of the special abyssal community with many calcareous sponges.

A new *Cladorhiza* from Southern California, USA

Henry M. Reiswig{ XE "Reiswig, Henry M." }^(1*); Welton Lee{ XE "Lee, Welton" }⁽²⁾

(1) Department of Biology, University of Victoria, and Natural History Section, Royal British Columbia Museum, P.O. Box 3020 Stn CSC, Victoria, B.C. V8W 3N5, Canada, hmreiswig@shaw.ca; (2) 6600 Mokelumne Ave., Oakland, CA, 94605 USA, fiddlesponge@cs.com

A very large new species of *Cladorhiza*, collected from 1,442 m depth on the San Juan Seamount by the ROV Tiburon in 2004, is strikingly bilateral in symmetry and feather-like in form. The specimen, 382 mm in total length, consists of a narrow stalk, 168 mm long by 8 mm thick, attached to hard substrate by a small disc, and with an elongate body, 235 mm by 68.5 mm total width (including filaments), with body and stalk overlapping slightly. The main body is triangular in section, with continuation of the stalk forming a flattened rigid keel along the abfrontal surface. The slightly concave frontal surface bears a continuous marginal fringe of about 400 filaments, 21 mm long by 0.7 mm thick at their base. A series of 13 fleshy lobes, project from the midline of the frontal surface. They average 12 mm in length and are spaced fairly regularly at a mean interval of about 18 mm. Major biologic processes are regionally separated. Male reproduction (spermatic cysts) is restricted to the tissues of the frontal surface of the main body, including the frontal lobes, with an estimated total number of 36,000 cysts for the entire specimen. Female reproduction (oocyte production, embryo development and larval maturation) occurs exclusively in abfrontal surface tissues in the cushions between the keel and the more fleshy main body. The specimen carries an estimated total of 482 developing embryos and larvae, each between 567 and 892 μ m diameter. Prey capture, apparently of exclusively small crustaceans 0.17 - 1.50 mm in length, is restricted to the marginal filaments. The total number of prey cysts on the entire specimen, based upon serial sectioning of three filaments, is estimated at between 4,000 and 8,000. The elegant bilateral symmetry attained by this species attests to the continuing experimentation with development patterns within Porifera.

The use of sponge bathymetric distribution patterns in defining depth zones in the Greater St Lucia Wetland Park off St Lucia on the East coast of South Africa

Toufiek Samaai{ XE "Samaai, Toufiek" }^(1*); Kerry Sink{ XE "Sink, Kerry" }⁽²⁾

(1) CSIR, Environmentek, Coastal Programme, P.O. Box X 17001, Congella, 4013, Durban, South Africa, tsamaai@csir.co.za; (2) SAIAB, Rhodes University, Coelacanth Programme, Grahamstown, South Africa

The deep reefs environment is one of the least known marine habitats despite the recognition that these environments support diverse and abundant biological assemblages. The African Coelacanth Ecosystems Program has conducted three submersible-based expeditions in the submarine canyons of the Greater St Lucia Wetland Park (GSWLP) in South Africa. Video footage, photographs and observer reports were analysed to examine sponge diversity patterns in different habitats of the deep reefs and canyons. Patterns in sponge community structure between the different depth zones were investigated and revealed the following: sponge fauna decrease between 110 and 300 m; the deeper areas of the vertical distribution are dominated by Lithistid, Astrophorid and Hexactinellid sponges, whereas the shallow areas between 10-100 m have an assortment of species belonging to a wide range of orders (example Poecilosclerida, Haplosclerida, Halichondrida, Astrophorida and Dictyoceratida). Patterns of sponge communities are evident at certain depth zones and were used as a proxy in defining depth zones along the Greater St Lucia Wetland Park (GSWLP). Four zones were recognised within the canyon region, based on the sponge community patterns, and these were the margins, upper slope, lower slope and thalweg. The canyon sponge community is distinct from that of the adjacent inshore reefs. The margin habitat supports the most diverse sponge fauna. These results highlight the importance of offshore marine protected areas in conserving representative deeper water habitats.

**Facets of diversity estimated from combined seafloor trawling and optical mapping
in the sponge fauna of Tasmanian canyons (SE Australia)**

Monika A. Schlacher-Hoenlinger{ XE "Schlacher-Hoenlinger, Monika A." }^(1, 2); Thomas A. Schlacher{ XE "Schlacher, Thomas A." }⁽¹⁾; Alan Williams{ XE "Williams, Alan" }⁽³⁾; John N.A. Hooper{ XE "Hooper, John N.A." }^(2*)

(1) Faculty of Science, Health & Education, The University of the Sunshine Coast Australia; (2) Centre for Biodiversity, Queensland Museum, PO Box 3300, South Brisbane, Qld 4101, Australia, johnh@qm.qld.gov.au; (3) CSIRO Marine & Atmospheric Research, PO Box 1538, Hobart, Tas 7001, Australia

Evidence continues to accumulate in support of the hypothesis that the deep-sea may represent one of the most speciose marine domains. However, the supporting data come almost exclusively from soft-sediment habitats sampled with grabs and cores; quantitative studies of deep-sea hard bottoms, especially those on abrupt topographies, such as seamounts and canyons, are scarce. Much of this asymmetry in the data coverage may stem from difficulties in sampling in these environments using traditional types of gear like dredges and trawls. Here we report results from a dredge survey of hard seabed associated with submarine canyons off Tasmania at depths from 95 to 525m that yielded a collection of 323 sponges. Site-to-site variation in yield was high, ranging from 1 to 46 sponges per sample. Demospongiae comprised the bulk of the fauna (92% of specimens) and were represented by 160 species, 68 genera, 40 families, and 10 orders, while Calcarea were much less diverse at 13 species. The diversity of the canyon sponge assemblages was extraordinarily high at local and regional spatial scales: i) species turnover (b diversity) was substantial both between sites in single canyons and between canyons, and ii) gamma-diversity ('seascape biodiversity') of the area (173 species) at least rivals that of other bathyal habitats in the region. High levels of beta diversity translated to a spatial mosaic of assemblage composition that does not appear to be structured by clear geographic drivers and affinities. High diversity is confirmed by features of regional scale affinities: initial comparisons indicate the canyon fauna may share fewer than 10% of species with assemblages on the adjacent shallow continental shelf and has low species overlap with sponge communities from other abrupt topographic features (seamounts) in the Tasman and Coral Sea. While not replacing the need to take physical collections, non-destructive photographic methods may provide an important component of future biodiversity assessments in bathyal environments. Here we provide an initial assessment of the scope to compare this physical collection with diversity estimates from high-resolution video data taken on the same survey.

Diversity of sponges in bathyal coral reefs of the North East Atlantic

Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽¹⁾; Marc S.S. Lavaleye{ XE "Lavaleye, Marc S.S." }⁽²⁾; Gerard C. Duineveld{ XE "Duineveld, Gerard C." }⁽²⁾; Conny Maier{ XE "Maier, Conny" }⁽²⁾; Fleur C. van Duyl{ XE "Duyl, Fleur C. van" }⁽²⁾

(1) Zoologisch Museum van de Universiteit van Amsterdam, P.O.Box 94766, 1090 GT Amsterdam, the Netherlands, soest@science.uva.nl; (2) Royal Netherlands Institute of Sea Research, P.O. Box 59, 1790 AB Den Burg, the Netherlands, duyl@nioz.nl

Bathyal (200-1200 m) coral reefs of the North East Atlantic built primarily by the stony coral species *Lophelia pertusa* and *Madrepora oculata* are a major hard substrate habitat situated in a predominantly soft bottom environment. These habitats are the focus of national (Moundforce, BIOSYS) and international (e.g. EU-HERMES) research projects. During 2004 and 2005 a sampling program using boxcores complemented by trawl and dredge attempts was carried out in the oceanic region west of Ireland (Porcupine Bank and Rockall Bank), where reefs are found at depths of 500-900 m on ridges flanking the Rockall Trough. Sponges dominate the suspension feeder community that dwells upon the mounds of dead coral branches fringing living coral thickets. Species richness of sponges on local reef mounds ranged from approx. 40 to approx. 95 species. Reef sponge beta diversity of the region amounts to approx. 140 species based on combination of three reef sites. Most species could be readily identified from bathyal sponge monographs of the early 1900s and many of them appear to be coral reef habitat endemics. Literature research yielded a richness of approx. 300 species for the whole of the North East Atlantic bathyal coral reef region (Azores, sea mount region W of Portugal, Gulf of Cadiz, Gulf of Biscay, Faroes, off Norway). Despite comparable species richness in individual reef mounds, local heterogeneity is high, with closely situated reefs showing dramatic differences in species composition and abundance. Adjacent non-reef areas (sandy gullies with stones) contained an impoverished sponge fauna with overall richness not exceeding 47 species and lacking habitat endemism. Bathyal reef sponges show similarities in the various adaptations of species to this habitat: regardless of locality, predominant forms are (1) thinly encrusting, often hairy, small patches, (2) hollow bladder-like forms, and (3) megabenthic highly siliceous forms. Among the latter, hexactinellids are dominant. Species of this group may show local high densities, such as observed for *Rossella nodastrella* (identity confirmed by K.R. Tabachnick), which may occur with 10 or more large individuals per square meter over considerable distances in one of the Rockall Bank reefs. Underwater videos of *Rossella* build-ups show that they are possibly the result of clonal processes. It remains to be determined how these megabenthic forms attain and maintain such large sizes in an environment in which food appears a limiting resource.

Seasonal and habitat correlation among freshwater sponges and the asiatic mytilid *Limnoperla fortunei* in an inner delta at Southern Brazil

Maria da Conceição M. Tavares{ XE "Tavares, Maria da Conceição M." }^(1*); Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }⁽¹⁾; Guilherme Hermany{ XE "Hermany, Guilherme" }⁽²⁾; Maria C.D. Mansur{ XE "Mansur, Maria C.D." }⁽³⁾

(1) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Rua Dr. Salvador França, 1427, Bairro Jardim Botânico, 90690-000, Porto Alegre, RS, Brazil, mcmtavares@hotmail.com; cvolkmer@fzb.rs.gov.br; (2) Programa de Pós-Graduação em Geociências, Instituto de Geociências Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91540-000, Porto Alegre, RS, Brazil, guilherme.hermany@terra.com.br; (3) Museu de Ciências e Tecnologia da PUCRS, Av. Ipiranga 6681, prédio 40, 90619-900, Porto Alegre, RS, Brazil, maria.mansur@puers.br

The roots of the water hyacinths *Eichhornia azurea* and *E. crassipes* were seen to be the preferred substrate of the assemblage of sponges which occur in the floating meadows at the Delta of the Jacui river, at the extreme south of Brazil (29°56' and 30°03' S and 51°12' and 51°18' W). At the time that the field work was started to investigate the seasonal variation of this sponge assemblage, the roots of the water hyacinths were seen to have been also occupied by the recently introduced asiatic mytilid *Limnoperla fortunei*. The invading animal, also sessile and filter feeding as the sponges, was thus included into the research. Four seasonal campaigns were carried out between August 2001 and June 2002 at the Delta floating meadows encompassing 12 sampling stations, representing the islands' embayments, the channels among the islands and the river mouths. At each station six *E. azurea* root portions staked at the bottom were sampled standing for the benthic habitat, and six *E. crassipes* root portions, standing for the pleustonic habitat. The roots were next dried and weighed. The 558 samples were observed under stereoscopic microscope for the extraction of all sponge and mytilid specimens. Only sponges with gemmules or isolated gemmules were considered aiming the counting of identified specimens. The samples were standardized considering the number of individuals of each species of sponges and bivalves per each gram of dry root. Descriptive statistics were used to determine the mean abundance of the population of *Limnoperla fortunei* and of the sponges from the different sampling locations and seasons. These differences were assessed in terms of statistical significance by means of randomization test using block design. Thus, in the comparison between seasons of the year, sampling sites were considered blocks, eliminating the spatial effect and vice-versa. The pairs which differed between themselves were established upon a contrast analysis, similar to the Scheffé test. Correlations between sponge species and *L. fortunei* were also searched for through randomization tests. *Heteromeyenia stepanowii*, *Trochospongilla paulula*, *T. minuta*, *Oncosclera navicella* and *T. lanzamirandai*, were detected in this order of abundance. Only juveniles and adults up to 2.0 cm in diameter of *L. fortunei* were seen to occur in the same substrates with the sponges. A positive correlation was seen among *H. stepanowii*, *T. paulula*, *T. minuta*, *O. navicella* and *L. fortunei*, indicating a preferential distribution in the same types of environments, *i.e.* the embayments of the islands situated farther of pollution sources and a larger abundance in autumn.

C. Volkmer-Ribeiro: Fellow researcher of CNPq; G. Hermany: Graduate fellow of CAPES

Diversity, evolution and classification of deep-sea carnivorous sponges
(invited lecture)

Jean Vacelet{ XE "Vacelet, Jean" }

Centre d'Océanologie de Marseille (CNRS-Université de la Méditerranée, UMR 6540), Station Marine
d'Endoume, 13007 Marseille, France, jvacelet@com.univ-mrs.fr

The poecilosclerid family Cladorhizidae numbered approximately 90 species at the end of the 20th century. A carnivorous habit of feeding has been demonstrated in a few species but is likely for all the representatives of the family. Several recent studies and new results have shown that the diversity of the family was underestimated. Most taxonomic studies have been performed on samples from dredging or trawling, which are more successful on mud substrates. Direct observations and collections from manned submersibles or Remote Operated Vehicles (ROV) on rocky substrates near active hydrothermal sites have provided well preserved specimens of these sponges, with a remarkably high proportion of new species, especially on the East Pacific Rise and in the North Fiji and Lau Basins where eight new species have been described. It is presently difficult to determine if the abundance and diversity of these carnivorous sponges are due to the presence of solid substrata linked to the hydrothermal sites, to the general organic enrichment around the sites, or to a special sampling effort by direct methods in these zones. Cladorhizid sponges are apparently absent from the rich animal communities, which thrive in the immediate environment of the active smokers, and thus they cannot be considered as true members of the hydrothermal fauna. They may, however, benefit from a general organic enrichment around the hydrothermal sites. Several other studies on specimens obtained from trawling in the Pacific or in the Atlantic have also increased the number of described species. Many species are known by very few specimens, and their variability is thus poorly known. This family and more generally the carnivorous poecilosclerids raise a taxonomic problem, which appears difficult to resolve with morphological characters. The recent results from deep Pacific specimens show that the genus *Abyssocladia*, which has been synonymized with *Phelloderma* (Phellodermidae), is a valid genus including seven species, which are carnivorous. This genus has been temporarily classified in the Cladorhizidae. The carnivorous poecilosclerids also include *Euchelipluma*, a representative of the Guitarridae. The microsclere complement in the diverse genera of the carnivorous sponges as presently known suggests that a carnivorous feeding habit appeared independently in several evolutionary lines of poecilosclerids. However, the relationship of the diverse genera with extant families of Mycalina and possibly of Myxillina remains ambiguous. It is suggested, given the important morphological adaptations of these sponges and their ambiguous relationships with extant families of poecilosclerids, that it would be premature to change drastically the classification before having informations from molecular data.

Freshwater sponges indicators to Brazilian coastal water bodies

Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }^(1*); Vanessa de Souza Machado{ XE "Machado, Vanessa de Souza" }⁽²⁾

(1) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul. Rua Dr. Salvador França, 1427. Bairro Jardim Botânico. 90690-000. Porto Alegre, RS, Brazil, cvolkmer@fzb.rs.gov.br; (2) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul. Rua Dr. Salvador França, 1427. Bairro Jardim Botânico. 90690-000

Surveys for freshwater sponges were performed at several water bodies along a north- south direction at the Brazilian costal line at the sites: The National Park of Lençóis Maranhenses, MA (2°20' - 2°45'S 42°45' - 43°30'W), the Mundaú-Manguaba lagoonal complex, AL (9°34' - 9°45'S 35°44' - 35°58'W), the National Park of Lagoa do Peixe, RS (31°20'00''S 51°05'00''W), the ponds system around the northern border of the Patos Lagoon, RS (30°28'21''S 51°16'31''W) and the Taim Ecological Station, RS (32°32' - 32°50'S 52°23' - 52°32'W). The surveys were performed by hand or gemmules were detected in sediments caught with an Ekman dredge. Fragments of specimens and whole gemmules were boilded in Nitric Acid aiming dissociation of the spicules and mounting of permanent slides for microcopic observation, species identification and illustration. The results obtained upon the sponge surveys allowed for the distinction of four different species-specific environments along this coastal border. The main fact considered was the dominant or the sole occurrence of a single sponge species at one particular habitat. The first group, with two sub-groups, is that of shallow ponds among dunes, one at the tropical belt (Lençóis Maranhenses) indicated by *Corvoheteromeyenia heterosclera* and the other at the temperate region (Parque Nacional da Lagoa do Peixe) indicated by *Racekiela sheilae*. The second is that of the mixohaline habitats of the lagoons (Mundaú-Manguaba) indicated by *Spongilla alba*. The third one stands for the shallower permanent lakes and ponds with an extensive colonization by macrophyte stands (Lagoa do Casamento) at the continental boundary of the coastal belt. This coastal environment is indicated by *Corvoheteromeyenia australis*. The fourth one is that of the freshwater muddy-acid coastal swamps, not too distant from the ocean border and practically covered by macrophytes (Estação Ecológica do Taim). *Ephydatia facunda* is the species to occupy this habitat with almost exclusiveness. The results presented aim to contribute to the identification of spicules/sponge species in sediment columns recovered at the Brazilian and South American coastal area. Freshwater sponge spicules have proved along the last decades to be an excelent tool in reconstruction of paleo environments. On the other hand determination of paleo ocean borders are an issue of utmost importance in what respects projections of timing and fluctuations of ascending sea levels. The study provides also taxonomic tools to the knowledge of the biodiversity of the studied environments since most of them belong to brazilian preserved areas.

C. Volkmer-Ribeiro: Research fellow of CNPq; V.S. Machado: Undergraduate fellow of FAPERGS

Freshwater sponges of the Atlantic Forest at Southern Brazil

Cecília Volkmer-Ribeiro{ XE "Volkmer-Ribeiro, Cecília" }⁽¹⁾; Carolina Coimbra Mostardeiro{ XE "Mostardeiro, Carolina Coimbra" }^(2*)

(1) Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul. Caixa Postal 1188, 90001-970. Porto Alegre, RS, Brasil, cvolkmer@fzb.rs.gov.br; (2) Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul. Caixa Postal 1188, 90001-970. Porto Alegre, RS, Brasil, ccmostardeiro@gmail.com

A survey for freshwater sponges is in progress at three protected areas at Rio Grande do Sul state: the State Park of the Tainhas River, the Ecological Station of Aratinga and the State Park of Itapeva. The results will contribute to the proposition of the Management Plan for these Conservation Units, which are inserted in the Biosphere Reserve of the Atlantic Forest. The first two areas pertain to the distribution domain of the native pine tree *Araucaria angustifolia*, whilst the third one to the coastal shrubby vegetation. The sponge survey is part of the whole biodiversity survey being carried by the Museum team with funding by the German bank KfW. The first expedition was carried out on January 24 - 28/ 2005 at the Ecological Station of Aratinga and the State Park of the Tainhas River. Prospections were done across rivers and brooks with examination of substrata that could bear encrusted sponges. The detected specimens were photographed alive and a few were sampled, dried and catalogued at the Porifera Collection of MCN. Permanent slide preparations of the dissociated spicules were next done aiming the taxonomic identification. *Oncosclera jewelli* (Volkmer, 1963) was the only species detected at River Tainhas, its distribution extending probably along the whole river, at the very least, inside the Park. The sponge was seen to conform to the original description of continuous, shallow, permanent crusts at the river rocky bottom. *Heteromeyenia insignis* Weltner, 1895 was detected at 'Arroio Corneta' a small rocky brook at the boundary of the Ecological Station of Aratinga. The sponge occurred as circular, fragile, green patches full of gemmules in a gentle shallow current of water, having its seasonal condition confirmed. The results come to extend the distribution of the two species in the *Araucaria angustifolia* domain confirming their status of endemics of this region, and of indicators of waters in natural conditions or very close to these. On the other hand, their occurrences in two protected areas consist in renewed arguments justifying the maintenance of these areas, in reason of the pointed endemisms and of the indication that these species operate on water quality, besides the fact that both species compose the Brazilian Red List of endangered species.

C. Volkmer-Ribeiro: Research fellow of CNPq; C.C. Mostardeiro: Undergraduate fellow of CNPq

The sponge fauna of the Anchialine Lakes of Kakaban and Maratua (East Kalimantan, Indonesia)

Nicole J. de Voogd{ XE "Voogd, Nicole J. de" }^(1*); Wallie H. de Weerd{ XE "Weerd, Wallie H. de" }⁽²⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽²⁾

(1) National Museum of Natural History, 'Naturalis', P.O. Box 9517, 2300 RA Leiden, The Netherlands, voogd@naturalis.nnm.nl; (2) Institute for Biodiversity and Ecosystem Dynamics (Zoological Museum), University of Amsterdam, P.O. Box 94766, 1090 GT, Amsterdam, The Netherlands

Marine lakes are biologically interesting marine ecosystems. Such lakes harbour small, isolated, rapidly evolving populations, endemic subspecies, and new species. Approximately 200 marine lakes are thought to be distributed worldwide, of which "Jellyfish Lake" from Palau (Micronesia) is well known. In the present study, the sponge fauna of four marine lakes located at the islands of Kakaban and Maratua, East Kalimantan, Indonesia were surveyed. The islands are thought to represent a lagoonal reef of a former barrier reef complex. These lakes were formed as the atoll raised and the former lagoon lost its connection with the sea making it anchialine ("pools without surface connection to the sea, containing salt or brackish water"). The lakes are still connected to the sea through a network of underground fissures and caves, and the lakes therefore have delayed fluctuating tides compared with the surrounding sea. The marine flora and fauna of these lakes is sparsely documented, although a high incidence of endemism is thought to exist. Sponges and seaweeds are the most abundant species and also the main space acquirers. Most sponges grow on tree branches and mangrove roots hanging in the water, although some are buried in the sandy bottom. Here, we present some preliminary results on the sponge diversity of these lakes. Approximately 100 sponge specimens were collected from the four lakes of Kakaban and Maratua, belonging to 45 species, 26 genera and 24 families. Most species were found in the largest lake of the island Kakaban, whereas the least species were found in the smallest lake of Maratua which did not harbour any unique species. Only eleven species were found in all lakes, such as *Cinachyrella australiensis*. Other abundant species belonging to genera *Suberites*, *Tethya*, *Haliclona* and *Spongia*. Interestingly, *Suberites* sp. is also known from other anchialine lakes such as those from Palau and Lake Satonda (near Sumbawa, Indonesia). Further comparison still has to be made in order to determine whether these species also occur in the surrounding sea, if the species prevalent in all lakes are indeed the same species, and if any new sponge species are recorded from these lakes.

Microbial nitrification in *Aplysina aerophoba*Kristina Bayer{ XE "Bayer, Kristina" }; Ute Hentschel{ XE "Hentschel, Ute" }^(*)

Research Center for Infectious Diseases, University of Wuerzburg, Röntgenring 11, 97070 Wuerzburg, Germany, kristina.bayer@mail.uni-wuerzburg.de; ute.hentschel@mail.uni-wuerzburg.de

Nitrification describes the catalysation of ammonia (NH₃) to nitrite (NO₂-) by ammonia-oxidizing bacteria (AOB) and subsequently to nitrate (NO₃-) by nitrite-oxidizing bacteria (NOB) for energy purposes. Indeed, nitrifying microorganisms have already been identified in tropical mangrove sponges. The aim of this study was to assess the potential for nitrification in *A. aerophoba* using a combined physiological and molecular approach. Whole sponges were incubated in experimental aquaria and the concentrations of ammonia, nitrite and nitrate was determined in the incubation water using colorimetric assays. Nitrate concentrations reached values of up to 0.5 µmol.g fresh weight⁻¹.day⁻¹ for *Aplysina aerophoba*. The increase in nitrate was inversely correlated to a decrease in ammonia concentration. The incubation of the so-called "low microbial abundance" sponges (*Dysidea avara*, *Tethya* sp.) resulted in significantly reduced nitrate excretion. Control experiments without sponges showed no accumulation of nitrate in the incubation water. Moreover, addition of the specific inhibitor nitrapyrine effectively inhibited nitrate excretion. Interestingly, a positive correlation between nitrate excretion and pumping activity of the sponges was observed (see contribution by F. Hoffmann, Germany for more details). By using a specific primer set for amplification of 16S rRNA genes of the betaproteobacterial clade of nitrifying bacteria, a number of sequences were recovered from *Aplysina* sponges. The sequences form an apparently sponge-specific sub-cluster within the marine *Nitrospira* group. Moreover, the amoA-gene, encoding the functional subunit of the ammonia monooxygenase of AOB was amplified and sequenced from sponge tissues. In conclusion, this study provides physiological and molecular evidence for the presence of nitrifying bacteria in *A. aerophoba* and contributes to an ongoing effort to link microbial diversity with function in these phylogenetically highly diverse, elusive and so far uncultivated marine sponge-associated microbial communities.

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Bacterial consortia in the marine sponge *Erylus discophorus* from the Northeast Atlantic

Nuno Conceição{ XE "Conceição, Nuno" }⁽¹⁾; Marise Almeida{ XE "Almeida, Marise" }^(1*);
Manuela Carolino{ XE "Carolino, Manuela" }⁽²⁾; Sandra Chaves{ XE "Chaves, Sandra" }⁽³⁾;
Mário Gadanho{ XE "Gadanho, Mário" }⁽³⁾; Rogério Tenreiro{ XE "Tenreiro, Rogério" }⁽³⁾;
Madalena Humanes{ XE "Humanes, Madalena" }⁽¹⁾

(1) Centro de Química e Bioquímica - Departamento de Química e Bioquímica da Faculdade de Ciências da Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal; (2) Centro de Ecologia e Biologia Vegetal-Departamento de Biologia Vegetal da Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal; (3) Centro de Genética e Biologia Molecular - Departamento de Biologia Vegetal da Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal

The increasing interest in the biotechnological production of sponge biomass for sustainable use is primarily due to the fact that many marine sponges produce and store pharmacologically-active metabolites. There is an ongoing discussion as to whether some of these compounds are produced by the sponge itself or by associated bacteria. Marine sponges are known for containing large amounts of bacteria embedded within the sponge tissue matrix or adsorbed in their vascular system. Some of these bacteria are transient, but others are true sponge-bacteria associations that are widely found and, in some cases, specific to the host. This fact gives support to the idea of symbiotic interactions. Crude extracts of the sponge *Erylus discophorus* present a remarkable anti-HIV, as well as, haloperoxidase activities. The preliminary characterization of this enzyme points to a non-heme, non-vanadium haloperoxidase. These types of haloperoxidases, apparently without a metal in the active site, were found, so far, only in microorganisms. It seems reasonable to think that the activity can be produced by the sponge itself or from associated microorganisms. The purpose of the present study was to identify bacteria permanently associated with the sponge *E. discophorus* collected in three different places in the northeast Atlantic using the 16S ribosomal DNA (rDNA) sequences. The tree created by phylogenetic analysis of the 16S rDNA partial sequences of sponge associated microorganisms revealed that those sequences were distributed through 3 bacterial phyla: Proteobacteria (only alpha class is represented), Acidobacteria and Bacteroidetes. The major diversity was detected for a sample from Ria de Ferrol, (Galicia, Spain), which has sequences in the 3 represented phylogenetic groups and forming groups of sequences closely related within each phylum. It's also the only sample that has microorganisms belonging to Acidobacteria. The closest sequence to these bands corresponds to a non-identified bacterium, described as a sponge symbiont. The majority of the sequences correspondent to a sample from Archipelago of Berlengas formed a very well individualized cluster in phylum Bacteroidetes. The closest sequence to it belongs to a non-cultivated organism, associated to corals. The sequences related to samples from Archipelago of Azores are very similar and formed a well-defined cluster although also including a band from the sample from Ria de Ferrol. They are phylogenetically closely related to genera *Brevundimonas* and *Caulobacter* of α -Proteobacteria. The specimens of the sponge *Erylus discophorus*, collected in geographically distinctive places present diversity in the microbial associated flora suggesting that α -Proteobacteria may be part of the intrinsic microbial flora of this sponge.

Sponge-associated prokaryotes: Abundance, community composition and their possible role in the nutrition of the host

Fleur C. van Duyl{ XE "Duyl, Fleur C. van" }^(*); Jasper M. de Goeij{ XE "Goeij, Jasper M. de" }; Astrid Hoogstraten{ XE "Hoogstraten, Astrid" }; Conny Maier{ XE "Maier, Conny" }; Martine M. van Oostveen{ XE "Oostveen, Martine M. van" }

Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

To increase our understanding of the role of sponge-associated prokaryotes in sponge functioning, we study the abundance and composition of the prokaryote community associated with coral reef sponges with molecular probes. Eubacteria represent 40-60% and Archaea 1-20% of the total prokaryote counts in the coral reef sponges tested. Prokaryote densities in sponges varied considerably between different species and between specimens of the same species (0.7 to $6 \times 10^8 \text{ cm}^{-3}$), and were always higher than prokaryote abundance in ambient water. The composition in tested sponges differs from that in the ambient water. To link abundance and composition of the associated prokaryotes to sponge nutritional functions, we study dissolved organic matter consumption and incorporation, bicarbonate fixation and nitrification activities by the sponges. There are indications that these functions may at least be partly mediated by sponge-associated prokaryotes. Several sponges were tested for their ability to consume dissolved organic matter, to incorporate leucine in proteins, to fix bicarbonate (or derived CO_2) and to nitrify. Preliminary results of counts and incubation experiments will be discussed.

Incidence, identity and importance of photosynthetic symbionts in shallow-water Coral Reef sponge communities

Patrick M. Erwin{ XE "Erwin, Patrick M." }; Robert W. Thacker{ XE "Thacker, Robert W." }^(*)

Department of Biology, University of Alabama at Birmingham, CH109 1530 3rd Avenue South,
Birmingham, Alabama, 35294-1170, USA, erwin@uab.edu; thacker@uab.edu

Symbioses between marine sponges and photosynthetic organisms (*e.g.* cyanobacteria) have been described from coral reef communities; however, the frequency of these associations and the ecological nature of interactions between hosts and symbionts often remain unresolved. In this study, we determined the number of sponge species harboring photosymbionts and assessed their prevalence in the shallow-water reefs of Bocas del Toro, Panama, by conducting line-intercept transects with measurements of chlorophyll *a* (chl *a*) concentration. Sponges were dominant members of these benthic communities, second in abundance only to stony corals. Twenty-five of the 67 species investigated (37.3%) exhibited high chlorophyll levels (>150 µg/g) and accounted for 35.6% of the observed sponge community. Association with photosymbionts is a common strategy among coral reef sponges and may represent an important source of primary productivity in oligotrophic coral reef environments. Two sponge species known to harbor the sponge-specific cyanobacterium *Synechococcus spongiarum* (*Aplysina fulva* and *Xestospongia subtriangularis*) were experimentally shaded to test the effects of reduced light availability on symbiont load and host growth. Six weeks of shading reduced the abundance of symbionts in both sponge species (>40% reduction in chl *a*). In *A. fulva*, control sponges exhibited over twice the growth of shaded sponges (by volume and mass). In *X. subtriangularis*, no significant difference in growth was observed between control and shaded sponges. These results suggest that the relationship between sponges and their cyanobacterial symbionts varies among host species, with some sponges strongly dependent on symbiont photosynthesis and others unaffected by short-term decreases in symbiont activity. The photosynthetic capacities of cyanobacterial symbionts were determined for *A. fulva* and *X. subtriangularis*, in addition to *Xestospongia* n.sp. and *Haliclona* n.sp., two sponges that harbor the filamentous cyanobacterium *Oscillatoria spongeliae*. Oxygen consumption (*i.e.* respiration) and production rates were calculated using relative change in dissolved oxygen concentrations over a 1 h incubation period in paired light and dark conditions. No significant difference was observed in respiration rates among the three species ($P=0.98$). *Xestospongia* n.sp. and *Haliclona* n.sp. exhibited higher oxygen production ($0.652 \text{ mg O}_2 \cdot \text{g sponge}^{-1} \cdot \text{hr}^{-1}$ and $0.408 \text{ mg O}_2 \cdot \text{g sponge}^{-1} \cdot \text{hr}^{-1}$, respectively) than *A. fulva* ($0.296 \text{ mg O}_2 \cdot \text{g sponge}^{-1} \cdot \text{hr}^{-1}$) and *X. subtriangularis* ($0.086 \text{ mg O}_2 \cdot \text{g sponge}^{-1} \cdot \text{hr}^{-1}$), although only with *Xestospongia* n.sp. was this difference significant (ANOVA, $P<0.05$). These results suggest the filamentous *O. spongeliae* is more abundant and/or more active than the single-celled generalist *S. spongiarum* and thus potentially more beneficial to the host sponge.

Stability and structure of microbial communities in marine sponges: Case studies with temperate and tropical sponges

Malcolm Hill{ XE "Hill, Malcolm" }^(*); Nathan Lemoine{ XE "Lemoine, Nathan" }; April Hill{ XE "Hill, April" }

Department of Biology University of Richmond Richmond, VA 23113 USA, mhill2@richmond.edu; nathan.lemoine@richmond.edu; ahill2@richmond.edu

Many species of marine sponge harbor dense and highly diverse symbiont communities and the role of these microbes has been discussed in a number of biological contexts. While a portion of the microbial community may be composed of transient members of the intrasponge habitat, it is clear that sponge specialists compose a substantial portion of the community. Several sponge hosts from different taxonomic groups, dissimilar habitats, and widely separated geographic locations harbor remarkably similar bacteria. We have begun to examine the structure and stability of sponge microbial communities in the face of environmental change. We examined community composition in *Chondrilla* sp. collected in different years from the same mangrove habitats in the Florida Keys. Total community DNA from *Chondrilla* was extracted from both the endosome and choanosome. Eubacterial 16S rRNA sequences were amplified from total community DNA. Only 5% of the species (*i.e.*, operational taxonomic units (OTUs)) uncovered in water samples surrounding the sponges were also found in *Chondrilla* indicating that a distinct community is found in the sponge. The clone libraries obtained from sponges collected in different years contained 21 and 23 OTUs respectively with approximately 25% overlap in RFLP patterns between years. The phylogenetic classification of these clones is ongoing. We have also compared changes in *Chondrilla* microbial symbionts after sponges were maintained in an aquarium for over 2 years with minimal attention. In a series of ongoing experiments, we have examined the ability of host sponges to pick up symbionts from ambient seawater using cell aggregates (*i.e.*, primmorphs) from *Halichondria bowerbanki*, *Microciona prolifera*, and *Haliclona loosanoffi*. Primmorphs cured of microbial symbionts were exposed to ambient seawater and water treated with tissue from adult sponges. TEM and DGGE data were collected to monitor the mechanism and extent of symbiont uptake. Results from these experiments may shed light on the dynamics of the host-symbiont association. Finally, a series of experiments were performed to compare symbiont communities from *Halichondria bowerbanki* grown under different (and stressful) thermal regimes. Data regarding changes in sponge morphology and the microbial community has been collected and we will discuss aspects of observed changes in response to elevated seawater temperatures. Sponge microbial communities offer opportunities to explore hypotheses generated by current community ecological theory and we are only beginning to understand the significance of these sponge-microbe associations.

Oxygen dynamics in sponges: Microbial life in a fluctuating chemical environment

Friederike Hoffmann{ XE "Hoffmann, Friederike" }^(1*); Hans Røy{ XE "Røy, Hans" }⁽¹⁾; Kristina Bayer{ XE "Bayer, Kristina" }⁽²⁾; Ute Hentschel{ XE "Hentschel, Ute" }⁽²⁾; Hans Tore Rapp{ XE "Rapp, Hans Tore" }⁽³⁾; Ole Larsen{ XE "Larsen, Ole" }⁽⁴⁾; Dirk de Beer{ XE "Beer, Dirk de" }⁽¹⁾; Antje Boetius{ XE "Boetius, Antje" }⁽¹⁾

(1) Max Planck Institute for Marine Microbiology, Celsiusstr. 1, D - 28359 Bremen, Germany, fhoffman@mpi-bremen.de; (2) Research Center for Infectious Diseases, University of Würzburg, D - 97070 Würzburg, Germany; (3) Department of Biology, University of Bergen, Bergen High-Technology Center, PO Box 7800, N-5020 Bergen, Norway; (4) DHI Water and Environment, Krusenbergs 31, D- 28857 Syke, Germany

Sponges can host vast amounts of phylogenetically and physiologically diverse microorganisms in their mesohyl. Descriptions of anaerobic microbes inhabiting sponge mesohyls have questioned the general assumption that sponge tissue is well-oxygenated due to continuous percolation by oxygen-rich water. We applied Clark-type oxygen-microelectrodes with tip diameters of 18-40 μm to investigate oxygen microenvironments in three sponge species: The Mediterranean shallow-water species *Aplysina aerophoba*, the cold-water species *Geodia barretti* from the Norwegian coast, and the Arctic deep-sea morphotype of *Tentorium semisuberites*. All species harbor dense populations of microbes. In *A. aerophoba* we observed highly fluctuating oxygen conditions, which were directly correlated with sponge pumping activity, while consistent tissue anoxia was found in *G. barretti*. In *T. semisuberites*, where Archaea dominated the microbial community, no signs of tissue anoxia could be measured. Due to pumping activity and a variety of metabolic processes, complex chemical gradients are present in sponge tissue, which create peculiar living conditions for sponge cells and specific habitats for the associated microbes. Detailed knowledge about the varying chemical microenvironments in sponges may aid in the development of successful protocols to cultivate sponge-associated microbial lineages or sponge cell lines.

Comparative analysis of bacteria possessing antimicrobial activities isolated from different marine sponges of Japan

Manmadhan Kanagasabhpathy { XE "Kanagasabhpathy, Manmadhan" }^(1*); Hideaki Sasaki { XE "Sasaki, Hideaki" }⁽²⁾; Soumya Haldar { XE "Haldar, Soumya" }⁽³⁾; Shinji Yamasaki { XE "Yamasaki, Shinji" }⁽³⁾; Shinichi Nagata { XE "Nagata, Shinichi" }⁽¹⁾

(1) Environmental Biochemistry group, Research Center for Inland Seas, Kobe University, 5-1-1 Fukaeminamicho, Higashinada-ku, Kobe 658-0022, Japan, manmadhan_k@yahoo.co.uk; (2) Department of Life and Environment Science, College of Science and Engineering, Iwaki Meisei University, 5-5-1, Chuodaiiino, Iwaki, Fukushima 970-8551, Japan.; (3) Laboratory of International Prevention of Epidemics, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1, Gakuen-cho, Sakai-shi, Osaka 599-8531, Japan

Marine sponges and the microbes living within them are important from an ecological and biotechnology point of view. The genus *Halichondria* attains importance in demosponges due to the production of secondary metabolites. Previous reports have revealed that marine sponges belonging to *Halichondria* sp. collected from Adriatic sea, North sea and Baltic sea harboured symbiotic and commensalic bacteria. To our knowledge, the antibacterial or bacteriocidal activities of marine symbiotic bacteria from Japanese *Halichondria* sp. have not been reported. In addition there were limited comparative studies on the phylogenetic identification of bacteria isolated from different sponge species especially from this genus *Halichondria*. Sponges belonging to *Halichondria* species are abundant and wide spread in Japan. The aim of this study is to describe microbial diversity in three selected Japanese marine sponges, isolate and describe novel microbes, determine the role of symbiotic microbes in the host, and also screen cultivated microbes for secondary metabolites. For this purpose, specimens of *Halichondria japonica*, *H. panicea* and *H. okadai* were collected from the intertidal zone of Awaji Island, Japan Sea and isolation of symbiotic bacteria were carried out using traditional methods. More than hundred bacteria were collected and stocked as pure cultures. Primary screening of antibacterial metabolite producing symbiotic bacteria was performed using a Gram positive *Staphylococcus aureus* and a Gram negative *Escherichia coli* by disc diffusion method. Bacteria which showed high antibacterial activity were further studied for their ability against (i) a set of fouling bacteria isolated from marine biofilm, (ii) some luminescent *Vibrio* and *Photobacterium* species and (iii) a panel of pathogenic bacteria. Comparative analysis of such novel bacterial diversity among these three sponges was performed by using 16S rRNA gene sequencing techniques. The results provided interesting features about the diversity and antibacterial patterns of *Halichondria*-associated microbes.

**Bioactivity profile of sponge-associated bacteria isolated from marine sponges,
Callyspongia sp. and *Sigmatocia* sp.**

Aron Premnath Lipton{ XE "Lipton, Aron Premnath" }; V.S. Pramitha{ XE "Pramitha, V.S." };
Joseph Selvin{ XE "Selvin, Joseph" }; A.J. Huxley{ XE "Huxley, A.J." }

Marine Biotechnology Laboratory, Vizhinjam Research Centre of CMFRI, Vizhinjam 695 521, Kerala,
India

Considering the possibility of a link between secondary metabolites and specific bacteria, investigations were carried out to infer whether such bioactivity could be due to the symbiotic association of the bacteria and the results are presented in this paper. Sponges, *Callyspongia diffusa* and *Sigmatocia carnosa* obtained as 'by-catch' in fishnets off Arokiapuram (near Kanyakumari, Tamil Nadu, South India: lat. 8°40'N, long. 76°00'E) were used. One sq. cm of epiphyte free internal mass of sponge was minced and ground in 2 ml sterile saline using a sterile motor and pestle and aliquots were serially diluted, plated in Zobell Marine Agar and incubated at 37°C for 18 h. The morphologically distinct bacterial colonies were segregated and grown in Zobell Marine Broth (HiMedia) in shaker at 80±5 rpm for 18 h. Cell free culture or Exocellular product (ECP) was obtained from each isolate by either centrifuging or filtration. The supernatant or filtrate was screened for antibiotic activity along with the methanol extracts of sponge by well plate method at 20 or 37°C. Heat stability of the extracts and ECPs were also evaluated. In *Callyspongia diffusa* three distinct colonies were obtained and their numbers ranged from 3.2 x10⁴ cfu /ml or 6.4 x 10⁴ cfu/sq cm. In *Sigmatocia carnosa*, two different types of bacterial colonies were noted with their numbers ranging from 8.8x10⁴ cfu/ml or 1.76x10⁵ cfu/ cm². The ECP of predominant bacterial isolates produced similar antimicrobial results as observed in the methanol extract of the host sponge, *Sigmatocia carnosa*. The growth of *Bacillus cereus*, *B. subtilis* and *E. coli* was inhibited by the ECPs of bacterial isolates from sponge and not by the methanol extract. The activity was totally lost when exposed to 60°C. The incubation temperature also influenced the antibiotic activity, as the *Callyspongia* extract was active at 20°C and not at 37°C. The predominant isolate from *Callyspongia* produced similar trend of inhibition as that of the methanol extract. These activity profiles of extracts of sponge and ECPs of associated bacteria prove the requirement of symbiotic association for synthesizing novel products.

Molecular census and comparison of cultured and uncultured microbial symbiont diversity from an ancient metazoan host, phylum Porifera

Jose Lopez{ XE "Lopez, Jose" }^(1*); Dedra Harmody{ XE "Harmody, Dedra" }⁽¹⁾; Angela Ledger{ XE "Ledger, Angela" }⁽¹⁾; Cheryl Peterson{ XE "Peterson, Cheryl" }⁽²⁾; Shirley A. Pomponi{ XE "Pomponi, Shirley A." }⁽¹⁾; Karen Sfanos{ XE "Sfanos, Karen" }⁽¹⁾; Peter McCarthy{ XE "McCarthy, Peter" }⁽¹⁾

(1) Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution 5600 US 1 North Ft Pierce FL 34946, Lopez@hboi.edu; (2) Historic Bok Sanctuary, 1151 Tower Blvd Lake Wales, Florida 33853

Our laboratory has characterized the culture-independent prokaryotic (mostly eubacterial) consortia of several different marine demosponge species from various oceanic regions using molecular genetics techniques. The primary goal was to begin a rudimentary profile of specific microorganismal associations (or "symbioses") within diverse sponge species. More than 700 partial small subunit (SSU) 16S rRNA gene segments (average size 450 bp) were characterized from twenty different culture-independent recombinant libraries derived from 10 diverse demospongiae host genera and families: *Corallistes*, *Pachestrella*, *Aplysina*, *Dercitus*, *Lissodendoryx*, *Characella*, *Neofibularia*, *Poecillastra*, *Tedania* and *Raspailiidae*. These hosts varied according to taxonomy, ecology, depth and geographic location. Sequence profiles and phylogenetic analyses comparing the culture independent SSU rRNA sequences with previously characterized sponge-derived cultured isolates indicated large divergences and the detection of *Spirochaetes*, *Planctomycetales*, *Deinococcus*, *Cyanobacteria*, *Riftia*, *Arcobacter*, *Chloroflexi*, *Thermales*, *Nitrospira*, *Acidobacter*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Archaea* and numerous matches to previously described "uncultured" lineages (SAR, JAWS series etc.), which were absent in the cultured isolate collection (Harbor Branch Marine Microbe Culture Collection - http://www.hboi.edu/dbmr/dbmr_hbmmd.html). Quantitative PCR (qPCR) experiments also verified greater relative abundances of selected uncultured 16S rRNA clones over cultured isolates. Although ubiquitous eubacterial taxa (soil clone C083, green non-sulfur bacteria) appear among several of the host sponges, many sponge associated microbes are distinct from surrounding environmental samples of sediments and ambient seawater. Moreover, some sponges appear to host specific symbiont taxa. Lastly, rarefaction analyses of specific libraries indicated that this limited snapshot of sponge-derived uncultured microbial symbiont diversity was not exhaustive, and that many more uncharacterized microbial taxa will be harbored by untested sponge species.

Effects of the sponge *Chondrilla nucula* (Hadromerida, Demospongiae) in the transformation of DIN species (ammonium, nitrite, and nitrate) in marine aquaria containing fish

Maria Millán{ XE "Millán, Maria" }⁽¹⁾; Maria Cristina Díaz{ XE "Díaz, Maria Cristina" }⁽²⁾;
Nydia Ozejo{ XE "Ozejo, Nydia" }⁽³⁾; Rojas Lisbetsida{ XE "Lisbetsida, Rojas" }⁽³⁾

(1) Escuela de Ciencias Aplicadas del Mar, Boca del Río, Edo. Nueva Esparta, Universidad de Oriente, Venezuela; (2) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com; (3) Fundación La Salle, Calle Colon Punta de Piedras, Edo. Nueva Esparta, Venezuela.

The transformation of dissolved inorganic nitrogen (DIN) species, in sea water, mediated by the sponge *Chondrilla nucula*, in aquarium containing fish is here evaluated. Three incubation treatments were carried out: unfiltered seawater as a control (I), unfiltered seawater with fish, and unfiltered seawater with fish and a sponge specimen (III). Three replicates for each treatment type were carried out. All experiments used 20 l of sea-water, and similar light and temperature conditions. Water was sampled from each experimental chamber at three time intervals (0, 2, and 4 hours). DIN analyses (ammonium, nitrite, and nitrate) were carried out twice for each water sample taken. Treatment I values for all three DIN species remained approximately constant. Treatment II shows a considerable accumulation of ammonia (18-62% increase), and a slight decrease of nitrite (28-29%), and nitrate (14-21%). Treatment III shows a significant reduction of ammonium concentrations (62-90%), while nitrite and nitrate increase considerably, 21-81%, and 21-120%, respectively. Differences between replicates of treatment II, and III may relate to variations on the ammonia release by fish, and sponge weight and stress. These results indicate that *C. nucula*, through its well known capacity to mediate nitrification, may be useful on the removal of reduced and toxic forms of DIN from marine aquaria. Further studies must be done to evaluate the usefulness of this sponge as a biofilter for marine aquaria in a long-term basis.

Antimicrobial activities of bacteria isolated from marine sponges of Rio de Janeiro coast, Brazil

Ana Paula Barbosa Moreira{ XE "Moreira, Ana Paula Barbosa" }⁽¹⁾; Guilherme Muricy{ XE "Muricy, Guilherme" }⁽²⁾; Maria do Carmo de Freire Bastos{ XE "Bastos, Maria do Carmo de Freire" }⁽¹⁾; Kátia Regina Netto dos Santos{ XE "Santos, Kátia Regina Netto dos" }⁽¹⁾; Marcia Giambiagi-deMarval{ XE "Giambiagi-deMarval, Marcia" }⁽¹⁾; Marinella Silva Laport{ XE "Laport, Marinella Silva" }^(1*)

(1) Departamento de Microbiologia Médica, Instituto de Microbiologia Prof. Paulo de Góes, Bloco I, CCS - UFRJ, Cidade Universitária - Ilha do Fundão - 21941-590, Rio de Janeiro, Brasil, apbmoreira@osite.com.br; mcbastos@micro.ufrj.br; santoskrn@micro.ufrj.br; marciagm@micro.ufrj.br; marinella@micro.ufrj.br; (2) Departamento de Invertebrados - Museu Nacional - UFRJ - Quinta da Boa Vista, s/no., São Cristóvão - 20940-040, Rio de Janeiro, Brasil, muricy@acd.ufrj.br

Numerous natural products from marine sponges show striking structural similarities to known metabolites of microbial origin, suggesting that microorganisms (bacteria, microalgae) are at least involved in their biosynthesis or are in fact the true sources of such metabolites. The bacterial community of fourteen marine sponges of Rio de Janeiro was screened and characterized using classical and molecular techniques. Sixty-three bacterial strains were isolated from BHI-agar, Marine-agar or Marine-agar in sea water, and phenotypically characterized. Most of the bacterial isolates were pigmented (yellow, orange or brownish); 85% of the strains were Gram-positive, cocci or rod in shape; sometimes, rod strains showing cellular inclusions similar to spore formations. Gram-negative strains were usually long or short rods and not spore-forming. Eight bacterial isolates from *Mycale microsigmatosa*, *Petromica citrina* and *Polymastia janeirensis* sponges showed antimicrobial activity against *Corynebacterium fimi* NCTC7547 or *Staphylococcus aureus* ATCC 29213. Partial sequence (about 500 bp) of the 16S rDNA of the eight strains was amplified by PCR and it will be sequenced to molecular identification of the bacteria at the species level. The sequence obtained will be analyzed with the queries generated by BLAST of GenBank Database.

Cross-species induction for the enhancement of antimicrobial activity produced by epibiotic bacteria isolated from Indian marine sponge *Pseudoceratina purpurea*

Shinichi Nagata { XE "Nagata, Shinichi" }^(*); Manmadhan Kanagasabhpathy { XE "Kanagasabhpathy, Manmadhan" }

(1) Environmental Biochemistry group, Research Center for Inland Seas, Kobe University, 5-1-1 Fukaminamicho, Higashinada-ku, Kobe 658-0022, Japan, nagata@maritime.kobe-u.ac.jp

Cross-species control of bacterial metabolism was first described in *Vibrio harveyi* where induction of bioluminescence by culture supernatant fluids of non-luminescent *Vibrio* species was reported. Our laboratory is currently investigating the potential of surface associated bacteria isolated from marine invertebrates against environmentally relevant strains. In our earlier study we have isolated four strains of Gram positive bacteria attaching the surface of a potent marine sponge *Pseudoceratina purpurea* from the shallow waters of Gulf of Mannar, India. These four bacteria belonging to genus *Bacillus* and *Virgibacillus* showed inhibitory activity against several marine strains tested. Current screening assays are inadequate for checking the antibacterial activity, because it was already reported that some bacteria which do not produce antimicrobial compounds in single culture do produce when other microbes challenge them, as would be the situation in marine environment. Until now very few attempts have been made in the above aspect of "cross-species induction". In this study antibiotic producing marine strains isolated from marine sponge *P. purpurea* were exposed to living cells of some human pathogenic bacteria as well as some fouling bacteria isolated from a natural biofilm. Marine fouling bacteria were used as inducer strains, because we assumed that these strains would induce the production of antimicrobial compounds specifically against these organisms. Our results have important implications on the new methods of screening bacteria that are producing antimicrobial compounds. Further the effect of mixed cultures of bacteria on antimicrobial activity will be discussed as this will give a clear picture of ecological relationship taking place on sponge surface.

**Use of molecular and conventional methodology to access microbial diversity in
*Hymeniacidon heliophila***

Raquel Peixoto{ XE "Peixoto, Raquel" }⁽¹⁾; Estevão Miranda{ XE "Miranda, Estevão" }⁽²⁾; Ana Paula Barbosa Moreira{ XE "Moreira, Ana Paula Barbosa" }⁽³⁾; Marinella Silva Laport{ XE "Laport, Marinella Silva" }⁽³⁾; Fábio Araújo{ XE "Araújo, Fábio" }⁽²⁾; Alexandre Rosado{ XE "Rosado, Alexandre" }^(1*)

(1) IMPPG/UFRJ- Departamento de Microbiologia Geral/CCS Bloco E-Cidade Universitária, Rio de Janeiro, CEP 21941-590, Brazil, asrosado@micro.ufrj.br; (2) Faculdade de Formação de Professores, UERJ, Brazil; (3) IMPPG/UFRJ - Departamento de Microbiologia Médica/CCS Bloco I-Cidade Universitária, Rio de Janeiro, CEP 21941-590. Brazil

The aim of this study was evaluate the microbial community structure associated to *Hymeniacidon heliophila* using molecular and conventional techniques. Three specimens were collected at Praia Vermelha, Rio de Janeiro. We used 3 different pieces of each one and surrounding water from the 3 points of sampling for culture independent approach and plating in Marine agar medium. Plating counts revealed a considerable number of bacterial colonies for each sample (about 10^3 - 10^4 CFU), with 13 different morphotypes. Those different isolates are being tested against some pathogenic bacteria to see their antagonistic potential. The 16S rDNA amplification and the DGGE analysis showed very different patterns between water samples and sponge samples. Sponge replicates and replicates of each sponge had the same dominant community structure in DGGE patterns. Some bacterial populations were dominant in sponge samples and could not be detected in water samples. Even more, other bacterial populations were also only observed in water samples. This preliminary result indicates a specific dominant bacterial community in sponge samples. Now we are sequencing the specific bands to identify the selected microorganisms and also evaluating the diversity of fungi and archaea associated with *H. heliophila* by PCR/DGGE. We will also perform the molecular characterization of the bacterial isolates with antagonistic activities by BOX/PCR fingerprinting.

A closer histological view on microbial life in Mediterranean sponges

Martin Pfannkuchen{ XE "Pfannkuchen, Martin" }^(*); Sabine Schlesinger{ XE "Schlesinger, Sabine" }; Anne Klöppel{ XE "Klöppel, Anne" }; Franz Brümmer{ XE "Brümmer, Franz" }

Biological Institute, Department of Zoology, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany, martin.pfannkuchen@bio.uni-stuttgart.de; sabine.schlesinger@bio.uni-stuttgart.de; anne.kloepfel@bio.uni-stuttgart.de; franz.bruegger@bio.uni-stuttgart.de

Sponges (Porifera) are sessile filter feeders with an enormous filtering capacity and an equally enormous energy consuming throughput resulting in most cases in difficulties in sponge cultivation eg. sufficient nutrient supply. We here show the employment and combination of electron microscopical, light microscopical and molecular biological techniques to approach the function of sponges and their microbial association and their variability during sampling and *ex situ* cultivation. To have a closer look at sponge associated microbes during cultivation of marine sponges, tissue samples were taken in-situ and during *ex situ* cultivation. Changes in the bacterial association were correlated to our histological observations and resulting physiology of the sponges eg. secondary metabolites like isofistularin aerophysinin I and aerophobin I II are measured and do not decrease during *ex situ* cultivation. Besides transmission electron microscopy (TEM) also paraffin embedding and cryosectioning techniques were applied. In addition, for specific bacterial identification fluorescent in-situ hybridisation (FISH) was used and, for the detection of cyanobacteria their autofluorescence. Additional DAPI staining showed also non autofluorescent bacteria. Transmission electron microscopy identified different bacteria by morphological structures. Combining the data sets resulted, we constructed a three dimensional model of bacterial distribution in sponge tissue and we found a substantially low bacterial load for the *in situ* condition we investigated. With the different microscopic techniques, especially the optimized fluorescence microscopy, we were able to overcome the main problems like high autofluorescence signal shift and specificity. What is even more, the different methods are controlling themselves.

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Vertical transmission of bacteria in Caribbean reef sponges

Susanne Schmitt{ XE "Schmitt, Susanne" }⁽¹⁾; Jeremy Weisz{ XE "Weisz, Jeremy" }⁽²⁾; Niels Lindquist{ XE "Lindquist, Niels" }⁽²⁾; Jonathan Cowart{ XE "Cowart, Jonathan" }⁽³⁾; Ute Hentschel{ XE "Hentschel, Ute" }^(1*)

(1) Research Center for Infectious Diseases, University of Wuerzburg, Roentgenring 11, 97070 Wuerzburg, Germany, susanne.schmitt@mail.uni-wuerzburg.de, ute.hentschel@mail.uni-wuerzburg.de; (2) University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557, USA, weisz@unc.edu, nlindquist@unc.edu; (3) Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC 28403, USA, jdc1234@uncw.edu

Settlement experiments were performed with various caribbean reef sponges to investigate the role of the reproductive stages (embryos and larvae) for vertical transmission of the microbial community associated with the host sponge. Larvae of viviparous sponges (*e.g. Ircinia felix*, *I. strobilina*, *Smenospongia aurea*) were caught from the water column immediately upon release. The reproductive stages of other sponges (*e.g. Agelas wiedenmayeri*, *Ectyoplasia ferox*, *Xestospongia muta*) that are excreted in form of a gelatinous sheet were taken up with a pipette shortly after spawning. The reproductive stages were transferred directly into tea buoies and allowed to settle for 3-4 days before recovery by SCUBA diving. Electronmicroscopical inspection revealed that large amounts of morphologically diverse bacteria were located in the center of sponge larvae while the outer rim appeared to be bacteria free. In the settled larvae, bacteria were found in between densely packed sponge cells. Denaturing gradient gel electrophoresis (DGGE) was performed on *I. felix* to characterize and compare the microbial community profiles of the adult sponge with that of its reproductive stages. Interestingly, the banding patterns of larvae released by the same adult were more similar than the banding patterns of larvae from different individuals. In total, over 200 bands were excised and sequenced. The phylogenetic diversity of adult *I. felix* and its larvae was equally high and included members of the phyla Alpha-, Gamma- and Deltaproteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, Bacteroidetes and a clade of uncertain taxonomic affiliation. 59% of the adult, 40% of the larval sequences and 16% of the sequences from settled larvae were composed of members of the sponge-specific microbial community described previously. In summary, it could be shown that a phylogenetically complex microbial consortium as an entity is transmitted vertically to the next sponge generation. These results help to gain a better understanding of the evolution of this possibly very ancient sponge bacteria association.

Strategies for the isolation and cultivation of sponge associated microbial endosymbionts

Joseph Selvin{ XE "Selvin, Joseph" }^(1*); Aron Premnath Lipton{ XE "Lipton, Aron Premnath" }⁽²⁾

(1) Department of Microbiology Bharathidasan University, Tiruchirappalli 620 024, India, selvinj@rediffmail.com; (2) Marine Biotechnology Laboratory Central Marine Fisheries Research Institute, Vizhinjam 695 520, Trivandrum, India, liptova@yahoo.com

The wider biosynthetic capabilities of marine sponges reported to be associated with the symbiotic microorganisms. Bacterial symbionts constitute for 30%-60% of the mesohyl, mostly occur as intracellular symbionts (endosymbionts). It has been estimated that 99% of sponge associated bacterial endosymbionts are uncultivable under laboratory condition using available media. The development of isolation methods is a state of art, since the microbes originated in a complex undetermined niche of sponge body. Therefore efforts were taken to develop various strategies for the successful isolation of bacterial endosymbionts. Preliminary studies indicated that the sponge-associated bacteria could be isolated in the medium supplemented with sponge extract. Sponge-associated antagonistic actinomycetes were isolated from two marine sponges (*Dendrilla nigra* and *Axinella donnani*) reported for potent biological activity, using modified Emerson agar enriched with respective host sponge extract. The isolation methods were effectively used for the selective isolation and cultivation of *Streptomyces* sp. (SBE5) from *D. nigra* and *Saccharomonospora* sp. (SBE9) from *A. donnani*. Both isolates showed antimicrobial activity as observed in the host sponges. The isolate *Saccharomonospora* was isolated first time from a marine sponge, though it has been reported from marine environment. However, this method was not always successful particularly isolation using marine agar (Himedia) due to the over grown plate by fast growing environmental contaminants. Therefore an alternate strategy was used to prevent the growth of fast growing and/or environmental contaminants in the isolation media. In this method, chosen antibiotic (nalidixic acid) was added to the culture media to prevent the growth of habitat bacteria. The morphotypes of sponge isolates were compared to the total community structure of habitat flora to preclude most common morphotypes. Considering the involvement of acyl homoserine lactones in bacterial colonization of many higher organisms, it was speculated that such quorum sensing signals could play a part in interactions between sponges and the dense bacterial communities living within them. The enrichment of autoinducer, N-(ketocaproyl)-DL-homoserine lactone in the media found increased the growth of new morphotype (SBE19) in the culture media from *D. nigra*. Though the enrichment methods were increased the possibility of isolating sponge endosymbionts, the commercial production of these media needs thorough understanding of nutritional requirement of bacterial endosymbionts.

Bioprospecting of sponge associated microbial endosymbionts

Joseph Selvin{ XE "Selvin, Joseph" }^(1*); Aron Premnath Lipton{ XE "Lipton, Aron Premnath" }⁽²⁾

(1) Department of Microbiology Bharathidasan University, Tiruchirappalli 620 024, India, selvinj@rediffmail.com; (2) Marine Biotechnology Laboratory Central Marine Fisheries Research Institute, Vizhinjam 695 520, Trivandrum, India, liptova@yahoo.com

Marine sponges are known to produce a wide range of natural products and bioactive secondary metabolites. However in many instances, the limited availability of sponge material might preclude the commercial production of bioactive compounds of potential pharmaceutical importance. The chemical ecology of bacterium sponge relationship has recently been recognized as a promising venture in the discovery of novel drugs from marine sponges. Antimicrobial agents of host sponges were isolated from associated bacterial endosymbionts. The antagonistic bacterial endosymbionts was presumed to be playing an active role in the host defense of marine sponges, particularly maintaining homeostasis in their habitat. Therefore the isolation of bioactive compounds from symbiotic bacteria could overcome the supply problem by providing a consistent yield using large-scale fermentation process, eliminating the need to harvest sponges from the natural bed. The secondary metabolites of sponges were widely varied with geographical location, substratum and growth stage of the sponges. This variation was earlier reported due to the variation of microbial endosymbionts in different environmental conditions and growth stages. Recent findings indicated that the host sponge obviously harbour uniform microbial endosymbiont(s) irrespective of environmental conditions. Similar actinomycetes were isolated from *Dendrilla nigra*, *Axinella donnani* and *Spongia* sp. collected from different locations along peninsular coast of India. Primers designed for the amplification of actinomycete specific 16S rRNA gene sequences used to demonstrate occurrence of uniform actinomycetes in the sponge tissues. *In vitro* findings revealed that all actinomycete strains contained broad spectrum antibacterial agents irrespective of collection centres.

Identification of a vertically transmitted microbial assemblage in the tropical sponge *Corticium candelabrum*

Koty Sharp{ XE "Sharp, Koty" }^(1*, 2); Boreth Eam{ XE "Eam, Boreth" }⁽¹⁾; Margo Haygood{ XE "Haygood, Margo" }^(1, 3)

(1) Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, CA 92037, USA; ksharp@ucsd.edu; (2) Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA; (3) Department of Environmental and Biomolecular Systems, OGI School of Science & Engineering, Mail Code OGI 100, Oregon Health & Science University, 20000 NW Walker Road, Beaverton, Oregon 97006-8921, USA, haygoodm@ebs.ogi.edu

Sponge-microbe associations, which play crucial roles in nutrient and carbon cycling in diverse marine environments, are spatiotemporally stable, but how these consistent associations are assembled and maintained from one sponge generation to the next is not well understood. Sponges also are a prolific source of diverse bioactive compounds with potential therapeutic and clinical value, many of which have long been suspected to be of microbial origin. Here we investigate the sponge *Corticium candelabrum*, a sponge from the tropical Pacific, which contains the tedanolides, complex polyketides with highly potent anti-tumor bioactivity. This study characterizes the diversity of microbial associates in *C. candelabrum*, focusing on microbes associated with embryos and larvae. Here we show that both archaea and bacteria are present in brooded *C. candelabrum* embryos. Further examination of the bacterial community using sequence-specific fluorescence *in situ* hybridization (FISH) demonstrates that at least three bacterial sequence types - one proteobacterium, an actinobacterium, and a group of strains from an uncharacterized clade of bacteria ("sponge clade 1"), are consistently present in specialized aggregates within embryos of the sponge. These three sequence types were present in all adult samples tested over a three-year period, collected across 100km in the Palau archipelago. FISH results suggest that they make up a small portion of the overall bacterial biomass in the embryos. *C. candelabrum* oocytes do not appear to contain bacteria, but bacteria are present in early stage embryos, and they remain associated with developing embryos and larvae after release into the seawater. One of three taxa examined, alpha-CC01, an alpha-proteobacterium most closely related to other known symbionts, is present in swimming *C. candelabrum* larvae after release from the adult. There is no indication of microbe digestion in any stage of embryonic development, and bacteria are extracellular in the embryos and larvae. This is the first identification of specific multiple microorganisms vertically transmitted by a marine invertebrate host, and results of this study provide evidence that sponges maintain specific associations with complex assemblages of microbes. These studies also highlight the importance of identifying bacteria and archaea associated with invertebrate embryos in focusing the search for microbes consistently associated with the host.

Filamentous cyanobacterial symbionts of Caribbean sponges

Robert W. Thacker{ XE "Thacker, Robert W." }^(1*); Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }⁽²⁾; Klaus Rützler{ XE "Rützler, Klaus" }⁽³⁾; Patrick M. Erwin{ XE "Erwin, Patrick M." }⁽⁴⁾; Steven J.A. Kimble{ XE "Kimble, Steven J.A." }⁽⁴⁾; Melissa J. Pierce{ XE "Pierce, Melissa J." }⁽⁴⁾

(1) Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA, thacker@uab.edu; (2) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com; (3) National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0163, USA, ruetzler.klaus@nmnh.si.edu; (4) Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA, erwin@uab.edu; sjkimble@uab.edu; mslissa@uab.edu

Filamentous cyanobacteria classified as *Oscillatoria spongeliae* have previously been reported from sponges distributed throughout the Indo-Pacific, including representatives of the dictyoceratid genera *Dysidea*, *Lamellodysidea*, *Lendenfeldia*, and *Phyllospongia*. Morphologically similar symbionts have also been reported from the Caribbean, associated with the dictyoceratid sponge *Hyrtios violaceus* (formerly *Oligoceras hemorrhages*). We investigated the filamentous cyanobacterial symbionts associated with two recently described species from the Caribbean coast of Panama, *Haliclona* n.sp. and *Xestospongia* n.sp. We amplified and sequenced cyanobacterial 16S ribosomal RNA genes from *H. violaceus*, *Haliclona* n.sp. and *Xestospongia* n.sp. We used the chlorophyll a content of *Haliclona* n.sp. and *Xestospongia* n.sp. as an index of symbiont abundance and also measured the photosynthetic and respiration rates of these associations. A phylogeny of 16S ribosomal RNA genes reveals that the symbionts of all three sponges are part of the *O. spongeliae* clade and that each sponge hosts a unique ribotype of the cyanobacterium. The *H. violaceus* symbiont is most similar to that of *Lendenfeldia chondrodes*, while *Haliclona* n.sp. and *Xestospongia* n.sp. host very similar symbionts (only 0.78% sequence divergence) that are most similar to the symbiont of *Phyllospongia papyracea*. Chlorophyll a content averaged $423 \pm 10 \mu\text{g} / \text{g}$ for *Haliclona* n.sp. and $248 \pm 8 \mu\text{g} / \text{g}$ for *Xestospongia* n.sp. Preliminary data on the photosynthetic and respiration rates of these associations reveal photosynthesis to respiration ratios of 2.1 for *Haliclona* n.sp. and 3.8 for *Xestospongia* n.sp., indicating that these symbionts can compensate for the respiration demands of their host sponges. Therefore, specialized associations with filamentous cyanobacteria can provide a valuable source of carbon to host sponges. These associations occur over a broader phylogenetic range of host sponges than previously described, including representatives of the orders Dictyoceratida and Haplosclerida.

**Simultaneous calcification and silification processes in a non-sclerosponge species:
The enigmatic *Hemimycale* spherules revisited**

Maria-J. Uriz{ XE "Uriz, Maria-J." }^(1*); Xavier Turon{ XE "Turon, Xavier" }⁽²⁾

(1) Centre d'Estudis Avançats de Blanes (CEAB). CSIC Accés Cala St Francesc, 14. 17330 Blanes (Girona). Spain, iosune@ceab.csic.es; (2) Department of Animal Biology (Invertebrates), University of Barcelona, 645 Diagonal Av. E-08028 Barcelona, Spain

The typical sponges known so far that produce simultaneously calcium-carbonate and silica-made skeletons are sclerosponges, which proliferated and built reefs in the late Permian (Paleozoic)-late Triassic (Mesozoic) and are currently reduced to cryptic confined habitats. Here we report on the simultaneous intracellular calcification and silification in an extant non-sclerosponge demosponge: *Hemimycale columella*. This species has been reported to contain high amounts of irregular calcified bodies (up to 55µm in size), the nature, origin and function of which remained enigmatic. In contrast, we have found only calcium carbonate spherulites, 1-1.5 µm in diameter, in living specimens of *H. columella*. Spherulites are produced intra-cellularly, in bacteriocyte-like cells. The large irregular forms previously reported seem to be the result of fixation procedures, which promoted calcium-carbonate dissolution and posterior precipitation, which may explain the absence of Mg found in these large bodies. Spherulites appear to be a particular type of symbiotic bacteria, since instances of spherulite bipartition are frequent and a spherical organic matrix is already visible before calcification starts. Both photosynthetic and heterotrophic bacteria have been reported to produce the appropriate micro-environment (a basic pH) favouring calcium-carbonate precipitation but this would be the first time that calcified bacteria are reported to live in symbiosis with sponges. Spherulites are in principle secreted in individual vacuoles. However mature bacteriocytes show multispherulitic vacuoles as a result of spherulite division. Spherulites are pseudospherical but also may have a compound shape as a result of partition in two/three/ and four fused spheres. Bacteria-full cells disaggregate and release the spherulites to the sponge mesohyle. The calcareous envelope of these microorganisms crystallises as Mg-calcite, as shown through X-ray microanalysis, although Mg is less abundant than in calcitic spicules. How the sponge manages this calcitic material, the possible benefits for the sponge, and the implications in skeleton evolution of sponges are considered.

Importance of sponge-cyanobacterial symbioses in temperate, nutrient-rich waters
(invited lecture)

Kayley M. Usher{ XE "Usher, Kayley M." }⁽¹⁾; Walter M. Goldberg{ XE "Goldberg, Walter M." }⁽²⁾; Simon Toze{ XE "Toze, Simon" }⁽³⁾; Jane Fromont{ XE "Fromont, Jane" }⁽⁴⁾

(1) University of Western Australia. Microbiology M502 School of Biomedical & Chemical Sciences 35. Stirling Highway Crawley 6009, Australia, kusher@cyllene.uwa.edu.au; (2) Florida Int Univ, Dept Biol Sci, Electron Microscopy Lab, Univ Pk Campus, Miami, FL 33199 USA, goldberg@fiu.edu; (3) CSIRO, Land & Water, Ctr Groundwater Studies, Private Bag PO, Wembley, WA6014. Australia; (4) Western Australian Museum, Perth, WA6000 Australia, jane.fromont@museum.wa.gov.au

How important are cyanobacterial symbioses to marine sponges living in temperate, relatively high-nutrient waters? A common assumption that symbioses with photosynthetic organisms are significantly more prevalent in oligotrophic waters of tropical and sub-tropical regions than in temperate waters has rarely been established quantitatively. Cyanobacterial symbioses occur in a large taxonomic range of sponges, contributing 80% of host carbon requirements in some instances, in addition to UV protection and biochemical defence. This significantly enhances host survival, growth and productivity. However, it is not known what percentage of marine sponges depend on cyanobacterial symbionts in temperate regions. Our investigation of sponge/cyanobacterial symbioses in temperate Western Australian waters suggests that these symbioses are abundant and important, possibly to an equivalent degree as in tropical regions. In addition, this research expands the understanding of the specificity and diversity of cyanobacterial symbioses with sponges, and has implications for the impact of climate change on temperate reef ecosystems.

Symbiotic bacteria of different species of *Oscarella* (Porifera, Homoscleromorpha, Plakinidae): Comparative ultrastructural studies

Andrey Vishnyakov { XE "Vishnyakov, Andrey" }^(1*); Alexander Ereskovsky { XE "Ereskovsky, Alexander" }⁽²⁾

(1) Department of Invertebrate Zoology, Biological and Soil Sciences Faculty, St. Petersburg State University, Universitetskaya nab., 7/9, 199034, Saint-Petersburg, Russia, vishnyakov@hotmail.com; (2)

Department of Embryology, Biological and Soil Sciences Faculty, St. Petersburg State University, Universitetskaya nab., 7/9, 199034, Saint-Petersburg, Russia, ereskovsky@hotmail.com

One of the specific features of the sponges is the presence of obligate endosymbiotic bacteria in their body. Symbiotic bacteria from six *Oscarella* species (adults, embryos and larvae) collected in Mediterranean (*O. lobularis*, *O. tuberculata*, *O. microlobata*, *O. imperialis*), Japan (*Oscarella* sp. 1) and Caribbean (*Oscarella* sp. 2) Seas were investigated by using of electron microscopy (SEM and TEM). In most cases symbionts are numerous and all sponge species are inhabited by at least two different symbiotic bacteria. Each sponge species has quite definite set of bacterial morphological types. The general feature of all *Oscarella* species is absence of intracellular symbiotic bacteria. Symbionts occupy the mesohyl of adult sponges, intercellular space in embryos and the larval cavity. Sometimes bacteria are in contact with mesohylar filaments or cells. In last case bacteria of some morphotypes produce specific for every morphotype blebs. All bacterial symbionts have no flagellum. Most symbiont morphs have bacterial cell wall which is characteristic for Gram negative bacteria. Cytoplasm content and nucleoid are variable. The data on bacterial symbionts supplemented by taxonomic results can be good characteristic for such species difficult to determine as *Oscarella* and their relatives.

Calcareous sponges (Porifera Calcarea) from Chile

Fernanda Correia Azevedo{ XE "Azevedo, Fernanda Correia" }⁽¹⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽²⁾; Michelle Klautau{ XE "Klautau, Michelle" }^(1*)

(1) Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Pau Brasil 211, CCS, 21941-590, Rio de Janeiro, RJ, Brazil; (2) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, 20940-040, Rio de Janeiro, RJ, Brazil

Sponges are one of the most important organisms of Chilean benthic fauna. Nonetheless, few calcareous species have been cited in the region. The present study describes and discusses the taxonomic composition of calcareous sponges from Chile. Analyses of qualitative composition as well as abundance and distribution by localities were made. A total of 37 specimens of Calcarea were collected by SCUBA in eight localities (Antofagasta, Algorrobo, Comau, Huinay, Tambor, Punta Choros, Punta Llonco, and Quintupeu) from 2003 to 2005. Depths varied from 6.5 to 25 m. Specimens were registered in the collection of the Museu Nacional do Rio de Janeiro (Brazil). A total of six species were found, four of the subclass Calcinea (*Clathrina* cf. *aspina*, *Clathrina* sp. 1, *Clathrina* sp. 2, and *Clathrina* sp. 3), and 2 of the subclass Calcaronea (*Sycettusa* sp. nov. and *Sycon* sp. nov.). The most abundant species was *Sycon* sp. nov. (16 specimens), followed by *Clathrina* sp. 3 (6 specimens). The presence of *C. cf. aspina* in Chile is very interesting. *C. cf. aspina* is part of a group of sponges whose skeleton is composed of triactines, tripods and tetractines with no spines or vestigial spines in the apical actine. A study on the biogeography of those sponges will be the next step of this work.

The need of *in situ* observation and documentation for the description of sponge species

Franz Brümmer{ XE "Brümmer, Franz" }^(1*); Martin Pfannkuchen{ XE "Pfannkuchen, Martin" }⁽¹⁾; Jochen Gugel{ XE "Gugel, Jochen" }⁽¹⁾; Andrej Jaklin{ XE "Jaklin, Andrej" }⁽²⁾

(1) Biological Institute, Department of Zoology, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany, franz.bruegger@bio.uni-stuttgart.de; martin.pfannkuchen@bio.uni-stuttgart.de; jochen.gugel@bio.uni-stuttgart.de; (2) Rudjer Boskovic Institute, Center for Marine Research, G. Paliaga 5, 52210 Rovinj, Croatia, jaklin@cim.irb.hr

Sponges are often soft bodied, many are very fragile, some are only a few millimetres in diameter or thickness and colours are generally not very persistent. For the collection of sponges it is recommended to freeze the samples or transfer them immediately in a fixative like ethanol or different aldehydes. There are also recommendations to dry the sponges on air in the sun. It is clear, that these treatments are often necessary and inevitable for the investigation and identification of samples and for establishing new species. Also the storage over decades is only possible with dried or chemically fixed material. All these treatments, however, can and in most cases do, result in alterations in shape, colour and in loss of certain and sometimes also prominent sample characteristics. If the worst comes to the worst, the characteristics that lead to the erection of a new species cannot be found *in situ* or the other way round. Thus, as our investigations demonstrate, important characters disappear during sampling and/or fixation and thus are not included in a subsequent taxonomic work! Scientific diving has been developed during the last decades into an important and most valuable technique for obtaining new insights in marine biology. The methods used under water for *in situ* observation and documentation and, most important, direct observation and sampling in a sustainable manner are more valuable than remote mechanical scraping and dredging. SCUBA diving is in contrary to the common belief, not dangerous at all, if performed under tolerable conditions by well trained and equipped staff. Of course, all the safety rules must be strictly obeyed. This "multi level strategy" allows us, however, to obtain coordinated information of the target species in a lot of different fields, e.g. ecology and biodiversity of the local habitats, behavior of submarine organisms, morphology and colour, geology, geomorphology and so on. Moreover, the relationships to other taxa depending upon the site can be also explored by diving in the first place. The possibility of submarine photos taken by the diver using modern photographic devices demonstrates that this procedure provides us with delicate characteristics profitable for the description and identification of specimen. Information on specimens collected and documented in this way shows morphological characters like colour, subectodermal canals, and even oscula with corresponding canal system virtually disappearing after e.g. mechanical stress due to other and ungentle methods. Some of our findings are: *Tethya* spp. - shrinkage, disappearance of oscules, change of colour; *Mycale tunicata* - shrinkage, disappearance of oscules and pinacodermal canal system, change of colour; *Suberites* spp. - shrinkage, loss of sediment cover, reduction of oscule diameter or even loss of oscule, change of colour. Our aforementioned results show the importance of *in situ* observation and delicate sampling performed by SCUBA divers for an undisturbed, complete and differential description of species.

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Description and taxonomic position of a new genus of Tethyidae from North Sulawesi (Indonesia)

Barbara Calcinai{ XE "Calcinai, Barbara" }^(1*); Giorgio Bavestrello{ XE "Bavestrello, Giorgio" }⁽¹⁾; Carlo Cerrano{ XE "Cerrano, Carlo" }⁽²⁾; Michele Sarà{ XE "Sarà, Michele" }⁽²⁾

(1) Dipartimento di Scienze del Mare, Via Breccie Bianche, 60131, Ancona, Italy, b.calcinai@univpm.it; bavestrello@univpm.it; (2) Dipartimento per lo studio del Territorio e delle sue Risorse, Corso Europa, 26, 16132, Genova, Italy, cerrano@dipteris.unige.it; sara@dipteris.unige.it

The area of Pacific Ocean, including also the Indonesian Archipelago, is considered a centre of evolutionary radiation, with a very high level of marine biodiversity. The high number of new marine genera and species that recently have been described from the Indonesian Archipelago supports this idea. In this paper we describe a new sponge species belonging to a new genus recorded in the Bunaken Marine Park (North Sulawesi, Indonesia). The sponge body is massive, often flattened, cushion-shaped with rounded lobes; some samples were observed with an evident oscular prominence. This species can be found from about 5 m to about 40 meters depth, and can reach more than 30 cm in diameter, and more than 15 cm in thickness. In the field, it is not easily visible owing to the presence of a coat of epibionts. In the sponge a definite collagenous cortex, orange in colour, 4-5 mm thick and a brilliant yellow medulla are distinguishable. The skeleton is radial in the sponge periphery and disarranged in the central part. In the cortex long subtylostyles are organized in fans of radiating macroscleres protruding through the sponge surface. Among the fans the megasters are densely distributed. In the choanosome the oxeas make bundles that in the peripheral part are organized in a clear radiating skeleton. In these bundles rare anatriaenes are always present. In the inner part of the choanosome the skeleton becomes confused and the radiating organisation is lost. The microscleres are large megasters and four types of micrasters, differently distributed in cortex and medulla. Megasters and micrasters of the first and second type are only present in the cortex. The micrasters are variously distributed around the lacunae and in the peripheral part, where they make a thin tangential stratum. In the medulla micrasters of the third and fourth types are variously dispersed. This species may be considered as representative of a new genus likely belonging to the Tethyidae and especially close to *Oxytethya* characterized by a main skeleton of oxeas. The occurrence of scattered triaenes, never recorded before in Tethyidae, may be considered as a further indication of the strong relationship between Tethyidae and Astrophorida and also raise the question of the position of this family.

Sponges (Porifera, Demospongiae) of Brazilian Antarctic Program - PROANTAR, Bransfield Strait, off D'Urville and Joinville Island

Maurício Campos { XE "Campos, Maurício" }^(1*,2); Beatriz Mothes { XE "Mothes, Beatriz" }⁽¹⁾;
Cléa Lerner { XE "Lerner, Cléa" }⁽¹⁾; João Luís Carraro { XE "Carraro, João Luís" }⁽¹⁾; Inga
Ludmila Veitenheimer-Mendes { XE "Veitenheimer-Mendes, Inga Ludmila" }⁽²⁾

(1) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul. Rua Salvador França 1427, 90690-000 Porto Alegre-RS, Brazil, mrcpoa@hotmail.com; (2) Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul. Av. Bento Gonçalves 9500, 91501-970 Porto Alegre-RS, Brazil

Sponges from Antarctica have many records, which were yielded by several expeditions since more than 100 years ago. Nevertheless some areas are not yet completely studied, like South Shetland Islands and neighbour areas. The achievement of new faunistic surveys in that continent will be greatly important, in order to associate your abundance with the environmental conditions, their annual changes and also to extend geographic and bathymetric records. Through "Programa Antártico Brasileiro" - PROANTAR, Porifera samples were gathered in the course of the 1980s decade, in areas covering the South Shetland Islands, Bransfield Strait and neighbouring islands, D'Urville and Joinville Islands, where the present study is focused. Such samples, coming from the IV expedition, were collected at 62°53'S-56°27'W / 63°01'S-54°49'W, between depths from 82 to 274 m, by using "beam-trawl" dredge; the samples are deposited in the Porifera collection of Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Brazil. The following species are so far identified: *Iophon terranova* Calcinai & Pansini, 2000, *Artemisina apollinis* (Ridley & Dendy, 1886), *Phorbas areolata* (Thiele, 1905), *Myxilla* (*Myxilla*) *mollis* (Ridley & Dendy, 1886), *Myxilla* (*Ectomyxilla*) *chilensis* (Thiele, 1905), *Mycale* (*Oxymycale*) *acerata* (Kirkpatrick, 1907), *Isodictya erinacea* (Topsent, 1916), *Haliclona* (*Gellius*) *rudis* (Topsent, 1901), *Haliclona* (*Gellius*) *phakelloides* (Kirkpatrick, 1907), *Haliclona* (?*Reniera*) *dancoi* (Topsent, 1901), *Hemigellius pachyderma* Burton, 1932 and *Microxina benedeni* Topsent, 1901. For the first time both D'Urville and Joinville Island had their sponge fauna extensively studied.

Sponges of Order Halichondrida (Porifera, Demospongiae) collected at Santa Catarina state coast, Brazil

João Luís Carraro{ XE "Carraro, João Luís" }⁽¹⁾; Alexandre Bondan{ XE "Bondan, Alexandre" }⁽¹⁾; Guilherme Rupp{ XE "Rupp, Guilherme" }⁽²⁾; Adriano Marenzi{ XE "Marenzi, Adriano" }⁽³⁾; Mauricio Campos{ XE "Campos, Mauricio" }⁽¹⁾; Rafael Eckert{ XE "Eckert, Rafael" }⁽¹⁾; Beatriz Mothes{ XE "Mothes, Beatriz" }⁽¹⁾; Mariana S. de Carvalho{ XE "Carvalho, Mariana S. de" }⁽⁴⁾; Cléa Lerner{ XE "Lerner, Cléa" }^(1*)

(1) Museu de Ciências Naturais, Seção de Zoologia de Invertebrados da Fundação Zoobotânica do Rio Grande do Sul. Av. Dr. Salvador França, 1427. 90690-000, Porto Alegre, RS, Brasil, joaocarraro_rs@yahoo.com.br; (2) Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A., Centro de Desenvolvimento Em Aquicultura e Pesca; (3) Centro de Ciências Tecnológicas da Terra e do Mar, Maricultura, Universidade do Vale do Itajaí; (4) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro

The infralittoral Porifera fauna of south Brazilian coast begins at Santa Catarina state because of the rocky coast, many islands and various substrates for settlement. Sponge samples have been collected since 1975 by research staff of "Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul (MCN-FZB)". Until 1989 only six species had been described for this area. After this time, collecting efforts were raised resulting in an increase of described species. Nowadays thirty one species are recorded for this area and some others are not yet published. The samples have been taken by hand collection, snorkeling and scuba diving. The collected material is deposited at Scientific Porifera Collection of MCN-FZB. The taxonomic study is based on the morphologic characters of shape and size of the sponge, color, consistency, surface, skeleton structure, shape and size of the spicules. The spicules have been photographed under Scanning Electronic Microscope and the skeletons under light microscope. Due to these collecting efforts six species of Order Halichondrida are now described for Santa Catarina state coast representing 19% of the whole knowledge species for this area and at least four species are southern boundaries enlargement or probably new species. Studied material is distributed in three families: (1) Axinellidae: *Axinella corrugata* (George & Wilson, 1919), *Dragmacidon reticulata* (Ridley & Dendy, 1886), *Dragmaxia* sp. and *Axinella* sp.; (2) Halichondriidae: *Ciocalypta alba* Carvalho et al. 2003, *Hymeniacidon heliophila* Parker, 1910, *Petromica citrina* Muricy et al. 2001 and *Halichondria* (*Halichondria*) sp.; (3) Dictyonellidae: *Scopalina ruetzleri* (Wiedenmeyer, 1977) and *Scopalina* sp. Present results increase participation of studied area as a pool of Halichondrida species only behind of Canal de São Sebastião area, São Paulo state, which have twenty four species recorded.

Towards a revision of Chilean Astrophorida (Demospongiae) - SE Pacific

Mariana S. de Carvalho{ XE "Carvalho, Mariana S. de" }⁽¹⁾; Gustavo Bastos{ XE "Bastos, Gustavo" }⁽¹⁾; Ruth Desqueyroux-Faúndez{ XE "Desqueyroux-Faúndez, Ruth" }⁽²⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(1*)

(1) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil, hajdu@acd.ufrj.br; (2) Museum d'histoire Naturelle, P.O. Box 6434, 1211 Geneva 6, Switzerland, ruth.faundez@mhn.ville-ge.ch

The Demospongiae of the SE Pacific, especially the Chilean coast, are poorly known, with only about 140 marine species recorded. This number contrasts with the large length of this coast (about 4265 km), stretching from subtropical northern ecosystems to subantarctic ones on its extreme south. Several sectors of the SE Pacific were not well sampled yet, as is the case of the Patagonian Channels and the north of Chile, as well as the Peruvian and Ecuadorian coasts. Only three species of the order Astrophorida were previously known from the continental Chile, viz. *Geodia magellani*, *Stelletta clarella* and *Stelletta phrissens*. In this study, apart from a revision of known records, additional material is being gathered. New specimens were obtained on 2004 and 2005 mainly through scuba diving, down to 30 m depth, with an intensive use of underwater photography as an aid for better taxonomic descriptions. Further images are accessible from www.guiamarina.com. These specimens are deposited in the MNRJ and MHNG Porifera collections. Other specimens were obtained from the IZUA, HSFS, MHNG, AHF-USC and ZMA collections. In total, 31 specimens of Chilean Astrophorida are now available to us for study, belonging to eight species, viz. *Asteropus* sp.; *Geodia magellani* (Sollas, 1886); *G. aff. magellani* 1, *G. aff. magellani* 2, *G. aff. magellani* 3; *Poecillastra* sp. 1; *Poecillastra* sp. 2; *Stelletta phrissens* Sollas, 1886; *Stelletta clarella* De Laubenfels, 1930; *Stelletta* sp. 1; *Stelletta* sp. 2, ranging from 12 to 180 m depth. *Poecillastra* sp. 1 appears to lack tetraxons completely and is thus a likely new species.

Morphological plasticity of *Chondrilla* aff. *nucula* (Porifera, Demospongiae)

Fernanda Cavalcanti{ XE "Cavalcanti, Fernanda" }⁽¹⁾; Carla Zilberberg{ XE "Zilberberg, Carla" }⁽²⁾; Michelle Klautau{ XE "Klautau, Michelle" }^(1*)

(1) Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Pau Brasil 211, CCS, 21941-590, Rio de Janeiro, RJ, Brazil, mklautau@biologia.ufrj.br; (2) Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Pau Brasil 211, CCS, 21941-590, Rio de Janeiro, RJ, Brazil

Chondrilla nucula Schmidt, 1862 is a morphologically simple species defined by the presence of spherasters in its skeleton. For a long time *C. nucula* was considered a cosmopolitan species. However, in 1999, a molecular study that included specimens from the Mediterranean, Caribbean Sea and the Southwest Atlantic Ocean demonstrated the existence of a species complex with at least four cryptic species, besides *C. nucula*. At that time, those four species were not formally described because the morphological differences observed (*e.g.* spicule size) were attributed to plasticity and did not reflect the molecular differences. Since then, authors working on *Chondrilla* species suggested that the skeleton organization should be used to differentiate species. In order to find stable morphological characters for the systematics of *Chondrilla*, we accompanied during one year (from October 2004 to October 2005) a population from Arraial do Cabo, RJ, Brazil. Ten to fifteen specimens were monthly collected and morphological characters, such as presence of pigments, spicule distribution, and surface characteristics were analysed. The size of the spicules was also measured to observe if it varied within a population, as demonstrated earlier for conspecific populations. Our results demonstrate that the skeleton of *Chondrilla* is extremely plastic. The distribution of spicules varied along the year. From December to April, spherasters were concentrated forming a band in the ectosome, with few spicules at the base. In June, the skeleton became disorganized, with the band disappearing and spicules becoming scattered throughout the body. In October, spherasters were concentrated at the base of the sponge. The skeleton disorganization seems to be related to the sponge reproductive cycle, since in September and October, *Chondrilla* was releasing gametes. Although the distribution of spicules was variable, spicules size was homogeneous along the year. The variation in the size of spicules appears to be influenced by the environment, while the organization of the skeleton seems to be related to the sponge reproductive cycle. This result contradicts the current opinion that skeleton should be the best morphological character for the systematics of *Chondrilla*.

Demospongiae (Porifera) of the shallow coral reefs of Maceió, Alagoas state, Brazil

Victor Ribeiro Cedro{ XE "Cedro, Victor Ribeiro" }⁽¹⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽²⁾; Monica Dorigo Correia{ XE "Correia, Monica Dorigo" }^(3*)

(1) Setor de Comunidades Bentônicas, LABMAR, Universidade Federal de Alagoas; (2) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro; (3) Departamento de Zoologia e Setor de Comunidades Bentônicas, LABMAR, Universidade Federal de Alagoas, mdc@fapeal.br

The Porifera fauna in the reef ecosystems at the coast of Alagoas state is still very little known. This work reports on the sponges of the coastal coral reefs of the state's capital, Maceió (population 900,000). Four sampling areas were selected, viz. (1) Maceió harbour, (2) Piscina dos Amores, (3) Ponta Verde and (4) Jatiúca reefs, in a S - N sequence. Distances were 2,300 m between (1) and (2), 2,000 m between (2) and (3) and 1,200 m between (3) and (4). Collections were made in 2004 and 2005 by wading at low tide and snorkeling. A total of 28 species were identified so far. These were *Amphimedon* aff. *complanata*, *A. viridis*, *Biemna microacanthosigma*, *Chalinula molitba*, *Chondrilla* aff. *nucula*, *Chondrosia collectrix*, *C. aff. reniformis*, *Cinachyrella alloclada*, *C. apion*, *Cliona* aff. *celata*, *C. varians*, *Dysidea etheria*, *Echinodictyum dendroides*, *Geodia corticostylifera*, *G. papyracea*, *Haliclona curacaoensis*, *H. manglaris*, *H. melana*, *Iotrochota birotulata*, *Ircinia strobilina*, *Mycale diversisigmata*, *M. microsigmatosa*, *Niphates erecta*, *Placospongia* aff. *melobesioides*, *Spirastrella coccinea*, *S. hartmani*, *Tedania ignis* and *Tethya* sp. nov. The number of species found in each station varied from 16 in the harbour reef to 24 at Ponta Verde reef. This is likely to reflect more extensive sampling on this easily accessible reef, coupled to the presence of a large subciaphilous and slightly eutrophic environment at Ponta Verde. *Amphimedon viridis* was very common on all four sampled areas. *Cinachyrella alloclada* was very common on three areas but just common on the fourth. Next, *Chondrilla* aff. *nucula*, *Haliclona manglaris*, *H. melana* and *Tedania ignis* were very common on two stations but just common on the other two. *Cinachyrella apion*, *Iotrochota birotulata*, *Mycale microsigmatosa* and *Spirastrella coccinea* were found in a single station each, where they were rare. *Mycale diversisigmata* is a first record for the southern Atlantic. These results, albeit preliminary, indicate the occurrence of a rich sponge assemblage in the Maceió coral reefs, and advise for stricter control on human impact on these urban reefs, some of which are usually visited by hundreds of people (local collectors of sea-food and tourists) on each low tide.

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Molecular phylogeography of Mediterranean and Eastern Atlantic sponges of the genus *Aplysina*

Pierre Chevaldonné{ XE "Chevaldonné, Pierre" }^(*); Christophe Lejeune{ XE "Lejeune, Christophe" }; Yohan Pillon{ XE "Pillon, Yohan" }; Charlotte Dehollain{ XE "Dehollain, Charlotte" }; Claire Lecroisey{ XE "Lecroisey, Claire" }; Caroline Rocher{ XE "Rocher, Caroline" }; Didier Aurelle{ XE "Aurelle, Didier" }; Anne Chenuil{ XE "Chenuil, Anne" }; Thierry Pérez{ XE "Pérez, Thierry" }; Nicole Boury-Esnault{ XE "Boury-Esnault, Nicole" }; Jean Vacelet{ XE "Vacelet, Jean" }

CNRS - UMR DIMAR, Station Marine d'Endoume, Centre d'Océanologie de Marseille, Rue de la Batterie des Lions, 13007 Marseille, France, chevaldonne@com.univ-mrs.fr

Aplysina aerophoba (Verongida: Aplysinidae) is a common Demospongiae of shallow waters (0.5 m to 20 m and more) exposed to sunlight in the Mediterranean Sea and NE Atlantic waters (Canary Is., Madeira, and the West African coast). A second species, *Aplysina cavernicola*, is virtually identical to the former, except for a slightly different color, the general lack of Cyanobacteria, and its ecology: it is found at the entrance of caves or under shaded overhangs, and seems to be a Mediterranean endemic. In areas such as Marseille (South of France), ecological differences are so sharp that the two species are easily identified. However in the Eastern Mediterranean and the Adriatic, this is not always the case: the ecological transition is not as sharp and it is often difficult to tell them apart. For this reason, some authors have proposed to synonymize both species, but this question has never been formally addressed. Molecular tools are obviously one way to search for an answer, although molecular markers universally appropriate for sponge species phylogeography have yet to be found. Mitochondrial Cytochrome Oxidase I (mtCOI) sequences could be obtained, and while this marker shows very low variability, it seems to indicate that the two species are indeed valid. The low divergence is due in part to the very low inter-species discrimination of COI in sponges, but it could also be due to a recent speciation of the two taxa. Possible evidence of hybrids is discussed for the Adriatic Sea. Other *Aplysina* species from the Caribbean display similarly low inter-specific distances. Genera within the family are however well separated with this marker. Ribosomal Internal Transcribed Spacer 2 (ITS-2) was then tested in order to find a more variable marker. Besides a problem with intragenomic variability, ITS-2 did not allow to better discriminate between the two Mediterranean species, although differences between Caribbean species are greater. Finally, 6 micro- and mini-satellite loci were tested for polymorphism, 2 of which looked promising. One minisatellite, although not polymorphic enough to assess intraspecific variability, could confirm the validity of the current Mediterranean species.

Clarification of taxonomic characters for Dictyoceratida

Steve Cook{ XE "Cook, Steve" }

Earth & Oceanic Sciences Research Institute Auckland University of Technology 24 St Paul Street Private
Bag 92006 Auckland, New Zealand, steve.cook@aut.ac.nz

Distinguishing between dictyoceratid families is relatively straight forward, but distinguishing genera and subgenera within some of those families can be difficult. Dictyoceratid genera are characterised by the presence, absence, structure and organisation, of skeletal elements, surface characters, choanocytes, collagen, cortical armour and general form. There are published instances of misunderstood characters leading to unnecessary and erroneous reassignments or generic mis-diagnoses, *e.g.* cortical armour. There are also situations where authors have 'tweaked' genera to accommodate new species, to the point where generic descriptions become too broad, such that the character boundaries of the genus become indistinct. These factors have led to confusion when attempting to allocate dictyoceratid specimens to some genera. Previous published work attempted to clarify some of these problems, but the aim here is to consolidate those clarifications of dictyoceratid characters into one entity. In addition, some of the images previously published in support of character definitions are poor, hence new and improved images are presented here.

New material of *Caminus spheroconia* Sollas, 1886 from Brazil (Geodiidae, Astrophorida, Demospongiae), with an identification key for the *Caminus* of the world

Bruno Cosme{ XE "Cosme, Bruno" }⁽¹⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽²⁾; Solange Peixinho{ XE "Peixinho, Solange" }^(1*)

(1) Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, Campus de Ondina, 40210-170, Salvador, BA, Brazil, peixinho@ufba.br; (2) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, 20940-040, Rio de Janeiro, RJ, Brazil

Caminus is a small geodiid genus comprising five species worldwide, viz.: *C. albus* Pulitzer-Finali, 1996, from Papua New Guinea, possessing oxeas over 2000 mm long, globose sterrasters and oxyasters without a centrum; *C. awashimensis* Tanita, 1969, from Japan, with oxeas smaller than 2000 mm long and strongyles, as megascleres, plus globose sterrasters and oxyasters; *C. chinensis* Lindgren, 1897, from Malaysia, with strongyles, globose sterrasters and oxyasters; *C. vulcani* Schmidt, 1862, from the Mediterranean, contains strongyles, flattened sterrasters and oxyasters; and *C. spheroconia* Sollas, 1886, from the Tropical western Atlantic, which has strongyles and globose sterrasters, but do not have either oxeas or oxyasters. These species are further characterized by the possession of volcano-shaped (obconical) habit, apical oscule, and calthrops-like orthotriaenes and spherules. The specimen studied was collected through scuba diving (10 m depth) at Todos os Santos Bay, Bahia state, and is deposited in the MNRJ and UFBA-POR collections. Standard protocols were employed for description of external morphology, as well as for the obtention of dissociated spicules and thick sections preparations, for study of the internal morphology. The spicule complement has been studied under light and electron microscopy. In this work we are re-describing *C. spheroconia* based on a new specimen, and we propose an identification key based on characters assessed from the literature. The specimen studied is a massive sponge measuring 31 x 15 mm in area, and 15 mm in thickness. When alive the external colour was dark brown and the internal one beige. The surface is slightly hispid on most parts, but smooth near the lobule. Consistency is hard, but compressible externally, soft and dense internally. The ectosome is organized in concentric layers with a radial overall structure. The thin external layer has spherules and centrotylote microhabds, and is situated on top of the layer of sterrasters, which is 200 µm thick and trespassed by short perpendicular channels. The calthrops-like plagiortriaenes are organized side by side with their cladomes supporting the ectosome. The choanosome is radially arranged, especially on the peripheral area, and more confused towards the central region. It is formed by ascending paucispicular or multispicular bundles of strongyles. Spherules and sterrasters are present all over the choanosome too, but young forms are more abundant near the central region of the sponge. The spicules of our specimen are plagiortriaenes, one category of strongyles, sterrasters, spherules and centrotylote microhabds. When it is compared to previous descriptions, plagiortriaenes replace orthotriaenes, and centrotylote microrhabds confirmed the observation made by Sollas, who interpreted this last spicule type as "a passage from an aster with few actines to an oxeate spicule with a central tylote enlargement".

Preliminary results of Poecilosclerid sponges from Berlengas Island (Portugal)

Javier Cristobo{ XE "Cristobo, Javier" }^(1*); Pilar Ríos{ XE "Ríos, Pilar" }⁽²⁾; Joana Xavier{ XE "Xavier, Joana" }⁽³⁾; Helena Gaspar{ XE "Gaspar, Helena" }⁽⁴⁾; Andrea Cunha{ XE "Cunha, Andrea" }⁽⁵⁾; Madalena Humanes{ XE "Humanes, Madalena" }⁽⁶⁾

(1) Estación de Biología Mariña da Graña, Universidade de Santiago de Compostela. Casa do Hórreo, Rúa da Ribeira 1, 15590 A Graña, Ferrol, Spain, fjcrisob@yahoo.es; (2) Travesía da Peregrina 9 P4 2ºK, 15220 Bertamirans, Ames, A Coruña, Spain, baprios@usc.es; (3) Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, P.O.Box 94766, 1090 GT Amsterdam, The Netherlands, xavier@science.uva.nl; (4) INETI. Instituto Nacional de Engenharia e Tecnologia Industrial. Estrada do Paço de Lumiar, Edifício F, 1649-038 Lisboa, Portugal, helena.gaspar@ineti.pt; (5) Universidade das Ações. Rua Mãe de Deus, 9501-855 Ponta Delgada. Portugal, joanaxavier@yahoo.com; andrea_cunha@linus-uac.pt; (6) Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016, Lisboa, Portugal, mhumanes@fc.ul.pt

Berlenga, Estelas and Farilhões Islands were declared a Nature Reserve in 1981. Located on the western coast of Peniche (Portugal), they have a high biodiversity and productivity because of upwelling phenomena. Also, the diversity of habitats, a rugged bathymetry, the special hydrodynamics of the area and the mixture of oceanic and littoral influences make the richness of the species be much higher than on the continent. However, this nature reserve has been seldom studied and more specifically, the Porifera fauna is hitherto unheard-of. To avoid this lack of knowledge, it has been planned a collaboration between the Universities of Lisboa, Açores and Santiago de Compostela as well as the Instituto Nacional de Engenharia e Tecnologia Industrial, in order to start a study about the Porifera populations, their ecological and taxonomic aspects and continue with the study about their chemical composition. In this work, the preliminary results of the study of the sponges belonging to the Order Poecilosclerida are released. This turns out to be one of the most abundant, especially in specific areas of the islands, where there are cave-dwelling environments that - thanks to favourable hydrodynamic conditions - give rise to a large settlement of sponges, especially in their encrusting and massive forms. Specimens were collected by Scuba diving in 6 different localities; 4 in the main island (Berlenga), 1 in Estelas Island and 1 in the depths of Farilhões Islands from 1 to 21 m.

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Demosponge fauna of the Berlengas Natural Reserve (Portugal): Diversity and zoogeographical affinities

Andrea Cunha{ XE "Cunha, Andrea" }⁽¹⁾; Joana Xavier{ XE "Xavier, Joana" }^(2*); Ana Costa{ XE "Costa, Ana" }⁽¹⁾; Javier Cristobo{ XE "Cristobo, Javier" }⁽³⁾; Pilar Ríos{ XE "Ríos, Pilar" }⁽³⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽⁴⁾; Madalena Humanes{ XE "Humanes, Madalena" }⁽⁵⁾

(1) Departamento de Biologia, Universidade dos Acores Apartado 1422 9501-855 Ponta Delgada Portugal, andrea_cunha@linus.uac; accosta@notes.uac.pt; (2) Institute for Biodiversity and Ecosystem Dynamics (IBED) University of Amsterdam P.O.Box 94766, 1090 GT Amsterdam The Netherlands, xavier@science.uva.nl; (3) Estacion de Biologia Marina da Grana Universidad de Santiago de Compostela Spain, fjcrisob@yahoo.es; (4) Zoologisch Museum University of Amsterdam P.O.Box 94766, 1090 GT Amsterdam The Netherlands, soest@science.uva.nl; (5) Departamento de Química e Bioquímica Faculdade de Ciências da Universidade de Lisboa Campo Grande 1749-016 Lisboa Portugal, mhumanes@fc.ul.pt

Located in the Atlantic, 10 km off the coast of Peniche (Portugal), the Berlengas Natural Reserve is a small archipelago comprising 3 islet groups (Berlenga Grande, Farilhões and Estelas). This archipelago shows an enormous diversity of subtidal habitats, such as rocky cliffs, mixed substrate bottoms and submerged/semi-submerged caves, some of which are included in the Habitats Directive as areas that require the designation of Special Areas of Conservation. These habitats are densely colonized by the main benthic invertebrates groups such as hydrozoans, bryozoans, demosponges, together with coralline and green/brown algae. In stark contrast to the diversity that gave origin to the several present conservation statuses, there is a scarcity of published data concerning the marine taxa. Here we present a preliminary study on the demosponge fauna diversity in this archipelago as a result of two sampling campaigns made in 2003 and 2005. In this study only the species belonging to the orders Homosclerophorida, Astrophorida, Hadromerida, Halichondrida and Haplosclerida are included. The 20 identified species are unequally distributed by the different orders. The best represented orders are the Halichondrida and Hadromerida with 4 and 6 species, respectively. For these species a morphological description is made and the autoecology presented. The main biogeographic affinities of the archipelago's demosponge fauna was analyzed, through a comparison with other geographical areas such as the Macaronesian archipelagos (Azores, Madeira, Canaries and Cape Verde), the Mediterranean and the Northeast Atlantic coasts. Using cluster analysis and multidimensional scaling (MDS), we determined the similarities between these regions. The Berlengas archipelago was shown to have a demosponge fauna more diverse than the oceanic islands of the Azores and Madeira. Although presenting the highest similarity with the Mediterranean, Berlengas seem to constitute a meeting point of the faunas from the Lusitanian, Mauretanian and Mediterranean regions.

***Halisarca* sp. nov. (Porifera, Demospongiae, Halisarcida): A new species from Caribbean mangroves**

Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }^(1*); Adriana Alvizu{ XE "Alvizu, Adriana" }⁽²⁾;
José Luis Palazón{ XE "Palazón, José Luis" }^(2,3)

(1) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com; (2) Universidad de Oriente N.E., adri_alvizu@yahoo.com; (3) Instituto de Ciencias Marinas de Andalucía Puerto Real, Cádiz, España, jluis.palazon@icman.csic.es.

A new *Halisarca* species, growing exclusively on submerged portions of aerial roots of *Rhizophora mangle* (red mangrove) stands, is here described. The species consist of a thin (1-2 mm thick), cream colored, smooth and slippery sponge that grows profusely on mangrove roots. Round and small oscules (1-3 mm), and delicate radial canals may be observed *in situ*. The species presents tubular choanocyte chambers, two types of spherulous cells (with large and small inclusions), non-flagellated pinacocytes, a thin cuticle, and fibrillar collagen. Although the type specimens are from "Laguna de La Restinga" National Park, Margarita Island, Venezuela, the species is abundant as well in other Caribbean localities such as: Bocas del Toro, Panamá, South Central Belize (Twin Cays, Pelican Cays, etc). Due to its abundance and overgrowth capabilities over other epifaunal species *Halisarca* sp. nov. probably plays a key role on the ecology of mangrove sub-tidal communities.

Mycale (Paresperella)* n.sp. (Mycalidae, Poecilosclerida, Demospongiae) from Bocas del Toro, Panamanian Caribbean, with a synopsis of other *Mycale (Paresperella)

Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }^(1,2*); Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽³⁾; Klaus Rützler{ XE "Rützler, Klaus" }⁽¹⁾

(1) Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0163, USA, ruetzler@si.edu; (2) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com; (3) Museu Nacional, UFRJ, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil, hajdu@acd.ufrj.br

Mycale (Paresperella) sp. nov. is an undescribed species found on mangrove roots in the Bocas del Toro region (Panamá). The species is a bright red to orange sponge that forms sprawling masses (up to 30 cm in length, 1-3 cm thick), from which digitate lobes (3-10 cm high, 1-3 cm thick) may branch. Transparent round oscules (1-1.5 cm in diameter), with white spicular strands are easily distinguished. The species presents a unique spicular combination of: substylostyles (I, II), styles (I, II), isochelae, anisochelae, toxas (I, II), and large serrated sigmas; being the second known *Mycale* species in the Caribbean where isochelae and anisochelae combine (after *M. angulosa*). Nearly 20 species assignable to *Paresperella* were previously reported worldwide: one Mediterranean (*serrulata*), two from the West coast of the USA (*psila* and *serratohamata* sensu Lambe), two Indian (*bidentata* and *serratohamata*), two Indo-Malayan (*moluccensis* and *sceptroides*), two from Japan-China (*macrosigma* and *undulata*), one from western Australia (*dichela*), two from southeastern Australia (*penicillium* and *repens*), one from New Zealand (*microsigma*), four from southwestern Africa (*atlantica* sensu Lévi, *curvisigma*, *levii* and *toxifera*), and one species from the northeastern Atlantic (*atlantica* sensu Stephens). *Mycale (Paresperella)* sp. nov. is the first representative of the subgenus in the Caribbean and possibly the only Tropical western Atlantic representative, since *M. (P.) spinosigma* has still a dubious status.

***Haliclona* sp. nov. and *Xestospongia* sp. nov.: Two new sponge species from Bocas del Toro (Panamá) with a unique cyanobacterial association**

Maria Cristina Diaz{ XE "Diaz, Maria Cristina" }^(1, 2*); Robert W. Thacker{ XE "Thacker, Robert W." }⁽³⁾; Klaus Rützler{ XE "Rützler, Klaus" }⁽¹⁾

(1) Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0163, USA, ruetzler@si.edu; (2) Museo Marino de Margarita, Blvd. El Paseo, Boca del Río, Margarita, Edo. Nueva Esparta, Venezuela, crisdiaz@ix.netcom.com; (3) Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA, thacker@uab.edu

Two undescribed species of the order Haplosclerida were found while surveying open reef and mangrove habitats in the Bocas del Toro region (Panamá). Both species have an encrusting growth form (only a few mm in thickness), grow copiously on shallow reef environments, and are of dark purple color, a consequence of a dense population of the cyanobacterial endosymbiont *Oscillatoria spongeliae*. *Haliclona* sp. nov. (Chalinidae) can be distinguished by its small oscules (1-2 mm in diameter) with radial, transparent canals, and its tan interior. The skeleton consists of paucispicular principal spicule tracts (20-40 µm in diameter) connected by mostly unispicular tracts or single spicules (oxea 130-190 x 2-9 µm). The species thrives on some shallow reefs, profusely overgrowing fire coral (*Millepora* spp.), soft corals, and scleractineans. *Xestospongia* sp. nov. (Petrosiidae) is dark purple, inside and out, and its oscules (1-2 mm in diameter) are on top of small, volcano-shaped mounds (1-2 mm high). The sponge is crumbly and brittle. It is found on reefs, coral rubble, and mangrove roots. Both species are currently being characterized genetically to determine similarity to other members of Chalinidae and Petrosiidae. This is the first report of haplosclerid sponges living in association with a *Oscillatoria*-type endosymbiont; previous records were restricted to associations with unicellular cyanobacteria. High rates of photosynthetic production of endobiotic *Oscillatoria spongeliae* (demonstrated elsewhere) could explain the abundance and overgrowth capability of these two sponges in the region's reef environments.

High diversity of *Crambe* Vosmaer, 1880 (Crambeidae, Poecilosclerida, Demospongiae) in the SE Pacific

Eduardo L. Esteves{ XE "Esteves, Eduardo L." }⁽¹⁾; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }⁽²⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(1*)

(1) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ; (2) Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524 - PHLC - Sala 205, 20550-013, Rio de Janeiro, RJ

The family Crambeidae is distributed worldwide. Nevertheless, only a few species belonging to the family have been described and only seven species of the genus *Crambe* Vosmaer, 1880 are so far recognized: *C. acuata* (Lévi, 1958) - SW Africa, W Indian Ocean and Red Sea; *C. crambe* (Schmidt, 1862) - Mediterranean and Canaries; *C. erecta* Pulitzer-Finali, 1993 - W Indian Ocean; *C. oamaruensis* (Hinde & Holmes, 1892) - New Zealand Eocene; *C. panamensis* Maldonado *et al.*, 2001 - tropical E Pacific; *C. tailliezi* Vacelet & Boury-Esnault, 1982 and *C. tuberosa* Maldonado & Benito, 1991 - Mediterranean. In the present work we describe three new species of the genus *Crambe* collected in fjords and channels of the southern-central coast of Chile. They were collected by scuba diving and photographed *in situ*. *Crambe* sp. nov. 1 (five specimens) is orange or pale yellow and encrusting, and differ from all other astroclone bearing species of the genus, viz. *C. acuata*, *C. erecta* and *C. panamensis*, by the presence of larger choanosomal tylostyles (up to 833 µm long) and desmas (up to 232 µm in diameter), a distinct size range of anchorate isochelae (20-36 µm long) and microrhabds (30-67 µm long), and occasionally raphide-like spicules. *Crambe* sp. nov. 2 (one specimen), also with astroclone desmas, is a yellow, encrusting sponge characterized by the presence of larger choanosomal tylostyles (up to 877 µm long) and desmas (up to 172 µm in diameter), and two types of anchorate isochelae in a distinct size range (unguiferate: 21-33 µm and spatulate: circa 32 µm long). *Crambe* sp. nov. 1 and *Crambe* sp. nov. 2 differ from each other mainly in the presence of smaller desmas, anchorate isochelae which are mostly unguiferate and with smaller claws (5-9 µm against 5-12 µm long), and in the absence of microspined microrhabds in the latter species. *Crambe* sp. nov. 3 (one specimen), with sphaeroclone desmas, is orange-red and encrusting and differ from all other living species with such desmas, viz. *C. crambe*, *C. tailliezi* and *C. tuberosa*, by the presence of larger choanosomal tylostyles (up to 769 µm long), ectosomal subtylostyles (up to 785 µm long) and desmas (up to 211 µm in diameter), and a single type of anchorate isochelae in a distinct size range (22-31 µm long) and microrhabds also in a distinct size range (22-43 µm long). This is the first record of the family Crambeidae for the west coast of South America, Southeastern Pacific, enhancing the number of species of the genus known from the Pacific Ocean from one to four. The proposed Tethyan origin of *Crambe* needs to be reevaluated on the basis of these new findings.

Higher biodiversity than thought before: *Suberites domuncula* in Rovinj/Croatia/Northern Adriatic Sea

Jochen Gugel{ XE "Gugel, Jochen" }(1*); Franz Brümmer{ XE "Brümmer, Franz" }(1); Andrej Jaklin{ XE "Jaklin, Andrej" }(2)

(1) University of Stuttgart, Institute for Biology, Department of Zoology, Pfaffenwaldring 57, 70569 Stuttgart, Germany, jochen.gugel@bio.uni-stuttgart.de; (2) Rudjer Boskovic Institute, Center for Marine Research, G. Paliaga 5, 52210 Rovinj, Croatia

Many authors follow Burton and others who recognize *Suberites domuncula* as the only species of a group of similar *Suberites*-forms (Demospongiae, Hadromerida, Suberitidae) in the Mediterranean Sea and neighboring Atlantic waters. Nevertheless in the northeastern Atlantic *Suberites ficus*, *S. virgulosus* and *S. suberia* are cited in the literature as occurring side by side with *S. domuncula*. In the northern Adriatic Sea within the area of Rovinj, Croatia, of these "species" only *S. domuncula* and *S. suberia* are occurring. Until now in the literature on Mediterranean biology mainly *S. domuncula* has been reported and *S. suberia* was a few times recorded as "*S. ficus*". The "species" are traditionally separated by their ability to build a symbiosis with hermit crabs and details of their spiculation. Recently we also could see slight morphological differences in the architecture of the skeleton. *S. domuncula* - symbiosis with a hermit-crab, only tylostyles, first canals outside the radial layer after 500-600 µm. *S. suberia* - symbiosis with a hermit crab, tylostyles and many centrotylote microrhabds, first canals inside the radial layer after 100-150 µm. *S. ficus* - no symbiosis with a hermit crab, tylostyles and very few centrotylote microrhabds, first canals outside the radial layer after 100-200 µm. *S. virgulosus* - no symbiosis with a hermit crab, styles and few tylostyles, many microrhabds, peripheral skeleton without clear layers. The results obtained from these sponges are only preliminary. To clarify how many species of this species-group are valid, especially so far lacking molecular observations (which we plan to do), would be helpful.

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Sponges (Phylum Porifera) associated to the fire-coral *Millepora alcicornis* Linnaeus, 1758 at the "parrachos" of Maracajaú, Rio Grande do Norte state, Brazil

Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(1*); Gustavo Bastos{ XE "Bastos, Gustavo" }⁽¹⁾; Simone Caires{ XE "Caires, Simone" }⁽¹⁾; Luiz F. Machado{ XE "Machado, Luiz F." }⁽¹⁾; Tatiane M. Garcia{ XE "Garcia, Tatiane M." }⁽²⁾; Wilson Franklin{ XE "Franklin, Wilson" }⁽²⁾; Helena Matthews-Cascon{ XE "Matthews-Cascon, Helena" }⁽³⁾

(1) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil; (2) Instituto de Ciências do Mar (LABOMAR), Universidade Federal do Ceará, Av. Abolição 3207, 60165-081, Fortaleza, CE, Brazil; (3) Departamento de Biologia, Bloco 906, Centro de Ciências, Universidade Federal do Ceará, Campus do Pici, Fortaleza, CE, Brazil

"Parrachos" is the local name for the tyde pools found on the reefs of Maracajaú, (Maxaranguape, Rio Grande do Norte state, northeastern Brazil). These pertain to the Recifes de Corais Environmental Protection Area, which determined the preservation of 32.500 ha of marine ecosystem, including nearly 6000 ha of coral reefs in the municipalities of Maxaranguape, Rio do Fogo and Touros, all at Rio Grande do Norte state. The "parrachos" are located between 05°00' and 05°20' S, and between 35°30' and 35°10' W, and comprise a series of patch-reefs less than 10m deep. They are about 60 km distant from Natal, the state's capital, but only 7 km away from the shore. Four species of *Millepora* are known from Brazil, *M. alcicornis*, stretching from 3 to 23 S, is the commonest. The study of epizooism has been suggested as a good surrogate for determination of coral health, and the establishment of lists of species associated to *M. alcicornis* in the "parrachos" will be a valuable tool in monitoring the anthropic effect on these intensively visited coral reefs. A total of 26 stations were selected, with sponges occurring in 16 of these. Species found were: *Calcarea* 1, *Calcarea* 2, *Cliona* cf. *mucronata*, *Geodia gibberosa*, *Haliclona* sp. 1, *Haliclona* sp. 2, *Mycale arenaria*, Spongiidae sp., *Stoebea* sp. and *Timea* sp. *Cliona* cf. *mucronata* and *Stoebea* sp. are likely new species, but additional material is necessary to allow their full taxonomic descriptions.

**A rosettes bearing *Clathria* (*Cornulotrocha*) sp. nov. from a SE Pacific fjord
(Chilean Patagonia) (Microcionidae, Poecilosclerida, Demospongiae)**

Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽¹⁾; Ruth Desqueyroux-Faúndez{ XE "Desqueyroux-Faúndez, Ruth" }⁽²⁾; Philippe Willenz{ XE "Willenz, Philippe" }⁽³⁾

(1) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil, hajdu@acd.ufrj.br; (2) Muséum d'histoire naturelle, CP 6434, CH-1211, Genève 6, Switzerland; (3) Department of Invertebrates, Section Malacology, CALMARS Group, Royal Belgian Institute of Natural Sciences (IRSNB/KBIN), Rue Vautier 29, B-1000, Brussels, Belgium

Only 140 species of sponges are known from over 5000km of Chilean coastline, which motivated us to conduct a series of collecting trips in the area. This work reports on a new species originating from the northern fjord region (ca. 42°S). *Clathria* (*Cornulotrocha*) sp. nov. was collected at 23m depth at Quintupeu Fjord, and is unique within this very large genus, for the possession of rosettes of palmate anisochelae. The new species is compared to the only other known *Clathria* (*Cornulotrocha*), viz. *C. (Cornulotrocha) cheliradians* n. comb.; to the single other *Clathria* known with anisochelae, viz. *C. (Thalysias) dubia* (Kirkpatrick, 1900); to other crustose *Clathria* from southern South America, the Subantarctic and Antarctic areas; and also to other sponges bearing rosettes; and is considered clearly distinct from all. The phylogenetic significance of rosettes is discussed, a likely adaptive value being discarded in view of the variable location of such structures in the distinct poecilosclerid taxa where they occur.

High sponge richness in the southwestern Atlantic (3 - 25°S)

Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(1*); Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾; Maíra V. de Oliveira{ XE "Oliveira, Maíra V. de" }⁽¹⁾; Ulisses S. Pinheiro{ XE "Pinheiro, Ulisses S." }^(1, 2); Suzi M. Ribeiro{ XE "Ribeiro, Suzi M." }^(1, 3); Cristina P. Santos{ XE "Santos, Cristina P." }⁽¹⁾; Eduardo Vilanova{ XE "Vilanova, Eduardo" }^(1, 4)

(1) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil; (2) Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia, Jequié, BA, Brazil; PhD student at Museu Nacional; (3) PhD student, Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, Niterói, RJ, Brazil; (4) PhD student, Laboratório de Tecido Conjuntivo, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

The Tropical western Atlantic has been classified historically as to comprise a richer northern component, the Caribbean/West Indian fauna, and a southern poorer, transitional component, known as the Brazilian fauna. While it is true that the vast majority of Brazilian shallow marine species have a Tropical western Atlantic distribution pattern which justifies the linking of both northern and southern components, this oversimplified picture hinders a comprehensive view of many underlying, unexpected and unexplored biological phenomena. Our recent findings have revealed repeatedly higher richness on the southern component of the Tropical western Atlantic. This has been observed for genera *Aplysina*, *Dysidea*, *Tethya* and *Timea*, and ongoing work suggests a similar pattern may also be found for *Monanchora* and *Mycale*. A further contradiction to the widespread concept of an impoverished Brazilian fauna stems from the observation that richness may not be decreasing gradually from north to south but, rather, apparent hotspots occur around 23-24°S, wherefrom nearly 200 sponge species are known. It is suggested that an important Gondwanan fauna must have been established in the south Atlantic before its connection to the western Tethys sea, and that important regional vicariant phenomena must have played pivotal roles in the shaping of this rich, autochthonous Brazilian marine sponge fauna. The increased effectiveness as a barrier to dispersal of large river outflow on periods of low sea level and shortened continental platform may have been one of these. Entirely contrasting explanations could stem from long-distance sub-equatorial (trans-Atlantic) and Antarctic offshoots dispersal, both of which suggesting the SW Atlantic to be a deposit for allochthonous species.

Cytochrome oxidase subunit I - Opportunities and limits for molecular species discrimination

Isabel Heim{ XE "Heim, Isabel" }^(*); Michael Nickel{ XE "Nickel, Michael" }; Franz Brümmer{ XE "Brümmer, Franz" }

University of Stuttgart, Biological Institut, Department Zoology, Pfaffenwaldring 57, 70569 Stuttgart, Germany, isabel.heim@bio.uni-stuttgart.de; michael.nickel@bio.uni-stuttgart.de; franz.bruegger@bio.uni-stuttgart.de

Recent molecular phylogenetic studies on higher-ranking sponge taxa have proven the capability of molecular characters either to resolve taxonomic problems or to support existing trees based on other markers, *e.g.* morphology, cytology or development. Nevertheless, a proper molecular character has to be chosen and eventually verified for each taxonomic question or level, one wishes to address. The aim of the present study was to establish a molecular marker for species discrimination in a variety of sponge genera. In preliminary species level studies on Mediterranean *Aplysina* spp. we encountered difficulties, using ITS-, 16S- and 12S-rDNA sequences in contrast to cytochrome oxidase subunit I, which is also used for 'DNA-barcoding' in other organisms. Consequently, we tested 17 species of three demosponge genera for intrageneric sequence variability, using the complete COI sequence. In addition we compared our results to three demosponge species used as outgroup. Our results show a divergence of intrageneric COI sequence variability for *Aplysina*, *Tethya* and *Suberites*. *A. aerophoba* Schmidt, 1862 and *A. cavernicola* Vacelet, 1959, both from the Mediterranean Sea, differed only in one base pair. *A. fistularis*, *A. cauliformis* and *A. fulva* (Caribbean Sea) displayed identical sequences and *A. archeri* differed in two bp from the other Caribbean species. Two nucleotide exchanges differentiate the Mediterranean and Caribbean group. *T. aurantium* (Pallas, 1776) and *T. citrina* (Sarà & Melone, 1965) from different regions in the Mediterranean Sea showed 66 bp exchanges. The species *T. wilhelma*, *T. gracilis* and *T. minuta*, found in German aquaria in 2001, differed in 9, 65 and 70 bp. And an unknown *Tethya* species from Pacific Ocean showed 5 bp differences to *T. californiana*. In the case of the Mediterranean species it is possible to differ exactly between both. *S. domuncula* differed from *S. massa* in 73 bp and from *S. ficus* in 13 bp. *S. massa* and *S. ficus* showed 69 bp exchanges. Our results show that the COI: 1. allows to differentiate sponge species, at least of the tested taxa, 2. facilitates to analyse intergeneric biogeographical interrelations and 3. has the potential to serve as a molecular marker on higher taxonomic levels *e.g.* family level. In addition, our results confirm that it is necessary to check it in every sponge genus if COI is suitable. Since all three tested sponge genera displayed differing intrageneric variability, differing nucleotide exchange rates can be assumed and thus differing rates of evolution. Taking this into account, COI sequencing is a valuable tool to resolve the molecular phylogeny of many sponge genera and most probably families.

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Sponge species distributions in marine bioregional planning (invited lecture)

John N.A. Hooper{ XE "Hooper, John N.A." }^(1*); Merrick Ekins{ XE "Ekins, Merrick" }⁽¹⁾;
Vicki Nelson{ XE "Nelson, Vicki" }⁽²⁾

(1) Biodiversity Program, Queensland Museum, PO Box 3300, South Brisbane, Queensland, 4100, Australia, john.hooper@qm.qld.gov.au; merrick.ekins@qm.qld.gov.au; (2) National Oceans Office, GPO Box 2139, Hobart, Tasmania, 7000, Australia, Vicki.Nelson@oceans.gov.au

It is often challenging trying to justify the significance of taxonomy, species distributions, historical biogeography and the other 'enabling sciences' in a political context because they appear to have little relevance to real-world problems. Biogeographic studies in particular are alluring as they attempt to interpret present species distributions in an historical context (and in geological time scales), but they have huge spatial scales and 'fuzzy boundaries' are so perceived to have limited (if any) practical value in solving the biodiversity crisis, or even as workable models to achieve conservation management outcomes. In contrast, 'bioregionalisation' (biogeographic regionalisation) is increasingly used by the international conservation planning and management bureaucracy to define, model, monitor and manage unique and/or representative biotic assemblages ('bioregions'). Bioregions are the reporting units for assessing the distribution and status of native ecosystems, their protection in a reserve system, conservation monitoring and evaluation, and ultimately for reporting on 'landscape [or seascape] health'. Many bioregions, especially those in the marine biome, are defined mainly by geomorphic features (geology, topography, climate), with few biological datasets. We recently applied a large dataset of 2248 sponge species from c.4000 localities along a transect across tropical and subtropical Australia (<http://www.oceans.gov.au/NMB.jsp>), to test the hypothetical IMCRA demersal bioregions (<http://www.deh.gov.au/coasts/mpa/nrsmmpa/pubs/imcra.pdf>). Analyses were both descriptive and numerical, the former using both GIS mapping of verified specimen point-data and predictive distributional modeling using the CAAB system (www.marine.csiro.au/caab). Numerical analyses examined patterns of taxonomic richness, species diversity, similarity in species composition, community structure and species turnover points using clustering, ordination and biodiversity statistical methods. Data were analysed at three taxonomic hierarchies (species to families) and at two spatial scales (localities, or α -scale diversity, and bioregions, or β -scale diversity). Briefly, only one species (*Clathria (Thalysias) vulpina*) occurred in 22 of the 34 smaller-scale localities, 19 species in 13-19 localities (0.01% of species), with the remaining 2228 species found in 12 or less localities (138 species (6%) in 6-12 localities, 713 (32%) in 2-5 localities, and 1377 species (61%) were rare, found in only single localities). Six peaks in species richness (biodiversity 'hotspots') were recorded across the tropical-subtropical transect, and three major sponge provinces were indicated (*i.e.* with 'hard' boundaries separating them), but with smaller ('soft' boundary) transition zones occurring within these larger units. Biodiversity hypothesis testing and multiple species turnover points were used to examine these crucial 'soft' bioregional boundaries and test the IMCRA demersal bioregions.

The 'Lithistid' Demospongiae of New Zealand

Michelle Kelly{ XE "Kelly, Michelle" }^(*); Lincoln Tubbs{ XE "Tubbs, Lincoln" }

National Centre for Aquatic Biodiversity & Biosecurity (NCABB), National Institute of Water and Atmospheric Research (NIWA) Ltd, Private Bag 109-695, Newmarket, Auckland, New Zealand,
m.kelly@niwa.co.nz

An inventory and revision of the 'lithistid' Demospongiae fauna of New Zealand has been completed after 10 years research on existing and new collections. The majority of specimens were recorded from the largest collection of sponges in New Zealand, at NIWA (incorporating the New Zealand Oceanographic Institute), and included voyages back to the 1960s. Significant recent collections were also examined from the Museum of New Zealand Te Papa Tongarewa, and NIWA's Seamount programme provided considerable new material from the Kermadec Ridge, Three Kings Islands, Cavalli Seamounts, the Bay of Plenty, and the Chatham Rise. All specimens were dredged from between 80 and 1700 m, but were most common between 200-800 m. With the exception of one specimen from the eastern edge of the Challenger Plateau on New Zealand's west coast, and a new species from the Ross Sea, Antarctica, all were found north of the southern edge of the Chatham Rise on New Zealand's east coast, and in New Zealand's most northern waters. Prior to this revision only a single species of lithistid sponge, *Aciculites pulchra* Dendy, 1924, was known from New Zealand waters. The fauna is now well represented by ten families and 32 species, 16 of which are new to science - Theonellidae (1 genus, 1 species), Phymatellidae (3 genera, 6 species), Corallistidae (2 genera, 7 species), Neopeltidae (3 genera, 4 species), Macandrewiidae (1 genus, 1 species), Pleromidae (1 genus, 3 species), Isoraphiniidae (1 genus, 1 species), Scleritodermiidae (3 genera, 5 species), Azoricidae (1 genus, 4 species). Prior to this work, two major regional faunas were known worldwide: the continental shelf and slope fauna of the tropical western Atlantic region, and the seamount fauna of the southwest Pacific including the seamounts of the New Caledonian Norfolk Ridge. In both locations lithistid sponges dominate the fauna between 150-1800 m but the structure and taxonomic composition of the communities differ considerably. With the description and revision of the New Zealand fauna, it is confirmed that the southwest Pacific fauna differs markedly from that of the tropical western Atlantic region where Theonellidae and Corallistidae dominate in terms of species. The southwest Pacific faunas contain genera from three families (Isoraphiniidae, Pleromidae, Phymatellidae) that are absent from the tropical western Atlantic and found only as fossils elsewhere.

**Two new marine sponges of the genus *Antho* (*Acarnia*) (Demospongiae:
Poecilosclerida: Microcionidae) from Korea**

Hyung June Kim{ XE "Kim, Hyung June" }; Chung Ja Sim{ XE "Sim, Chung Ja" }^(*)

Department of Biology, Hannam University, 133, Ojungdong, Daedukgu, Daejeon, 306-791, Korea,
cjsim@hannam.ac.kr

Two new marine sponges, *Antho* (*Acarnia*) sp. 1 and *A. (A.)* sp. 2 of family Microcionidae, genus *Antho*, were collected by fishing nets off Jeju Island, Korea, from 1978 to 1994. The genus *Antho* is defined by choanosomal skeleton modified to a basal or axial renieroid reticulation of acanthose or occasionally smooth styles and/or strongyles. The subgenus *Acarnia* is characterized by (acantho)tylostrongyles forming the renieroid skeleton, less frequent acanthostyles, and a special category of echinating acanthostyles, overlap the main skeleton. *A. (A.)* sp. 1 is closely related to *A. (A.) inconstans* in spicules types. But they are different in spicules size and growth forms. *A. (A.)* sp. 1 has many large isochaelae, but *A. (A.) inconstans* does not have large isochaelae. Also, *A. (A.)* sp. 2 resembles *A. (A.) inconstans*. However, thick and slender styles of *A. (A.)* sp. 2 are larger than those of *A. (A.) inconstans*. Growth form is branch but encrusting in *A. (A.) inconstans*. Additionally, these two new species are similar in growth form. *A. (A.)* sp. 1 has large isochaelae. However, *A. (A.)* sp. 2 does not have large isochaelae. Also the slender style of *A. (A.)* sp. 1 is smaller than that of *A. (A.)* sp. 2.

***Aplysina aerophoba* and *Aplysina cavernicola*: Phenotypic variability or two different species?**

Anne Klöppel{ XE "Klöppel, Anne" }; Franz Brümmer{ XE "Brümmer, Franz" }^(*)

Technical Biology/Zoology, University of Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart,
anne.kloepfel@bio.uni-stuttgart.de; franz.bruegger@bio.uni-stuttgart.de

Due to our *in situ* observations and *ex situ* transplantation experiments, we would like to revive an old question: Are *Aplysina aerophoba* and *Aplysina cavernicola* two different species or not? Both *Aplysina* species are common in the Mediterranean Sea. Phenotypic variability within one species makes it difficult to differentiate them morphologically both *in situ* and *ex situ*. The main morphological characteristics distinguishing *A. aerophoba* from *A. cavernicola* are: surface characteristics, digitations, lateral projections and colour. Aquarium observations showed that they possibly do not represent two separate species as indicated by bibliographical data. Thus, we performed transplantation experiments in two separate aquaria differing in light exposure, nutrients and current. Thereby specimens are able to change appearance like colour within only a few weeks when exposed to different ecological conditions. Surface characteristics can alter even within a few minutes. After a certain time, an *A. aerophoba* individual that has been identified based on morphological characteristics, can then be not clearly defined as this species. Furthermore, it showed surface characteristics typical of *A. cavernicola* after such a procedure. Therefore, differences can also be related to the physiological status of the sponges or are an adaptive response towards environmental variations. Thus, we presume that *A. aerophoba* and *A. cavernicola* possibly are not two different species but ecological variants adapted to special environmental conditions. Morphological differences represent probably two separate ecological forms and not two separate species. The existence of two different species of the genus *Aplysina* in the Mediterranean Sea should be re-examined using further taxonomic criteria other than morphology, such as biochemical characteristics (secondary metabolites) as well as, a so far lacking, appropriate molecular marker. Ecological measurements and field observations will be continued to accompany laboratory research.

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Fauna of dictyoceratid sponges in Korea

Kyung Jin Lee{ XE "Lee, Kyung Jin" }^(1*); Chung Ja Sim{ XE "Sim, Chung Ja" }⁽²⁾

(1) Natural History Museum, Hannam University, 133 Ojungdong, Daedukgu, Daejeon, 306-791, Korea, kjlee89@hanmail.net; (2) Department of Biology, Hannam University, 133 Ojungdong, Daedukgu, Daejeon, 306-791, Korea, cjsim@hannam.ac.kr

The present study deals with the taxonomy of dictyoceratid sponges (Porifera: Demospongiae) in Korea. The materials consist of the specimens collected from 40 localities including Islands and coastal areas of the East Sea, West Sea and South Sea of Korea from 1969 to 2005. Twenty eight species of dictyoceratid sponges are identified. All of which are classified into eleven genera of four families. Of these species, eight are candidates to new species: *Spongia* sp. 1, *Spongia* sp. 2, *Psammocinia* sp., *Semitaspongia* sp., *Cacospongia* sp., *Aplysinopsis* sp., *Luffariella* sp. and *Euryspongia* sp. Two species, *Hippospongia anomala* and *Scalarispongia scalaris*, are new records to the Korean fauna. The following seventeen species are recorded from Korean waters: *Spongia officinalis*, *S. zimmocca*, *Psammocinia mammiformis*, *P. mosulpia*, *P. jejuensis*, *P. samyangensis*, *P. wandoensis*, *P. bergquisti*, *P. gageoensis*, *P. lobatus*, *P. rubra*, *P. conulosa*, *P. ulleungensis*, *Sarcotragus maraensis*, *S. gapaensis*, *Bergquistia coreana* and *Smenospongia coreana*.

**A new *Mycale* Gray, 1867 with raphidotoxas from the Southern Brazilian coast
(Demospongiae, Poecilosclerida, Mycalidae)**

Cléa Lerner{ XE "Lerner, Cléa" }^(1*); João Luís Carraro{ XE "Carraro, João Luís" }⁽¹⁾; Beatriz Mothes{ XE "Mothes, Beatriz" }⁽¹⁾; Maurício Campos{ XE "Campos, Maurício" }⁽¹⁾; Alexandre Bondan{ XE "Bondan, Alexandre" }⁽¹⁾; Guilherme Rupp{ XE "Rupp, Guilherme" }⁽²⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽³⁾

(1) Museu de Ciências Naturais, Fundação Zoobotânica do RS, Rua Dr. Salvador França 1427, 90690-000, Porto Alegre, RS, Brasil, cblerner@fzb.rs.gov.br; (2) Centro de Desenvolvimento em Aquicultura e Pesca, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A., Florianópolis, SC, Brasil; (3) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, RJ, Brasil

Approximately 200 species of *Mycale* are known around the world, found in major marine habitats. The current scheme of classification for *Mycale* is based on the arrangement of the ectosomal skeleton and presence of particular types of microscleres. Four species of *Mycale* with raphidotoxas are known: two have ectosomal skeletal specializations (subgenus *Aegogropila*) and two have not (subgenus *Carmia*). A species with raphidotoxas and no ectosomal skeletal specialization associated with polychaete tubes was collected in the southwestern Atlantic and is compared with other species possessing raphidotoxas and without an ectosomal specialization, viz. *M. (C.) magnirhaphidifera* Van Soest, 1984 and *M. (C.) raphidotoxa* Hentschel, 1912. Specimens were collected manually, at about 3 m depth at Canto Grande Bay (27°13'S - 48°30'W, Bombinhas), state of Santa Catarina, growing over farmed *Nodipecten nodosus* (Linnaeus, 1758). Specimens are deposited in the Museu de Ciências Naturais Porifera Collection, preserved in alcohol, under MCNPOR 6517 and MCNPOR 6957. The studied material is characterized by having long raphidotoxas, three categories of anisochelae, stout C-shaped sigmas and toxas, in addition to mycalostyles. The color was orange *in situ* and specimens were thinly incrusting and very fragile. This species differs from *M. (C.) magnirhaphidifera* in having toxas, stout sigmas and orange color. From *M. (C.) raphidotoxa* it differs in having larger raphidotoxas (up to 460 µm vs. up to 300 µm), toxas, one category of smaller anisochelae and orange color.

New records of marine sponges for the southeastern Pacific (23 - 56°S)

Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }⁽¹⁾; Ruth Desqueyroux-Faúndez{ XE "Desqueyroux-Faúndez, Ruth" }⁽²⁾; Mariana S. de Carvalho{ XE "Carvalho, Mariana S. de" }⁽³⁾; Philippe Willenz{ XE "Willenz, Philippe" }⁽⁴⁾; Eduardo L. Esteves{ XE "Esteves, Eduardo L." }⁽³⁾; Verena Häusserman{ XE "Häusserman, Verena" }^(5,6); Günter Försterra{ XE "Försterra, Günter" }^(5,6); Alejandro Bravo{ XE "Bravo, Alejandro" }⁽⁶⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }⁽³⁾

(1) Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, PHLC, sala 205, Maracanã, 20550-013, Rio de Janeiro, Brazil; (2) Muséum d'histoire naturelle de Genève, CP 6434, CH-1211, Geneva 6, Switzerland; (3) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil (MSC and ELE - PhD students); (4) Royal Belgian Institute of Natural Sciences, Department of Invertebrates, 29 Rue Vautier, B 1000, Brussels, Belgium; (5) Huinay Scientific Field Station, Casilla 462, Puerto Montt, Chile; (6) Universidad Austral de Chile, Departamento de Biología Marina, Campus Isla Teja, Casilla 567, Valdivia, Chile

As far as sponge faunas are concerned, some of the world's poorest sampled continental shores are those of South America, both Atlantic and Pacific. The last 15 years saw a major reversal on commitment towards a better understanding of Tropical SW Atlantic sponge biodiversity. Accompanying biogeographic questions motivated the onset of an inventory of sponge diversity on a couple of neighboring provinces, *viz.*: Patagonian, Magallanic and Chilean-Peruvian, urged too by the lack of active sponge systematists on Argentina, Chile, Peru and Ecuador. On year 2004 the new inventory of Chilean sponges by our team started, aiming to check how far from the truth the list of 140 species known for the area was, and how well the distributions of these species were known. Some 30-35 localities have since been sampled, mainly through scuba diving, stretching from 23 to 56°S, but also from the intertidal to about 2000 m depth (UACH collection). Collected specimens are deposited in the MNRJ (Rio de Janeiro) and the MHNG (Geneva) collections. Vouchers preserved for electron microscopy are deposited in the RBINSc collection (Brussels). From nearly 600 Chilean specimens in the MNRJ collection, 3 are hexactinellids, 25 calcareous, and the remaining are demosponges. A preliminary taxonomic sorting of these samples revealed many new genus-level and family-level records for the SE Pacific, suggesting that an assessment of biogeographic affinities of this fauna may be quite premature at this stage. New family-level records are those of Cladorhizidae, Clathrinidae, Crambeidae and Dictyonellidae. New genus-level records revealed so far are *Amorphinopsis*, *Asbestopluma*, *Clathrina*, *Crambe*, *Echinochalina*, *Phakellia*, *Rhabderemia* (*Rhabderemia*) and *Scopalina*.

Revision of *Calycosoma* and finding of *Lophocalyx* in the Atlantic Ocean (Porifera, Hexactinellida)

Larisa L. Menshenina { XE "Menshenina, Larisa L." }; Konstantin Tabachnick { XE "Tabachnick, Konstantin" }^(*)

Institute of Oceanology of Academy of Sciences of Russia, Nahimovky 36, RU-117218 Moscow, Russia,
tabachnick@mail.ru

Two relative genera, *Calycosoma* and *Lophocalyx*, are revised. Specimens from the Antarctic, which were earlier included into a single species of *Calycosoma* (*C. validum*) obviously belong to *Lophocalyx* (new species). Five new species of *Lophocalyx* found in different regions of the Atlantic Ocean are described here. The genus *Lophocalyx* had been previously described as present only in the Indian and West Pacific Oceans. The diagnoses of *Calycosoma* and *Lophocalyx* are overlooked.

New records and one new species of calcareous sponges (Porifera) of Cagarras Archipelago, Rio de Janeiro, Brazil

Leandro C. Monteiro{ XE "Monteiro, Leandro C." }^(1*); Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾; Michelle Klautau{ XE "Klautau, Michelle" }⁽²⁾

(1) Departamento de Invertebrados - Museu Nacional/UFRJ. Quinta da Boa Vista s/no, São Cristóvão. 20940-040 Rio de Janeiro, RJ Brazil; (2) Laboratório de Biologia de Porifera, Departamento de Zoologia, Instituto de Biologia, Bloco A, CCS, UFRJ, Ilha do Fundão. 21941-590 Rio de Janeiro, RJ Brazil

The Cagarras Archipelago is located 5 km South of Ipanema Beach, in Rio de Janeiro, Brazil. It has three major islands (Cagarra, Palmas, and Comprida), and four islets. Despite the high number of divers in the Archipelago waters and the close proximity to the major touristic city in Brazil, its biodiversity is poorly known. In the present study we describe and discuss the taxonomic composition of calcareous sponges from Cagarras Archipelago. A total of 52 calcareous sponges were collected by SCUBA diving between 2001 and 2005. Seven species were found. Three of them were already known to the archipelago: *Clathrina aurea*, *C. conifera*, and *Paraleucilla magna*. Three are new records to the archipelago and also to: Rio de Janeiro city (*Clathrina aspina*), Southeastern Brazil (*Grantia kempfi*), and South America (*Leucandra rudifera*). One is a new species: *Leucandra* sp. nov. *Clathrina aspina* is white, with cornus composed of anastomosed tubes with asconoid organization; skeleton with triactines, tripods and tetractines in which the apical actine has vestigial spines or is smooth. *Grantia kempfi* is white, sac-shaped, with an apical osculum, syconoid aquiferous system, cortex with triactines and diactines, ostiolar triactines different from the cortical triactines; choanoskeleton with triactines and tetractines; subatrial skeleton with triactines; atrial skeleton with triactines and tetractines. *Leucandra rudifera* is white, sac-shaped, with an apical osculum, leuconoid aquiferous system, cortex with triactines, diactines and microdiactines; choanoskeleton with triactines; atrial skeleton with tetractines, triactines and anchors. *Leucandra* sp. nov. is white, massive, with folds and oscula located at the top of short projections, leuconoid aquiferous system, cortex with diactines and trichoxeas; choanoskeleton with triactines; atrial skeleton with tetractines. This species differs from other species of *Leucandra* by the spicular size and composition. In Cagarras Archipelago, the seven species of *Calcarea* represent 11.7% of the 60 estimated species for this site. This number is very high when compared to the world wide estimation of 5% for *Calcarea* species.

First records of Porifera from Trindade Island, Brazil, with description of four new species

Fernando Moraes{ XE "Moraes, Fernando" }^(1*); Michelle Klautau{ XE "Klautau, Michelle" }⁽²⁾;
Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾

(1) Departamento de Invertebrados, Museu Nacional - Universidade Federal do Rio de Janeiro. Quinta da Boa Vista, s/no. São Cristóvão, Rio de Janeiro - RJ, Brazil 20940-040; (2) Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro. Av. Pau-Brasil 211, CCS. Rio de Janeiro - RJ, Brazil 21941-590

Trindade (20°30' S - 29°20' W) is a volcanic oceanic island, located 1140 km east from the city of Vitória, Southeastern Brazil. It has a great environmental, scientific, economic and strategic value. Despite its importance, the local sponge fauna had never been studied before. During two scientific expeditions carried out in February and August 2003, 71 sponge samples were collected by snorkeling and SCUBA diving, from 0 - 30 m depth. The specimens were preserved in ethanol 70% and deposited in the Porifera collection of the Museu Nacional (Rio de Janeiro, Brazil). Nine sites were sampled in the island, including different habitats, such as tide pools and both sheltered, and exposed rocky coasts. Approximately 700 underwater, terrestrial, and aerial photographs were taken and 150 minutes of video images were recorded, showing specimens *in vivo* and the sampled habitats. These photographs and video images helped taxonomic description and identification of species, and were useful to characterize the studied area and to analyze ecological features of species. Here we describe and identify 23 sponge species from Trindade Island (19 Demospongiae, and four Calcarea). Of these, 17 morphotypes were identified to species level, and 6 to genus. Poecilosclerida was the most diverse order, with 5 species (*Clathria* sp. 1, *Clathria* sp. 2, *Ectyoplasia ferox*, *Crella* (*Grayella*) sp., *Monanchora* sp.), followed by Haplosclerida, with 3 species (*Aka coralliphaga*, *Petrosia weinbergi* and *Chalinula molitba*), Dictyoceratida, with 3 species (*Ircinia felix*, *I. strobilina* and *Cacospongia* sp.), Chondrosida, with 2 species (*Chondrosia collectrix* and *Chondrilla* aff. *nucula*), Verongida, with 2 species (*Verongula gigantea* and *Aiolochoiria crassa*), Clathrinida, with 2 species (*Clathrina* sp. n. 1 and *Clathrina* sp. n. 2), Leucosolenida, with 2 species (*Leucandra rudifera* and *Paraleucilla* sp. n.), Homosclerophorida, with 1 species (*Plakinastrella microspiculifera*), Astrophorida, with 1 species (*Erylus* sp. n.), Agelasida, with 1 species (*Agelas* sp.), and Halichondrida, with 1 species (*Topsentia ophiraphidites*). Four species are new to science: three Calcarea (*Clathrina* sp. n. 1, *Clathrina* sp. n. 2, and *Paraleucilla* sp. n.), and 1 Demospongiae (*Erylus* sp. n.). These 3 species of calcareous sponges are endemic to Trindade Island, and *Erylus* sp. n. was only recorded from São Pedro e São Paulo Archipelago, an oceanic island off the Northeast Brazilian coast.

Pachastrellidae Carter, 1875 from South Brazilian coast

Beatriz Mothes{ XE "Mothes, Beatriz" }^(1*); Manuel Maldonado{ XE "Maldonado, Manuel" }⁽²⁾;
 Rafael Eckert{ XE "Eckert, Rafael" }⁽¹⁾; Cléa Lerner{ XE "Lerner, Cléa" }⁽¹⁾; Maurício Campos{
 XE "Campos, Maurício" }^(1, 3); João Luís Carraro{ XE "Carraro, João Luís" }⁽¹⁾; Rafael Metri{ XE
 "Metri, Rafael" }⁽⁴⁾; Ricardo Capitoli{ XE "Capitoli, Ricardo" }⁽⁵⁾

(1) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul. Rua Salvador França 1427, 90690-000 Porto Alegre-RS, Brazil, bmothes@fzb.rs.gov.br; (2) Centro de Estudios Avanzados de Blanes (CSIC). Acceso Cala St. Francesc 14, Blanes 17300, Girona, Spain; (3) Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul. Av. Bento Gonçalves 9500, 91501-970 Porto Alegre-RS, Brazil; (4) Programa de Pós-Graduação em Zoologia, Universidade Federal do Paraná. Centro Politécnico Jardim das Américas 81531-990 Curitiba-PR, Brazil; (5) Fundação Universidade de Rio Grande, Departamento de Oceanografia, Av Itália Km 8 96201-900 Rio Grande-RS, Brazil

Here we present preliminary results on the abundance and taxonomic distribution of pachastrellid demosponges from South Brazilian bottoms. Our approach to the pachastrellid fauna is based on material collected by two oceanographic cruises ("Recursos Vivos da Zona Econômica Exclusiva", in 2001; "Projeto Rio Grande do Sul" I, off Rio Grande do Sul state, in 1968) using 2 different methods (trawling nets and dredges). The sponge collection was supplemented with additional material gathered by scuba diving at "Ilha do Arvoredo" (27°16'11"S - 48°22'12"W and 33° 30'00"S - 52°47'00"W). Therefore, the studied fauna contains representatives of both bathyal bottoms (170 to 284 m deep) and shallow, sublittoral bottoms (0 to 6 m deep). Upon collection, sponges were fixed in formalin (4%), then transferred to ethanol (96%) for preservation, and deposited at the Porifera Collection of the Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul. Preliminary taxonomic investigations suggest occurrence of four putative new species among the collected material, though further comparative examination is required before the status of "new species" can be corroborated definitively. Two of the putative new species belong to the genus *Pachastrella*, one to *Characella*, and one to *Stoeba*. The latter one provides the first record of the genus *Stoeba* in Brazilian waters, and it is apparently able to excavate calcareous nodules.

Marine sponges from Potiguar Basin, Northeastern Brazil

Guilherme Muricy{ XE "Muricy, Guilherme" }⁽¹⁾; Josivete P. Santos{ XE "Santos, Josivete P." }⁽²⁾; Paulo J.P. Santos{ XE "Santos, Paulo J.P." }⁽²⁾; Márcia F. Rocha{ XE "Rocha, Márcia F." }⁽³⁾; Eduardo L. Esteves{ XE "Esteves, Eduardo L." }^(1, 2)

(1) Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, muricy@acd.ufrj.br; (2) Departamento de Zoologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, Brazil; (3) CENPES - Centro de Pesquisas e Desenvolvimento Leopoldo Americo Miguez de Mello, PETROBRAS, Rio de Janeiro, Brazil

In this study we describe and discuss the taxonomic composition of the sponge fauna (Phylum Porifera) collected in Potiguar Basin (Northeastern Brazil) in May 2003, by BPOT 2 Campaign, within the "Potiguar Basin Environmental Assessment Project" of CENPES/PETROBRAS. Besides the qualitative composition of the sponge fauna, we also analyzed the abundance, frequency, spatial distribution, and both ecological and economic importance of the sponge species present in the area. Collections were made in 116 stations through dredging and trawling on board of the RVs "Astro Garoupa" and "Pegasus I". A total of 143 sponge specimens were collected, representing 65 species of 41 genera and 23 families of Demospongiae and 2 species of 2 families of Calcarea. Six species were new records for Brazil, and 11 were new records for Rio Grande do Norte state. At least 2 species are new to science. The most diverse families were Petrosiidae and Aplysiniidae, with 7 species each. The most diverse genera were *Aaptos*, *Oceanapia*, and *Aplysina*, with 4 species each. The most abundant and frequent species were *Aiolochoiria crassa*, *Topsentia ophiraphidites*, *Ircinia strobilina*, *Hyattella cavernosa*, *Drummacidon reticulatus*, and *Ptilocaulis walpersi*. Only 9 out of 116 sampling sites (6.9%) presented sponges. The sites richest in sponge species were stations BPot04, BPot05 and BPot33, with 21 species each. Species richness was higher in deeper sites, although it was highly heterogeneous within the study area. Several species present in Potiguar Basin are ecologically important, as they bear a diverse associated fauna: *Aiolochoiria crassa*, *Aplysina lacunosa*, *Ircinia strobilina*, *Agelas dispar*, etc. Many species also produce bioactive secondary metabolites with pharmacological importance. The biological activities of these compounds include anti-HSV1, anti-histaminic, cytotoxic, antibacterial, anti-inflammatory, and hemo-agglutinating activities. These examples demonstrate the great economic potential of the sponge fauna from Potiguar Basin.

New observations on *Clionaopsis platei* Thiele, 1905 (Clionaidae, Hadromerida, Demospongiae)

Marcos P.C. de Oliveira{ XE "Oliveira, Marcos P.C. de" }⁽¹⁾; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }⁽¹⁾; Amanda B. de Oliveira{ XE "Oliveira, Amanda B. de" }⁽¹⁾; Eduardo Hajdu{ XE "Hajdu, Eduardo" }^(2*)

(1) Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, PHLC, sala 205, Maracanã, 20550-013, Rio de Janeiro, Brazil; (2) Museu Nacional, Departamento de Invertebrados, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/n, 20940-040, Rio de Janeiro, RJ, Brazil

Clionaopsis Thiele, 1905 remains monotypic and ill-known. A single specimen has been referred to since the original description of the species. The genus is easily diagnosed within the Clionaidae as the only taxon bearing large oxeas as one of the main megascleres, which renders it atypical within the whole Hadromerida. Their perforating nature is not known for sure. Here we report on an additional five specimens collected on central-southern Chile, which extend the species known morphologic variability and geographic range. Two specimens were photographed *in situ* and are virtually indistinguishable from *Cliona chilensis* Thiele, 1905 as regards external morphology. All studied specimens were large, massive and intensely yellow when alive, and all were collected as if they were *Cliona chilensis*. Spicule micrometries found for the megascleres were: oxeas, 340-530/8-32 µm; tylostyles, 220-350/5-21 µm. The alleged two categories of spirasters (I: thin-long-sinuuous and II: stout-short-bent once or twice) vary considerably in abundance. The smaller category is also highly variable morphologically and an extensive study under SEM will be necessary for its detailed description. Originally described from Calbuco, at ca. 41°45'S, the geographic range of the species is extended here to the north, to 23°28'S (Península de Mejillones, Antofagasta), and to the south, to 44°S (Isla Laurel, Guaitecas Archipelago). The species appears to be an endemic of the Chileno-Peruvian Province, but its finding at 44°S makes it likely that its range may extend into the Magallanic Province too.

The *Agelas* from the Caribbean Sea

Fernando J. Parra-Velandia{ XE "Parra-Velandia, Fernando J." }^(1*,2); Sven Zea{ XE "Zea, Sven" }⁽¹⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽²⁾

(1) Departamento de Biología and Centro de Estudios en Ciencias del Mar - CECIMAR, Universidad Nacional de Colombia, INVEMAR, Cerro Punta de Betín, A.A. 10-16, Santa Marta, Colombia, fernando.parra@gmail.com; szea@invemar.org.co; (2) Institute for Biodiversity and Ecosystem Dynamics (IBED) & Zoological Museum, University of Amsterdam, P.O. Box 94766 1090 GT Amsterdam, The Netherlands; soest@science.uva.nl

The *Agelas* genus comprises a group of tropical reef sponges that contains long-lived species distributed throughout the Mediterranean Sea, the Indo-Pacific Ocean, and the Caribbean Sea. This group is characterized and defined by the presence of an unique type of spicule verticillated acanthose style-like megasclere and by the presence of a fibroreticulate skeleton of primary ascending spongin fibres invariably cored and echinated by spicules, and interconnecting secondary (occasionally tertiary) fibres only echinated by spicules. This genus includes sponges of diverse growth forms, *e.g.*, thickly encrusting, massive, globular, branching, fan shaped and tubular. Despite almost all major taxonomical works for this area include the common species from the genus and that some recent detailed works have added new species, there is still a great deal of taxonomic confusion, even for the more common species. Here we did a detailed review of *Agelas* species present in the Caribbean Sea using classic taxonomic tools. According to our results the genus *Agelas* in the Caribbean Sea has at least thirteen valid species; nevertheless, their identification is still obtained by combination of characters. Also we found that microscopic characteristics like skeleton arrangement, number of verticills or spicules length are subject to such variation that they can only be used as taxonomic tools by comparison with other species in the context of the same area, requiring even so a degree of familiarity with the genus' regional variation. The distribution and richness of these species in the Caribbean area shows a gradient north to south, which can be indicating the relative geologic stability of the Bahamian platform against the relatively greater dynamics of the Caribbean.

New records of tetractinellids sponges from Bahia, Brazil with description of a new species of *Acanthotetilla* Burton, 1959

Solange Peixinho { XE "Peixinho, Solange" }^(1*); Júlio Fernandez { XE "Fernandez, Júlio" }^(1,2);
Bruno Cosme { XE "Cosme, Bruno" }⁽¹⁾

(1) Universidade Federal da Bahia - Rua Barão de Geremoabo, s/n, Ondina, 40170 - 290 - Salvador/Bahia/Brazil; (2) Faculdades de Tecnologia e Ciências - Rua Luis Viana Filho, 8812. Paralela, 41820 - 785 - Salvador/Bahia/Brazil

Four Tetractinellids are presented in this work, including a new species, *Acanthotetilla* sp. nov. (Spirophorida), and three new records, *Cinachyrella kuekenthalli* Uliczka, 1929 (Spirophorida), first record from Bahia state coast, *Stelletta beae* Hajdu & Carvalho, 2003 (Astrophorida), first record from the Northeast Brazilian coast and *Stelletta kallitetilla* (Laubenfels, 1936), registered for the first time from the Southwestern Atlantic. The Spirophorida were mostly collected on the Bahia state northern coast in sandy substrate with gravel predominance, from 22 to 35 m depth, between 12°44'59.7S/38°04'05.5W and 12°50'23.0S/38°11'22.1W, using Holme and Petersen dredges. The Astrophorida were collected in Todos os Santos Bay, between 12°58'18"S/38°31'10"W and 12°54'S/38°39W, from zero to 10 m depth, in rocky substrate. The genus *Acanthotetilla* has disjunct or restricted distribution. The new species is encrusting and made up of one single category of oxeas and two categories of sigmaspires, prototriaenes and acanthoxeas, the last one including a longer category with 20-31 spine whorls. *Acanthotetilla* sp. nov. differs from the currently known four species of the genus particularly by the presence of a second category of acanthoxea and its encrusting habit. The genus *Cinachyrella* has distribution restricted to the tropical/subtropical regions of the world. On the Brazilian coast three species were previously registered, among them, *Cinachyrella kuekenthalli*, from the Pernambuco state coast. *Stelletta* is distributed in the Atlantic, Indian and Pacific Oceans, except in cold regions, with seven valid species registered in Brazilian waters. *Stelletta beae* was described on south and southeast Brazilian coasts. The comparative study detected the presence of one single category of orthotriaene with great size variation, instead of the two categories reported in the original description. *Stelletta kallitetilla* (Laubenfels, 1936) was redescribed, being in accordance with the descriptions available in the literature, except for the quantity of oxeas, which are abundant, and not rare, in our material. Although the sponge biodiversity of the Todos os Santos Bay and other localities on the Bahia state coast are notable, the number of species described and/or designated does not reach one hundred. As a result, this study contributes to increase the knowledge on tetractinellids sponges from Bahia.

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Sponge biodiversity of Rathlin Island

Bernard Picton{ XE "Picton, Bernard" }^(*); Claire Goodwin{ XE "Goodwin, Claire" }

Ulster Museum, Department of Botany and Zoology, Botanic Gardens, Belfast, BT9 5AB, Northern Ireland,
bernard.picton@magni.org.uk

Rathlin Island lies six miles off the North coast of Northern Ireland at the mouth of the Irish Sea. It is subject to strong tidal conditions and has sea cliffs which reach up to 200 m in depth. It is exceptionally rich in sponges, yet prior to this survey its sponge fauna was poorly studied. An intensive survey of the sponge fauna was conducted using scuba divers. One hundred and twenty sponge species were recorded; this amounts to approximately 50% of the species known to occur in the British Isles. Thirty two of the species were first records to the UK or previously undescribed species. On initial inspection, it appears that there may be new species of *Polymastia*, *Sphaerotylus*, Hemiasterellidae, *Axinella*, *Halicnemis*, *Crella*, *Antho*, *Clathria* (*Microciona*), *Lissodendoryx*, *Halisarca*, *Oscarella*, *Eurypon*, *Hymedesmia*, *Stylostichon*, *Phorbas*, *Plocamionida* and *Hymeraphia*. Digital photography was used to document the appearance of the sponges *in situ* before sampling. We discuss the survey methodology used and its contribution to the subsequent taxonomic work.

The demosponges dwelling in the coral reefs from Kham Islands, the eastern coast of the Gulf of Thailand

Sumaitt Puthakarn{ XE "Puthakarn, Sumaitt" }^(1*); Pichai Sonchaeng{ XE "Sonchaeng, Pichai" }⁽²⁾; Rob W.M. van Soest{ XE "Soest, Rob W.M. van" }⁽³⁾

(1) Institute of Marine Science, Burapha University Bangsaen, Chonburi 20131 Thailand, sumaitt@bims.buu.ac.th; (2) National Science Museum, Technopolis, Klong 5, Klong Luang, Pathum-thani 12120, Thailand, pichai@nsm.or.th; (3) Zoologisch Museum of the University of Amsterdam P.O.Box 94766, 1090 GT Amsterdam, The Netherlands, R.W.M.vanSoest@uva.nl

This paper presents the species diversity and distribution of demosponges dwelling in the coral reefs from Kham Islands, the eastern coast of the Gulf of Thailand. The field surveys were conducted at 13 sites of Kham islands, over the period December 2002 and February 2003. Most of the investigations were carried out by SCUBA diving during daytime and random observation throughout the reefs. The results yielded 37 species of demosponges from 11 Orders, 25 families and 30 genera. The most abundant and common sponges in this area are: *Iotrochota baculifera* Ridley, *Chondrilla australiensis* (Carter), *Paratetilla bacca* (Selenka), *Xestospongia testudinaria* (Lamarck) and *Oceanapia sagittaria* (Sollas).

About the presence of *Mycale (Mycale) tylotornota* Koltun, 1964 (Porifera, Demospongiae, Poecilosclerida) in Antarctic waters: Redescription and spicular iconography

Pilar Ríos{ XE "Ríos, Pilar" }^(1*); Javier Cristobo{ XE "Cristobo, Javier" }⁽²⁾

(1) Travesía da Peregrina 9 P4 2ºK, 15220 Bertamirans, Ames, A Coruña, Spain, baprios@usc.es; (2) Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela. Casa do Hórreo, Rúa da Ribeira 1, 15590 A Graña, Ferrol, Spain, fjcrisob@yahoo.es

From the 12th of January to the 7th of February 1995 the second Spanish expedition BENTART, whose main aim was the study of the Antarctic benthos, was carried out. The samplings were carried out to the South and North of Livingston Island, Deception Island and Bransfield Strait, where samples from 38 stations between 24 and 1,019 m depth were taken. In one of these stations, between Livingston Island and Deception Island and at 600 m depth, a sample of the sponge *Mycale (Mycale) tylotornota* Koltun, 1964, that had not been named again after its original description, was collected. It is a sponge with an erect bearing, which is made up of reticula of compact polyspicular and very ramified fibres that may easily break. Among them, the organic matter with brown colour in alcohol smashes easily. The exterior of the sponge has a reticular formation of much finer fibres, with approximately 1 mm diameter. Dimensions: 14 x 10 x 5 cm. The specimen is quite decaying because of the effect of the trawls, but in some areas, it can be seen a very thin ectosome with a smooth outer surface and with some oscula of 2 cm diameter and irregular outline. The choanosomal skeleton is made up of scattered multispicular bundles of tylotornotes and raphides. The ectosomal skeleton is made up of spicule bundles of the choanosome that fork and form bunches that slightly jut out the surface of the sponge. Among these spicules, the anisochelae and the raphides are abundant. The megascleres are either straight or slightly curved tylotornotes, with a small enlargement in both ends that end with a tip or a mucron. The microscleres are two categories of anisochelae and raphides. In his original description, Koltun considers that the external habitus of this species is very similar to that of *Mycale (Oxymycale) acerata* Kirkpatrick, 1907. The type of microscleres and to some extent, the macroscleres also indicate the close relationship of these two species. The clearest difference between both species can be found in the morphology of the megascleres, in the large anisochelae and in the raphides, that in the case of *Mycale (Oxymycale) acerata* gather together in trichodragmata on the contrary to *Mycale (Mycale) tylotornota*, where they are isolated. Although the specimen studied by Koltun also came from the Southern Shetlands, its bathymetrical grade is extended, as it had been previously collected at 370 m depth.

***Phorbas* sp. nov. (Porifera, Demospongiae, Poecilosclerida) of Bellingshausen Sea (Antarctic)**

Pilar Ríos{ XE "Ríos, Pilar" }^(1*); Javier Cristobo{ XE "Cristobo, Javier" }⁽²⁾

(1) Travesía da Peregrina 9 P4 2°K, 15220 Bertamirans, Ames, A Coruña, Spain, baprios@usc.es; (2) Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela. Casa do Hórreo, Rúa da Ribeira 1, 15590 A Graña, Ferrol, Spain, fjcrisob@yahoo.es

During the Antarctic summer of 2003 the third Spanish expedition was carried out in order to study the sea benthos. Twenty five stations were sampled and 13 of these samplings were carried out at the platform of the Bellingshausen Sea, between 490 and 2043 m depth. In the station 10 and by means of an Agassiz trawl, two specimens of a new species were collected: *Phorbas* sp. nov. It is a sponge that is made up of flat irregular cylindrical and anastomosed branches. Dimensions: 7 x 1 x 0.15 cm. Few elliptical oscula, which are irregularly scattered. Soft and flexible consistence. Smooth and velvety texture. Ivory colour *in vivo*, it keeps the same colour in alcohol. The choanosomal skeleton is made up of a bundle of main acanthostyles that are cemented with sponging and from which some thorny secondary acanthostyles come out. The microscleres are sigmata and isochelae. The ectosome is made up of arched isochelae. The tornotes form bunches of 8 to 10 spicules that either jut out in almost a third of their length or may be isolated in a tangential position. The main spicules are acanthostyles of two categories and tornotes, and the microscleres are anchorate chelae, arched isochelae and sigmata. Eight different species belonging to the genus *Phorbas* Duchassaing & Micheloti, 1864 are considered to exist in the Antarctic region and nearby areas. Among all of them, the closest species to the new one is *P. nexus* (Koltun, 1964), whose morphology and spicular iconography is similar, although some differences that can be constantly observed in Bellingshausen Sea have been found. Apart from having observed a second size of acanthostyles that Koltun does not describe but is illustrated, some arched isochelae and sigmas that had been neither described nor drawn by the author were described. In the samples of *Phorbas* sp. nov. other spicular kinds are frequent, although they had been quoted neither in the original description of *P. nexus* nor in the study that had been carried out for the paratype by Hooper. In the case of the arched isochelae, it could be justified by the peripheral arrangement of the sponge, but no explanation can be found for the lack of sigmas, as they have a big size and may be found in the choanosome. Consequently, it is considered that the presence of arched isochelae and sigmas are enough differentiating characteristics to affirm that they belong to a species that is different from that collected by Koltun and that they are, therefore, a new species. Moreover, the presence of this category of chelae confirms its inclusion in the genus *Phorbas*.

Poecilosclerida (Porifera, Demospongiae) from Peter I Island (Bellingshausen Sea, Antarctic) with description of a new species of *Isodictya*

Pilar Ríos{ XE "Ríos, Pilar" }^(1*); Javier Cristobo{ XE "Cristobo, Javier" }⁽²⁾

(1) Travesía da Peregrina 9 P4 2°K, 15220 Bertamirans, Ames, A Coruña, Spain, baprios@usc.es; (2) Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela. Casa do Hórreo, Rúa da Ribeira 1, 15590 A Graña, Ferrol, Spain, fjcrisob@yahoo.es

Peter I Island is a very remote island just in the middle of the Bellingshausen Sea that is seldom visited by the different scientific expeditions that have approached the Antarctic. Here we made a report about some samples of Poecilosclerida sponges that had been collected during the R/V Hesperides cruise in the Spanish expedition Bentart 03. In spite of its rough morphology, four different sampling stations on the four sides of the island between 86 and 370 m depth were prospected by using different kinds of trawls (Agassiz, Box corer, rock sled and trawl) apart from Scuba diving and a R.O.V. This work has the aim to contribute to a better knowledge of the island biodiversity. A total of 47 specimens of the Order Poecilosclerid belonging to 10 species of 5 genera have been collected. Two species of the genus *Myxilla* Schmidt, 1862: *Myxilla* (*Burtonanchora*) *pistillaris* Topsent, 1916 and *Myxilla* (*Burtonanchora*) *lissostyla* Burton, 1938 have been identified, both in the east station of the island at 86 m depth. Two species of the genus *Tedania* Gray, 1867: *Tedania* (*Tedaniopsis*) *charcoti* Topsent, 1907 and *Tedania* (*Tedaniopsis*) *tantula* (Kirkpatrick, 1907). The first one was collected in the west station of the island and the other one in the east station. *Chondrocladia* cf. *clavata* Ridley & Dendy, 1886, a specimen from the north station of the island at 370 m. Two species of the genus *Mycale* Gray, 1867: *Mycale* (*Mycale*) *gaussiana* Hentschel, 1914 and *Mycale* (*Oxymycale*) *acerata* Kirkpatrick, 1907. Both are very abundant in Antarctic waters and have been collected in 3 or 4 sampled stations of the island. Three species of the genus *Isodictya* Bowerbank, 1864: *Isodictya kerguelensis* (Ridley & Dendy, 1886) and *Isodictya setifera* (Topsent, 1901) - that had been widely quoted in the Antarctic bibliography - were collected. *Isodictya* sp. nov. was also collected from the station on the West of the island and its specimen was taken by means of a box corer trawl at 208-210 m depth, in a bottom whose sediment was made up of mud with some sand.

**Redescription of *Stylopus fristedti* Topsent, 1916, (Porifera, Poecilosclerida)
collected for the first time after its original description**

Pilar Ríos{ XE "Ríos, Pilar" }^(1*); Javier Cristobo{ XE "Cristobo, Javier" }⁽²⁾

(1) Travesía da Peregrina 9 P4 2ºK, 15220 Bertamirans, Ames, A Coruña, Spain, baprios@usc.es; (2)
Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela. Casa do Hórreo, Rúa da
Ribeira 1, 15590 A Graña, Ferrol, Spain, fjcrisob@yahoo.es

The Bentart 03 campaign was the third one in a series of expeditions that have been carried out by Spain in the Antarctic Ocean with the aim of studying the biodiversity of the benthic ecosystem and its environmental conditions. It took place between the 24th and the 3rd of March 2003. Twenty five stations were covered and 8 of them were carried out in the area of the Antarctic Peninsula. In Paradise Bay, at 104 m depth and in a substratum made up of sand with some mud and little gravel, it was collected only one specimen of *Stylopus fristedti* Topsent, 1916, epibiont of another sponge *Tedania (Tedaniopsis) tantula* (Kirkpatrick, 1907), whose presence had not been considered again since Topsent had described a specimen from very near waters along the NE coast of the Petermann Island, in Lemaire Channel, between 40 and 70 m depth. This species has a clear hymedesmioid skeleton and its spicules are acanthostyles of two categories and tylotes. It lacks microscleres. In Sistema Porifera, *Stylopus* is considered as a subgenus of *Hymedesmia*. The author of these subgenera (Van Soest) considers that the number of species of *Hymedesmia* is very big and suggests the use of two subgenera at convenience: *Hymedesmia* for species with microscleres and *Stylopus* when they lack microscleres. He also expresses his doubts as he considers that this classification is probably artificial. In this work, we follow the opinion of other authors that maintain the validity of both genera, as the lack or presence of microscleres can be considered as a generic character, since when these exist, they have a clear function in the spicular architecture of the sponge.

Sponges of genus *Myxilla* Schmidt, 1862, collected in Antarctic waters by Spanish Antarctic expeditions

Pilar Ríos{ XE "Ríos, Pilar" }^(1*); Javier Cristobo{ XE "Cristobo, Javier" }⁽²⁾

(1) Travesía da Peregrina 9 P4 2°K, 15220 Bertamirans, Ames, A Coruña, Spain, baprios@usc.es; (2) Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela. Casa do Hórreo, Rúa da Ribeira 1, 15590 A Graña, Ferrol, Spain, fjcrisob@yahoo.es

From 1994 to 2003 different Spanish expeditions have been carried out to the Antarctic and in some of them Porifera were collected, whose subsequent taxonomic study was carried out at the Universidade de Santiago de Compostela. As a result of this research, six species have been identified, among which some were considered in the summary of Sará *et al.* Among them, two new species are quoted for the first time after their original description and in the case of five of them, their bathymetrical range is extended. *Myxilla* (*Myxilla*) *elongata* Topsent, 1916 was collected in Trinidad Island at 141 m depth, at Bransfield Strait at 647-672 m and at Gerlache Strait at 46 m. *Myxilla* (*Burtonanchora*) *asigmata* Topsent, 1901 originates at Bransfield Strait, between 1192 and 1379 m depth. *Myxilla* (*Burtonanchora*) *magna* Topsent, 1916 was collected in Trinidad Island, between 141 and 233 m depth and in Paradise Bay at 104 m depth. Boury-Esnault & Van Beveren's criteria is followed and *M. magna* Topsent, 1916 is considered to be a valid species which is characterized by the fact that it has styles that make up the main skeleton with a polygonal structure, tylotes with thorny ends that are arranged in a tangential way in the ectosomal skeleton, anchorate chelae and sigmata of two sizes that constitute a thick layer on the ectosome. *Myxilla* (*Burtonanchora*) *pistillaris* Topsent, 1916 was collected in Pedro I Island at 86 m depth. So far it had only been quoted by Topsent from Graham Land at 92 m depth, from the second French expedition to the Antarctic. *Myxilla* (*Burtonanchora*) *lissostyla* Burton, 1938 is a sponge, whose presence seems to be very abundant in the Antarctic Peninsula and Bellingshausen Sea area, as 21 specimens have been collected in 5 different stations: in Livingston Island between 15 and 30 m depth, at Bransfield Strait between 1182 and 1400 m, in Pedro I Island at 86 m and at Margarita Bay at 517 m. *Myxilla* (*Ectomyxilla*) *hentscheli* Burton, 1929 on the contrary must be a very unfrequent species in Antarctic waters, as this is its first quotation after its original description, where it had been initially quoted by Hentschel from Gauss Station between 350 and 385 m depth and subsequently collected in Trinidad Island at 214 m.

A new species of *Cinachyra* (Tetillidae; Spirophorida) collected by the project REVIZEE score central in Espírito Santo state, Brazil

Pablo Rodriguez Dominguez Rodriguez{ XE "Rodriguez, Pablo Rodriguez Dominguez" };
Guilherme Muricy{ XE "Muricy, Guilherme" }^(*)

Laboratório de Porifera/ Departamento de Invertebrados - Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, 20940-040, Rio de Janeiro, RJ, Brasil

The project REVIZEE SCORE Central, including the Benthos campaigns II, V and VI, from 1997 to 2003, collected 72 specimens of sponges of the family Tetillidae (Class Demospongiae, Order Spirophorida). Two of these specimens belong to the genus *Cinachyra*, which are Tetillidae with cortex reinforced by auxiliary oxeas, with flask-shaped porocalices. So far, only a single valid species has been recognized with certainty as belonging to this genus (*Cinachyra barbata*), and most records of *Cinachyra* species belong to the genus *Cinachyrella*. Both specimens collected by the project REVIZEE were found at 500 m depth along the Vitória-Trindade Chain, in the Espírito Santo state, Central Brazilian coast. *Cinachyra* sp. n. differs from *Cinachyra barbata* by the possession of only one category of protriaenes, choanosomal anatriaenes, and the loss of the basal mass. Furthermore, choanosomal anisostrongiloxeas (2075-3305-4300 x 43 µm) and cortical oxeas (425-650-850 x 34 µm) are smaller than those of *Cinachyra barbata*. *Cinachyra* sp. n. is the first record of the genus *Cinachyra* for the Brazilian coast.

Speciation and biogeography of the sponge genus *Latrunculia*, within the Southern Ocean

Toufiek Samaai{ XE "Samaai, Toufiek" }^(*); Michelle Kelly{ XE "Kelly, Michelle" }; Mark J. Gibbons{ XE "Gibbons, Mark J." }

CSIR, NRE, Marine and Coastal Pollution, P. O. Box. 17001, Congella, Durban

The genus *Latrunculia* contains some 20 species from the Southern Hemisphere. It forms a monophyletic group defined by four synapomorphies. A cladistic analysis of the genus was performed using 33 morphological characters. Under the hypothesis of polymorphism for multistate characters, the phylogenetic analysis produced 728 equally parsimonious trees of 120 steps with a consistency index of 0.533, a retention index of 0.579 and a homoplasy index of 0.475. A successive weighting procedure was applied resulting in obtaining one cladogram with a consistency index (CI) of 0.600. This tree was used to represent the phylogenetic hypothesis of the *Latrunculia* within relationship. Results of the cladistic analysis support most of the current systematic classification of the genus. The historical biogeography of *Latrunculia* was analysed using the BPA protocol with PAUP. Frequent extinction events during the evolution of the genus, since at least the early Jurassic, may explain the inferred disjunct sister-group distributions. Two major vicariant events are suggested that account for the current distribution of the genus. The first relates to the breakup of the supercontinent Pangaea, and the second to the breakup of Gondwana.

Biodiversity “hotspots”, patterns of richness and endemism, and distribution of marine sponges in South Africa based on actual and interpolation data: A comparative approach

Toufiek Samaai{ XE "Samaai, Toufiek" }^(1*); Kerry Sink{ XE "Sink, Kerry" }⁽²⁾; Ruwen Pillay{ XE "Pillay, Ruwen" }⁽³⁾

(1) CSIR, Environmentek, Coastal programme, P.O. Box X 17001, Congella, 4013, Durban, South Africa;

(2) SAIAB, Rhodes University, Coelacanth Programme, Grahamstown, South Africa; (3) School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, P/B X54001, Durban, South Africa

“Hotspots” of biodiversity at a meso-scale diversity were examined for marine sponge populations throughout the temperate, subtropical and tropical South Africa. Actual and interpolated (predicted) data sets are presented in a comparative manner to compare the diversity patterns of South African Demospongiae species and their distribution in contiguous 5° grid sections, to demonstrate current knowledge of the sponge fauna in various coastal regions. Richness and endemism varied considerably between marine areas and grid sections, for species at all spatial scales. Richness and endemism were equally effective indicators of biodiversity “hotspots”, but differed in patterns between the two datasets. Species richness appears to be fairly unevenly distributed along the coast, and marked peaks occur in the south of the country, the greatest being in the area surrounding Cape Town (Sector 11); almost certainly the consequence of a disproportionately intense sampling effort at these locations. Levels of apparent endemism varied considerably between sectors, ranging from zero (Sector 1) to 71 species (Sector 11), with an average of 57% apparent endemic species recorded for all 35 regions based on the actual data, vs. 20% apparent endemics for the predicted data. The Bray-Curtis analysis (without transformations) showed no clear pattern of faunal relationships between the 35 selected sectors for the actual dataset, but the predicted data set showed three distinct faunal groups, the West coast fauna extending from Cape Point northwards, a South coast fauna, and an East coast fauna.

Global environmental changes: 'Boring' sponges call for more attention

Christine Hanna Lydia Schoenberg{ XE "Schoenberg, Christine Hanna Lydia" }

Centre for Marine Studies, The University of Queensland, Research Rd., St. Lucia, QLD 4072, Australia,
christineaway@gmx.net

Research on bioeroding sponge ecology temporarily slowed down in the 1980s and 1990s, but recently receives growing attention. This is partly due to the response of bioeroding sponges to globally changing conditions and to intense research efforts investigating coral reef demise caused by *e.g.* climate change. The sponges' roles in changing environments will be explained and some highlights of the author's most recent research on an Australian symbiotic bioeroding sponge will be shown. Due to anthropogenic impacts, the world's oceans are moving towards eutrophication, increased sea surface temperatures and acidification. This has led to significant disturbances and major phase shifts, *e.g.* coral mass mortalities due to thermal bleaching events and the putative increased incidence of diseases affecting benthic organisms. Some bioeroding sponges appear to benefit from such conditions, and the abundance of a bioeroding sponge community on the Great Barrier Reef grew by 50% when compared before and after two severe bleaching events. Two sponges that have become more common are *Aka mucosa* (Bergquist, 1965) and the symbiotic *Cliona orientalis* Thiele, 1900. Both sponges can invade live coral. The latter has been found to grow faster and erode more with increased food supply, and it is exceptionally resistant against bleaching. The sponge's hardiness may be explained by the fact that it contains a unique, relatively robust G-clade *Symbiodinium*, and that these symbionts are moved around within the sponge depending on environmental conditions. Given the fact that sponges are the major endolithic bioeroders in many tropic environments, we can predict rising levels of global bioerosion if present trends continue. This would lead to decreases in complexity in environments such as reefs, lower biodiversities, reduction of availability of habitats, and in effect to economic losses of the fisheries and the tourism industries.

Revision of Eastern Pacific *Geodia* Lamarck, 1815 (Porifera, Astrophorida) with new approaches on the classification of Geodiidae

Carla M.M. da Silva { XE "Silva, Carla M.M. da" }^(1*); Maurício Campos { XE "Campos, Maurício" }⁽²⁾; Beatriz Mothes { XE "Mothes, Beatriz" }⁽²⁾

(1) Laboratório de Biologia de Porifera, Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Geremoabo, s/n, CEP 40170-290, Ondina, Salvador, BA, Brasil, carlamms@ufba.br; (2) Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, RS, Brazil, bmothes@portoweb.com.br

The genus *Geodia* Lamarck, 1815 (Porifera, Astrophorida) has Tethyan distribution, with about 130 species in the tropical-subtropical regions of Atlantic, Pacific and Indian Oceans. An extended review of 34 species from Western Atlantic and Eastern Pacific, started in 1998, has detected 18 species or varieties for the Eastern Pacific Region, all of them described many years ago (1873-1910). This is the first redescription based on extensive SEM analysis. The previous records from this region were described originally as *Geodia agassizi* Lendenfeld, 1910, *G. breviana* Lendenfeld, 1910, *Sidonops bicolor* Lendenfeld, 1910, *G. acanthylastra* Lendenfeld, 1910, *G. mesotriana* Lendenfeld, 1910, *Sidonops mesotriana* var. *megana* Lendenfeld, 1910, *S. mesotriana* var. *microana* Lendenfeld, 1910, *G. ovis* Lendenfeld, 1910, *Sidonops angulata* var. *microana* Lendenfeld, 1910, *S. angulata* var. *megana* Lendenfeld, 1910, *S. angulata* var. *orthotriana* Lendenfeld, 1910, *S. californica* Lendenfeld, 1910, *Geodia ataxastra* var. *latana* Lendenfeld, 1910, *G. ataxastra* var. *angustana* Lendenfeld, 1910, *Sidonops oxyastra* Lendenfeld, 1910, *Geodia media* Bowerbank, 1873, *G. micropora* Lendenfeld, 1910, and *G. amphistrongyla* Lendenfeld, 1910. The examined material was collected at 0-1m or dredged from 37 to 245m depth, from the SE Alaska (54°40'N/126° W) to Easter Island (27°09'S/109°27'S), carried out under auspices of "Albatross" Cruise (1888, 1889), U.S. Fish Commission Steamer Albatross (1904) and SEPBOB - Anton Bruun Cruise (1966). Dissociated spicule mounts, thick sections and SEM stubs preparation followed the methodology used in previous descriptions of the genus. The revision proposed here has followed the International Code of Zoological Nomenclature, not considering varieties, and keeping only the specific level names. Eleven species of *Geodia* are now recorded from Eastern Pacific, most of them restricted to deep waters: *Geodia agassizi*, *G. breviana*, *G. bicolor* comb. n., *G. mesotriana* comb. n., *G. angulata* comb. n., *G. californica* comb. n., *G. ataxastra*, *G. oxyastra* comb. n., *G. media*, *G. micropora* and *G. amphistrongyla*. The comparative analysis detected some groups of specimens originally assigned to *Sidonops*, *Synops* and *Geodia* that exhibit identical and special details (size and form) in the spicule set, particularly the microscleres. In our opinion, the oscula distribution patterns are extremely variable and are not solid characters to define genera in Geodiidae. This spicule identity seems not to be associated with the aquiferous system structure. This condition was also observed in Brazilian and Caribbean sponges of the same genera. Our results indicate the plasticity of the oscule arrangement in this family and expose the spicule set and structure as one of the most relevant characters to indicate phylogenetic affinities among genera and species of Geodiidae.

Sponges from Ilha Grande Bay (Porifera, Demospongiae), Southwest Atlantic, detected by rapid assessment protocol

Carla M.M. da Silva{ XE "Silva, Carla M.M. da" }^(1*); Bruno Cosme{ XE "Cosme, Bruno" }⁽¹⁾; Fernanda Casares{ XE "Casares, Fernanda" }⁽²⁾; Gisele Lôbo-Hajdu{ XE "Lôbo-Hajdu, Gisele" }⁽³⁾; Joel Creed{ XE "Creed, Joel" }⁽²⁾

(1) Laboratório de Biologia de Porifera, Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Geremoabo, s/n, CEP 40170.290, Ondina, Salvador, BA, carlamms@ufba.br; (2) Laboratório de Ecologia Marinha Bêntica, Departamento de Ecologia, IBRAG, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, PHLC, Sala 220, CEP 20550-013, Rio de Janeiro, RJ, jcreed@uerj.br; (3) Laboratório de Organização Genômica de Porifera, Depto de Biologia Celular e Genética, IBRAG, Universidade do Estado do Rio de Janeiro, PHLC, Sala 205, Rio de Janeiro, RJ

The Biological Diversity Convention requires that signatory nations carry out species inventory lists to monitor changes and to elaborate plans for the conservation of biodiversity. In 1999, the Ilha Grande Bay was recognized as a priority due to its rich fauna and flora which remained poorly known, and unique position between the two largest metropolis from South America. Ilha Grande Bay is located off Angra dos Reis at the Southern coast of Rio de Janeiro state (23°05'-23°14'S/44°05'-44°23'W). The faunistic inventories were based on the Rapid Assessment Protocol (RAP). Forty four collection sites were chosen along 323 km of shore, covering representative habitats from that region. Sponges were collected by SCUBA dive at the infralittoral (1-15 m depth), from October to December 2003. The studied specimens were deposited at the Museu de Zoologia da Universidade Federal da Bahia (UFBA) collection. The most abundant sponges from the benthic communities were quantified through 50 x 50 cm quadrats subdivided in 10 x 10 cm. The Ilha Grande RAP Project aims to catalog the benthic marine fauna and flora focusing the appliance of this knowledge on the social-economic development of local human populations. This work was meant to fulfill a gap in the understanding of sponge diversity in the Tropical to Subtropical transition zone known as The Paulista Biogeographic Province. Until now, only two interspersed scientific reports were published about sponges from that area. This is the first extensive study for porifera at Ilha Grande Bay seashore. Results include 19 species distributed in 17 families and 8 orders of Demospongiae. Poecilosclerida and Halichondrida were the most abundant orders. The sponge community of Ilha Grande Bay has species in common with the Brazilian shore and Southeast region, such as *Amphimedon viridis*, *Drummacidon reticulatus* and *Chondrilla* aff. *nucula*. Nevertheless, samples identified as *Desmacella* sp.1, *Dysidea* sp.1 and *Iotrochota birotulata* are new occurrences for the region or even new records for the Brazilian coast and/or science. The follow up of this inventory, mapping the actual sponge diversity of the studied area, will produce data for biogeographic and phylogenetic studies on Porifera from the Brazilian coast.

Redescription of *Geodia glariosa* (Sollas, 1886) with new records along the Brazilian coast

Carla M.M. da Silva{ XE "Silva, Carla M.M. da" }^(1*); Meiryelen Vieira da Silva{ XE "Silva, Meiryelen Vieira da" }⁽²⁾

(1) Laboratório de Biologia de Porífera, Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Geremoabo, s/n, CEP 40170-290, Ondina, Salvador, BA, Brasil, carlamms@ufba.br; (2) Departamento de Biologia Celular e Genética, IBRAG, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, PHLC, 2º Andar, Maracanã, Rio de Janeiro, RJ

The redescription of *Geodia glariosa* (Sollas, 1886) is a sequence in the review of all species of the genus *Geodia* Lamarck, 1815 registered from Western Atlantic, initiated in 1998. *Geodia glariosa* is one of the five endemic species from the Brazilian coast, and its geographical distribution before this work was restricted to two localities, Bahia and Santa Catarina, determining a disjunct distribution pattern. The examined samples were collected from Pernambuco to Santa Catarina state coast, between 12 and 50 meters depth, by scuba diving or expeditions like CEZ - Zoological Studies Commission/UFRJ, Brazil and H.M.S. "Challenger", England. Dissociated spicule mounts, thick sections and SEM stubbs preparation follows the methodology used in previous descriptions of the genus. The type-material showed an ectocortex formed by radial bundles of stout oxeas, located above the sterrasters, provided of sand grains inclusions, which are typical characteristics not observed in any other species of the genus described till now, confirming the original description. However, in some specimens collected at rocky shores, such characteristic does not appear, possibly due to the substitution of sand grains for dead diatoms carapaces, abundant in the crevices, and that also constitute siliceous material used by the sponges. An additional category of megascleres, the plagiotriaenes, mentioned at the first time in this study, was detected in all examined samples. *G. glariosa* is common in rocky substrate, and has numerous barnacles (*Balanus* sp.) or bivalves attached on its surface (as the Schizosintype from Bahia). The gap in the distribution of the species along the Brazilian coast is almost completely fulfilled in this study through the record of collected samples at Bahia [Porto Seguro and Cumuruxatiba], Espírito Santo [Santa Cruz] and São Paulo [São Sebastião] states. The comparative study also allowed to amplify the geographical distribution of the species to Pernambuco coast. The sample recorded by Topsent to the Gasconha Gulf in Spain, at the Eastern Atlantic, is not considered as con-specific with *Geodia glariosa*, due to the fact that the American and European Atlantic coasts are disjunct distribution areas, with an important barrier for dispersal, effective for the sponge fauna, the mid Atlantic deep-waters.

Revision of *Auloplax* gives ground to place it in the order Aulocalycoida, family Aulocalycidae (Porifera, Hexactinellida)

Konstantin Tabachnick{ XE "Tabachnick, Konstantin" }

Institute of Oceanology of Academy of Sciences of Russia, Nahimovky 36, RU-117218 Moscow, Russia,
tabachnick@mail.ru

A new species of *Auloplax* found off Loyalty Islands and off New Caledonia is described. *Hexactinella filholi* Topsent is considered to be a lower synonym of *Auloplax auricularis* Schulze, *Dactylocalyx patella* Schulze - to be another representative of *Auloplax* and the species 'patella' may be considered to be the elder synonym of 'auricularis'. The latest placement of *Auloplax* in the reconstructed family Dactylocalycidae by Reiswig (in the *Systema Porifera*) is baffling and unsupported. This genus is undoubtedly a representative of Aulocalycoida-Aulocalycidae. The Dactylocalycidae, with two representatives *Dactylocalyx* and *Iphiteon*, requires further investigation and their diagnosis should be changed as real body construction of tubes is likely absent in these two genera. They seem to have true schizorhysal channelization as Tretodictyidae and Diapleuridae.

Hexactinellida collected by the French R.V. "Marion Dufresne" off the Brazilian coast

Konstantin Tabachnick{ XE "Tabachnick, Konstantin" }⁽¹⁾; Larisa L. Menshenina{ XE "Menshenina, Larisa L." }⁽²⁾

(1) Institute of Oceanology of Russian Academy of Sciences, Nahimovsky 36, Moscow, Russia, tabachnick@mail.ru; (2) Moscow State University, Physical Faculty, Vorobjovi Gory, Moscow, Russia

Description of hexactinellid sponges collected by the French expedition R.V.'Marion Dufresne' in 1987 voyage 55 is given. Three species are new: *Lophocalyx* sp.n. and two species of *Hyalonema*: *H. (Prionema)* sp.n. and *H. (Leptonema)* sp.n. Other hexactinellids: *Farrea* sp., *Sarostegia* aff. *oculata*, *Aphrocallistes* aff. *beatrix*, *Dactylocalyx* aff. *subglobosus* and *Euplectella suberea* were previously known to be widely distributed in the Atlantic ocean.

***Aplysina fulva* (Pallas, 1776) and *Aplysina cauliformis* (Carter, 1882) from shallow coral areas of Isla Colón, Panamá**

Diego F. Valderrama{ XE "Valderrama, Diego F." }^(1*); Juan C. Márquez{ XE "Márquez, Juan C." }⁽²⁾; Marco A. Romero{ XE "Romero, Marco A." }⁽²⁾

(1) Universidade Federal do Rio de Janeiro, Instituto de Biologia - Departamento de Zoologia, Laboratório de Biologia de Porífera, Av. Pau Brasil, 211 - CCS - Bloco A, sala A0-100, Ilha do Fundão - Rio de Janeiro - RJ - Brasil, diego_valderrama@lycos.com; (2) Universidad Nacional de Colombia (Departamento de Biología y Centro de Estudios en Ciencias del Mar-CECIMAR), INVEMAR, Cerro Punta de Betín, AA 10-16, Santa Marta, Colombia, jcmárquez@invemar.org.co; elmarcuspe@hotmail.com

The taxonomy of *Aplysina* (Nardo, 1834) at the species level is difficult and recent molecular evidence using ITS-2 and COI implied that all Caribbean species should be conspecific. This study surveyed the morphological plasticity of *Aplysina fulva* and *A. cauliformis*, the two *Aplysina* "ramose" species from the Caribbean, in two shallow coral areas of Isla Colón (Punta Caracol and Bocas del Toro), in Panama. Field sponge observations and collections were done by free diving within two 50 m areas between 1 and 6 m depth. Further observations of sponge fragments were done in a tank with running sea water, and sponge fibers were studied with a light microscope. A great morphological variation was found between *Aplysina* "ramose" individuals. *Aplysina fulva* ranged from creeping and highly anastomosed thin branches, in pebbles dispersed within sand (<1.5 m depth), to creeping and ramified branches, most with tubular oscules, in small coral conglomerates (1.5-5 m depth); both morphotypes were characterized by a mustard to yellowish brown colour, high branch flexibility and strong pigment oxidation (turning black) when taken out of the water. *Aplysina cauliformis* showed body morphology similar to sympatric *A. fulva* individuals (between 1.5-5 m depth) having, in contrast, light purple colorations with pink, blue and yellow shades, and slow oxidation out of the water. Two additional *Aplysina* "ramose" morphotypes - showing erect branch growing, hard consistency and difficult tearing - were found at 5-6 m depth: *Aplysina* sp1. aff. *cauliformis* included low and not ramified erect dark purple individuals, with slow oxidation out of the water; instead, *Aplysina* sp2. aff. *cauliformis* included tall and highly ramified groups of erect yellowish brown individuals, showing great size and branch diameter, and a less quick oxidation out of water (black shading over ectosome and choanosome colour tones). Neither external (*i.e.* branch diameter, consistency, surface, oscule morphology and distribution), nor internal characters (fiber, pith and reticule diameter), but colour and oxidation, could segregate *Aplysina fulva* from *A. cauliformis*. Following this sense, the common occurrence of tubular oscules at the base or over these species' branches, may suggest a closer relationship between "ramose" and "tubular" *Aplysina* species that share similar patterns of coloration and oxidation (*i.e.* *A. cauliformis*-*A. archeri*, *A. fulva*-*A. fistularis*), than between *Aplysina* species that share just similar patterns of body growth. This trend has been observed at a larger area and depth scale (meters to hundred of meters) in many Caribbean areas (*i.e.* Colombia, Venezuela, Bahamas) by other researchers. A search for new cytological and molecular characters for *Aplysina* species taxonomy and phylogeny is strongly encouraged to resolve this hypothesis and help define *Aplysina* species.

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