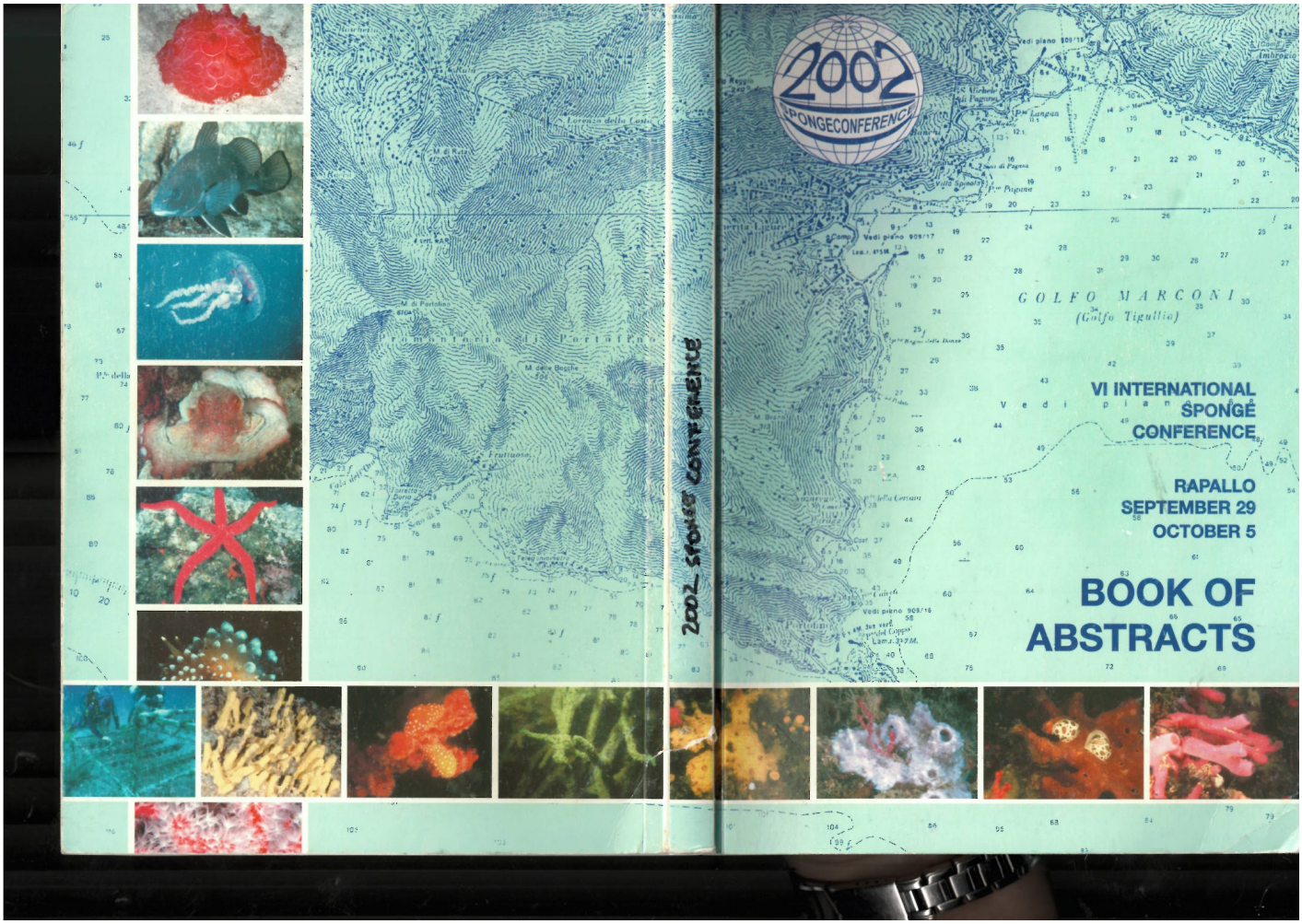
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SD-BRA AND SD-TBX: FIRST T-BOX TRANSCRIPTION FACTORS FROM PORIFERA

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During evolution, the appearance of the mesoderm, which is involved in the differentiation and formation of tissues in adult bilaterians, parallels with the appearance of the two perpendicular body axes. The effectors of bilaterians regional specification and pattern formation comprise an evolutionary-conserved set of genes; among those, several encode transcription factors related to the superclasses of homeoproteins (Hox and non-Hox genes), winged-helix, T-box, Wnt-pathway and zinc-finger proteins.

Interestingly, members of all these families have been isolated from Cnidarians, the most wide studied group of non bilaterian metazoans. Homologs to Forkhead, Brachyury, Wnt and Homeobox genes are regulated during their apical patterning. In contrast, the expression patterns of the Cnidarian Hox genes suggest that their apical-basal axis differentiation do not parallel the anterior-posterior of bilaterians.

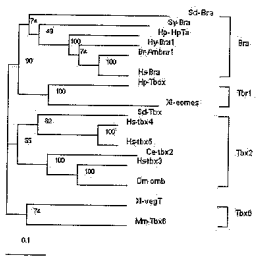
Knowledge of developmental mechanisms involved in pattern formation and morphogenesis in sponges can clarify the origin and ancestral function of these evolutionary-conserved pathways.

In sponges the basic elements for the differentiation of omni/pluripotent cells to distinct somatic cells through morphogenetic events as cell-cell and cell-matrix adhesion, as well as cell migration, have been identified. However, the existence of those major regulatory genes causing the establishment of a polar patterning in bilaterians remains to be elucidated. Until now only a few non-Hox and one highly diverged Hox-like gene had been isolated from sponges.

Here we report the isolation and characterization of two members of the T-box family in the sponge *Suberites domuncula*: Sd-Bra and Sd-tbx. T-box genes conform a family of transcriptional regulators that share a highly conserved region that binds to DNA, the T-box domain. The first member discovered was the Brachyury or T-gene from mice; heterozygous mutant animals have a short tail, while homozygous animals die in utero and lack the notochord and posterior mesoderm. Homologs of Brachyury have been isolated from all groups of metazoans, conforming the Brachyury subfamily.

Although Brachyury is responsible for the differentiation of the notochord and the formation of the posterior mesoderm in vertebrates, its expression around the original gut opening in invertebrates suggests a more ancestral function. All other T-box genes are grouped into different subfamilies, and are involved in type specification and morphogenetic movements during development.

Using the T-box domains for homolgy comparisons and for phylogenetic tree constructions, we can include Sd-Bra into the Brachyury subfamily, and Sd-tbx into the subfamily named Tbx2. Interestingly, members of Tbx2 subfamily have been found until now only in chordates, where they are involved in limb specification.



Phylogenetic tree of the T-box gene family, constructed by using the neighbor-joining algorithm, showing the families to which the two new members from *Suberites* can be included. The numbers in the nodes mean the percentage of times each node was supported. (Sd, *Suberites domuncula*; Sy, *Syon raphanus*; Hp, *Hemicentrotus pulcherrimus*; Hy, *Hydractinia sulgaris*; Br, *Branchiostoma floridae*; Hs, *Homo sapiens*; Xl, *Xenopus laevis*; Ce, *Caenorhabditis elegans*; Mm, *Mus musculus*).

THE ROLE OF EPIBIONT SPONGES AND THEIR MICROBIAL SYMBIONTS, IN NITROGEN LIMITED *RHIZOPHORA MANGLE* STANDS

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In Twin Cays, Belize, the subtidal epibiont community of *Rhizophora mangle* is dominated by sponges. Previous studies show that the presence of live sponges on *R. mangle* prop roots increases root biomass relative to spongeless roots. As red mangroves along the fringes of the cays are severely nitrogen limited, this could be due to nitrogen-fixing processes mediated by symbiotic microbes within sponge tissue. Using previously nitrogen-enriched and control transects from another study, epibiont sponges were sampled from red mangrove prop roots. Bacterial genes were then isolated from *Haliclona implexiformis*, one of the most abundant members of this sponge community. I used RFLP analyses to identify dominant members of the sponge bacterial community and 16S rRNA sequences to differentiate among bacterial species. Four bacterial species dominated *H. implexiformis* sponges from the nitrogen transect. I am now characterizing the dominant bacteria in *H. implexiformis* collected from the control transect and I will compare these to data from the nitrogen transect. In future studies I will identify and predict ways in which epibiont sponge bacterial symbionts could affect nitrogen cycling. These numerically abundant bacteria might play important roles in mangrove community ecology.

GENERAL COMMENTS ON SPECIES INVENTORY, FISHERIES, CULTURE AND SOME COMMUNITY FEATURES OF THE PORIFERA IN CUBA

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Up to now, 255 species have been reported from Cuba. Confirmed commercial species existing in Cuba are *Hippospongia lachne*, *Spongia obscura*, *S. pertusa*, *S. barbara* and *S. graminea*. *Spongia obliqua* and *S. tubulifera* are considered here as dubious records. Since 1959 to 2000, national commercial sponge extraction varied between 7.5 tons (1966) and 81 tons (1997), with an annual average of 42.5 tons. The highest average annual sponge production in Cuba was reported in the period 1920-1929 (500 tons). *Hippospongia lachne* has been historically the main commercial sponge in the Golfo de Batabanó (southwest of Cuba) till 1994, when it began to be substituted by a group of species of *Spongia*. However, *Spongia* species always dominated fisheries in the north central part of Cuba. Pilot experiences on sponge culture in Cuba (using horizontally suspended lines on sea grass beds) have been successful, but not implemented as a well-established economic activity in Cuba. Commercial sizes (15 cm in diameter) were attained after 18 months and faster growth rates were observed at the line level closest to the bottom (approximately 40 cm from the bottom). Most dominant sponge species for the most outstanding marine habitats of Cuba are given, as well as for a situation of pollution around Havana City. As expected, highest sponge diversity was found in coral reefs and inshore hard grounds. The highest values of community heterogeneity H' (very close to 3.5 natural bells) were found in the coral reefs of Cayo Esquivel (Archipelago Sabana-Camagüey) and Rincón de Guanabo (east of Havana City), in both cases at 20 m depth.

HALOPEROXIDASE FROM THE MARINE SPONGE *ERYLUS DISCOPHORUS*

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Bromine and iodine are known to accumulate in sponges since the nineteenth century. Bromine and iodine concentrations are variable although they present higher values for the non-siliceous sponges. The lack of spicules (low Si) was associated with a higher need of protection of these organisms against predators through the production of specific substances. A wide variety of halogenated metabolites are produced by marine organisms, namely by sponges, and many of them have properties of interest (e.g. antibiotic, antiviral, etc.).

An inverse relationship between the halogen content and the haloperoxidase activity has been found. These haloperoxidase enzymes are believed to be responsible for the production of halogenated metabolites.

A halogenating enzyme was reported in 1979 for the marine sponge *Iotrochota birotulata*, without further characterization.

On specimens of *Erylus discophorus* (Schmidt, 1862), collected from the Portuguese west coast in the Berlengas National Park, extraction, purification and characterization of a haloperoxidase was performed.

This enzyme is stable at high saline concentrations and has a molecular mass around 130 kDa, with subunits of 65kDa. The maximum haloperoxidase activity is around pH 5.0. The typical Soret band of heme haloperoxidases is absent, and there is no vanadium dependence of the haloperoxidase activity.

The enzyme also shows peroxidase activity (guaiacol oxidation), iodoperoxidase activity (triiodide formation) and bromoperoxidase activity (monochlorodimedone bromination).

SPONGE DIVERSITY IN DARWIN HARBOUR, NORTHERN TERRITORY, AUSTRALIA

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Darwin Harbour, situated to the west and south of the city of Darwin, is a drowned river valley with several elongated arms and covering and area of 1000 Km^2 at high tide. The harbour is macrotidal with two high and two low water levels every 24 hours and a maximum tidal range of 7.8 m. The water body is naturally turbid and carries a high level of nutrients. Water quality is mainly affected by seasonal rainfall (mean annual 1661 mm) and by the strong tidal action.

Sponges are one of the most abundant and diverse invertebrates groups represented in Darwin Harbour; indeed the area is located in one of the "hot spots"of sponge biodiversity within Australia (Hooper *et al.*, 2002).

The number of sponge species present within the limits of the harbour and in the surrounding areas is uncertain. Records of the Australian Bioinformatic facility (Environment Australia) indicate that a total of 126 sponge species are present in Northern Territory waters. Recent inventories (Hooper *et al.*, 2002), on the other hand estimate that a total of 274 species are present in the Darwin and Cobourg Peninsula regions. Further, 205 different sponge species only from Darwin Harbour are registered in the collections of NT museum. The discrepancy in all these estimates is a reflection of the great number of species that has either not been recorded yet in the literature or remain undescribed for this area.

An intensive collecting program within the limits of Darwin Harbour was initiated in May 2002, with the objective of fully documenting the diversity of sponges present in the area. So far 15 sites (including intertidal reef flats, reefs, coral heads and sand/rock flats, wharf piles and wrecks) have been surveyed using approximately equal effort. Each species has been photographed *in situ*, identified to the lowest possible level, databased and incorporated into the collections of NT Museum. Preliminary results indicate that approximately 230 species are represented in the Darwin Harbour alone. This suggests that sponge fauna in this area is amongst the richest in the Australian tropics as has been previously indicated (Hooper *et al.*, 2002). It also raises questions as to which environmental factors support this high diversity on such a small local scale. It is intended to expand collection efforts outside the limits of Darwin Harbour to fully document and inventory the sponge fauna of NT waters and to provide reliable estimates of the sponge richness of this area.

ULTRASTRUCTURE OF *ERYLUS DISCOPHORUS* (SCHMIDT, 1862) (DEMOSPONGIAE, ASTROPHORIDA)

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Sponges of the genus *Erylus* are able to produce a variety of biologically active compounds with pharmacological importance. However the biological significance of these compounds and the cells that produce them are unknown. Cytological and ultrastructural knowledge of the sponge organization is necessary for evaluation of this problem and is also required for the taxonomic characterization of the genus. In the sequence of the study of bioactive compounds of *E. discophorus*, we have made a preliminary ultrastructural study of this species.

Four samples of *E. discophorus* were collected by scuba diving at depths between 4 and 12 m in the coasts of Arrábida and Bedenga Island. The samples were maintained in sea water for 1 to 2 hours until processing. For light microscopy (LM), samples were fixed in 10 % formol in sea water or Bouin and embedded in paraffin. Sections were stained with H+E, Masson's trichromium and PAS. For transmission electron microscopy (TEM), small fragments were fixed in cacodylate buffered glutaraldehyde in sea water. After removal of spicules with treatment with hydrofluoric acid 5 % for 1 hour, the fragments were post-fixed in osmium tetroxyde and uranyl-acetate, and embedded in epon-araldite. Thin sections made with glass or diamond knives were contrasted with uranyl acetate and lead citrate, observed and photographed in a jeol 100S electron microscope.

In the ectosome, granular cells constitute the main cell type. These cells are intimately associated with collagen fibres and have oval secretory granules suggesting that they are responsible for laying down the dense collagen framework of the ectosome. Dense granules that give the sponge an irregular brown colour and large PAS+ granules several micrometers in size are also found in these cells which. The cells are interconnected through an extended network of slender cellular processes.

Choanocyte chambers may be opened with wide lumens or almost closed with small lumens. Choanocytes are either flattened with a conical cell body or cuboidal. Wandering cells migrate into the choanocyte chambers and degenerate in the lumen by a fragmentation process.

Spherulous cells with large homogeneously dense granules were found in the ectosome and choanosome surrounding the choanocyte chambers. The latter are often degranulated. The location of these cells suggests that they may serve a defence purpose.

ULTRASTRUCTURAL FEATURES OF THE CHOANOCYTES OF *THYMOSIA GUERNEI* TOPSENT, 1895 (DEMOSPONGIAE, CHONDROSIDA)

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Thymosia guernei is a species with NE Atlantic distribution, tentatively included in the family Chondrillidae on the basis of morphological characteristics. Cytological and ultrastructural data have been used to evaluate affinities among species of this family, and demonstrated the importance of these characters for the taxonomy of sponges with reduced skeleton and variable characters. We have studied the ultrastructure of 3 specimens of *T. guernei* from Portugal (Berlenga Natural Reserve) and report observations on the presence of intercellular junctions and granules of a probable secretory nature in the choanocytes. Three specimens of *T. guernei* were collected in Berlenga Island at a depth of 4-8 m by scuba diving. The specimens were maintained in sea water for 1 to 2 hours until processing. Small fragments were fixed in cacodylate buffered glutaraldehyde in sea water, post-fixed in osmium tetroxyde and uranyl acetate, and embedded in epon-araldite. Thin sections were contrasted with uranyl acetate and lead cytrate, observed and photographed in a Jeol 100s electron microscope. Choanocytes are truncated conical cells closely apposed to each other laterally. Intercellular junctions, resembling septate junctions in some favorable sections, connect the lateral surfaces. Intercellular septate junctions have been rarely observed in sponges, but include observations in modified choanocytes of *Clathrina*, in several cell types of Hexactinellids, between spongocytes of *Ephydatia fluviatilis* and between sclerocytes of *Sycon*. The choanocytes have well developed nucleoli and display different types of dense granules, some of probable lysosomal significance, others of secretory nature, suggesting that choanocytes of this species may engage in the synthesis of intercellular components. The well developed cellular junctions may be important for the secretory activity by delimiting a basal membrane domain specialized for secretion and by providing a barrier that prevents diffusion of the secretory products into the choanocyte chamber. Other prominent cell types observed in this species were spherulous cells found in large numbers in the mesohyle and microgranular cells, also called grey cells.

SUPEROXIDE DISMUTASES FROM TWO MARINE SPONGES OF THE PORTUGUESE COAST

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Antioxidative enzymatic defenses have evolved to protect the organisms against reactive oxygen species. Superoxide dismutase enzymes (SOD) play a critical role in the dioxygen metabolism and are widely distributed in all organisms. These enzymes have been tested as biomarkers of pollutant impact on living organisms in aquatic systems.

Although the presence of these enzymes in sponges is not surprising, no reference was found for characterization of SOD enzymes from these organisms.

As part of a survey on sponges of Berlenga National Park (western Portuguese coast) the SOD activities were studied. Almost all the specimens showed some activity with the exception of the *Erylus discophorus* (Schmidt, 1862) species where SOD activity was not detected.

The extraction, purification and preliminary characterization of SOD enzymes from two marine sponges, *Spongia agaricina* and *Adocia* sp. is reported.

The enzymes were extracted with phosphate buffer (50 mM, pH 5.8) followed by an ammonium sulphate precipitation step (80 % saturation). After dialysis, the extracts were purified through ionic and gel filtration chromatographies.

SOD activity was measured by the inhibition of the autooxidation of adrenaline in carbonate buffer. The purified enzymes present a violet colour and are inhibited by cyanide, indicating that there is probably a CuZn SOD.

GAMETE STRUCTURE AND FERTILIZATION PROCESS IN THE SPONGE *AMPHORISCUS KUEKENTHALI* (CALCISPONGIA, CALCARONEA) FROM THE WHITE SEA

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The gamete structure and the fertilization process in the White Sea sponge *Amphoriscus kuekenthali* (Calcispongia, Calcaronea) have been studied at light and ultrastructural levels. *A. kuekenthali* has an anatomical organization of syconoid type. Alike to what happens in other calcareous sponges, in the studied one the fertilization is conducted with special carrier cells. Gatenby (1920) was the first to give a coherent description of this process in *Grantia compressa*. At present the carrier-cell fertilization is found in series of Calcaronea species. But up to now ultrastructure of free spermatozoa in calcareous sponges is unknown. We managed to fix a specimen of *A. kuekenthali* with mass release of spermatozoa and to study their structure. Mature spermatozoa of the studied sponge show a unique organization. At light microscopy they appear as spherical cells (about 4 μm in diameter) with the main space occupied by a strongly compacted nucleus (about 3,3 μm in diameter) surrounded by a thin cytoplasm layer. Electron microscopy observations show that flagellum and acrosome are lacking. The nucleus of the mature spermatozoon is a complex structure consisting in several morphological units: 1) a dense, strongly compacted mass of chromatin 2) usually two electron-dense bodies apparently of protein nature 3) an intranuclear fibrillar layer situated between the chromatin mass and the nuclear envelope. This spermatozoon complex nucleus is surrounded by another fibrillar layer and by an irregularly thin cytoplasm layer, which contains a large amount of different membrane structures, several electrondense granules and mitochondria.

Ready for fertilization oocytes of *A. kuekenthali* reach 75 μm in diameter and have a large nucleus (about 35 μm in diameter) with a nucleolus (about 6 μm in diameter). Their cytoplasm contains diverse uniformly scattered inclusions among which the most numerous are inclusions with fibrillar contents that are typical of oocytes of calcareous sponges. (Such inclusions have been described for all studied Calcaronea). During vitellogenesis the complex of nurse cells is being formed from the choanocytes located above oocytes.

The specific fertilization process in *A. kuekenthali* might be put on account of its sperm's unique organization. During the mass sperm release any cell of the nurse cells complex can seize a spermatozoon transforming itself, *in situ*, into a carrier cell. The transformation of the seized spermatozoon into a spermiocyst is a rapid process because the mature spermatozoon of *A. kuekenthali* has all the necessary structures for making the spermiocyst. The electron-dense spermiocyst capsule is formed by the intranuclear fibril layer of the spermatozoon complex nucleus. This layer swells and becomes more homogeneous. The spermiocyst capsule deeply penetrates into the ooplasm towards the oocyte nucleus. The other morphological components of the spermatozoon's complex nucleus (the compact electron-dense chromatin mass, one or two electron-dense protein bodies) are clearly seen inside the space limited by the spermiocyst capsule penetrating into the oocyte. The electron-dense chromatin mass lies in a deep part of this formation, while the protein bodies lie in part adjoining the carrier cell. During fertilization the protein bodies flatten and look like electron-dense plates. The spermiocyst capsule gradually resolves in the ooplasm. This process is correlated with the destruction of the oocyte nucleus envelope. Then the oocyte starts meiotic divisions.

MULTIPLE DEFENSIVE ROLES FOR BROMOPYRROLE ALKALOIDS FROM CARIBBEAN *AGELAS* SPONGES

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Sponges of the genus *Agelas* (Family Agelasidae) are important components of Caribbean coral reef communities because they are abundant in a variety of habitats from shallow back-reef to deep-reef slope. Chemically, *Agelas* spp. are distinct because they contain primarily brominated pyrrole-2-carboxylic acid derivatives. The ecological roles of these compounds have only recently been examined. Previous studies have determined that *Agelas* sponges are chemically defended from fish predation by bromopyrrole alkaloids: *A. clathrodes* and *A. wiedenmayeri* by 4,5-dibromopyrrole-2-carboxylic acid and oroidin, *A. conifera* by sceptrin. Here, we expand our understanding of chemical defense strategies in this common and diverse sponge genus. In addition to the previously investigated sponges *Agelas conifera* and *A. Wiedenmayeri*, a detailed chemical analysis of the secondary metabolites of 92 samples of *A. cerebrum, A. cervicornis, A. clathrodes, A. dilatata, A. dispar* and *A. sceptrum* has been performed. *Agelas cervicornis* and *A. dispar* contained the same two major metabolites as *A. clathrodes* and *A. wiedenmayeri*, whereas extracts of *A. cerebrum, A. dilatata* and *A. sceptrum* comprised a mixture of dimeric compounds dominated by sceptrin, similar to *A. conifera*. The content of the defensive metabolites in crude extracts of these eight species was analysed and quantified by HPLC methods in order to prove if the required active concentration for antipredatory effects on fish feeding is present in the sponge tissue. Furthermore, brominated pyrrole alkaloids were tested in laboratory and field assays for effects on bacterial attachment and overgrowth by neighbouring sponges. The presented data suggest that bromopyrrole alkaloids fulfil multiple ecological functions in the chemical defense of Caribbean *Agelas* sponges.

HABITAT AMPLIFICATION BY HEXACTINELLID SPONGES IN BRITISH COLUMBIA

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Mats of rossellid sponge spicules occur in the Antarctic, and are reported to play a primary role in determining the species composition of sponges and associated fauna.

A similar mat is associated with three rossellid species at depths of 30 to 110 m in the Strait of Georgia, British Columbia. The surrounding region is primarily a mud substrate with a local outcropping of rock. These rossellids and an associated fauna are only found on the mat and on rock. At least one rossellid species harbors a well developed surface fauna as well as sediment. The epifauna is at least partially removed when the outer layer of spicules is periodically shed as reported by others. Two species of hexactinosans also occur here as well as on the rock cliffs of nearby fjords. In contrast to the rossellids, the hexactinosan surfaces are free of attached fauna and sediment. However, when all or a portion of these hexactinosans dies, the *post mortem* skeleton becomes populated by many species of sponges and other organisms. The biota on these hexactinosans was assessed from submersibles, trawls, and grab samples in B.C. fjords and on the recently described sponge bioherms in Hecate Strait. The development of a diverse epifauna and the growth of the sponge bioherms is dependent on the skeleton of dead hexactinosans remaining intact in the water for perhaps decades. Indirect evidence from one fjord indicates a minimum age of 30 years for a *post mortem* skeleton in anoxic waters.

FOSSIL SPONGES IN THE NATURAL HISTORY MUSEUM, LONDON

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Since 1753, when the sale of Sir Hans Sloane's Collection to the nation resulted in the foundation of the British Museum, many important specimens and manuscripts have been donated to or purchased by what is now The Natural History Museum. Using specimens, publications and contemporary manuscripts, this poster aims to highlight some historically and taxonomically important specimens added to the fossil sponge collection over the past 250 years, and the work of the early palaeontologists who described them.

Although some fossil sponges are from collections dating back to the 18th Century, such as those in the Thomas Pennant collection (although this was not donated until 1912), many more are from collections that were either purchased or donated in the 19th Century. These include sponges of historical importance, such as those collected and figured by William Smith (purchased 1816 and 1818), who was the first person to use fossils to identify strata and produce a large-scale geological map; and specimens figured by J. S. Bowerbank (1869-1876). The earliest taxonomically important specimens are those that were figured from the 1820's onwards and which represent the first attempts at understanding the British fossil sponge fauna, especially the rich fauna of the Cretaceous Chalk. Collections described by G. A. Mantell (1822) and J. Toulmin Smith (1847, 1848) were purchased in 1865 and 1869 respectively. Part of Toulmin Smith's manuscript collection, including his scrapbook on ventriculitids, is also held by the Museum. In 1883 G. J. Hinde published a catalogue of fossil sponges in the British Museum. This was the first attempt to monograph British fossil sponges and took into account the knowledge and ideas on taxonomy published in continental Europe by eminent palaeontologists such as K. A. von Zittel. Many specimens figured by G. J. Hinde throughout his career are held in the Museum collection, either donated at various times by Hinde himself or by his widow following his death in 1918. G. J. Hinde's collection, along with material purchased from A. Schrammen, are probably the two most important 20th Century acquisitions. The collection also contains significant material of Palaeozoic stromatoporoids described by H. A. Nicholson, and Mesozoic stromatoporoids by R. G. Hudson.

SCIAPHILIC SPONGE ASSEMBLAGES IN A SUBMARINE PASSAGE ON SW COAST OF DUGI OTOK ISLAND (CROATIA, ADRIATIC SEA)

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The Croatian Adriatic coast is mostly formed by karstified limestone and a number of submerged karst phenomena (e.g. caves) could be find there. Certain areas are especially rich in such phenomena like some parts of SW coast of Dugi Otok Island, middle Dalmatia. Among various submerged karst phenomena there, a simple shallow submarine passage near Brbinjscica cove was chosen as a model for studying relation between light intensity and sponge assemblages. The passage (44° 03' 21" N; 14° 59' 13" E) lays along the coast in SE to NW orientation and is 45 m long, 8 to 12 m deep and 6 to 10 m wide. There is an opening (15 x 6 m) at the top of the middle portion of the passage. Noticeable current is present in it almost all the time. It can be assumed that temperature oscillations and current velocity (availability of food) are homogeneous throughout the passage. In order to quantify the area covered by sponges, photos of 34 squares (40 x 40 cm) were taken and subsequently analyzed with ImageJ program (public domain Java image processing program inspired by NIH Image program). Samples for spicule preparations were taken also. Intensity of light was measured using a stowaway light data loggers (Onset Computers). Among 213 identified benthic species, 44 species of sponges were recorded in the passage. Four distinct biological zones were identified: biocoenosis of photophilic algae (not taken into account in this work), pre-coralligenous aspect of the coralligenous biocoenosis (PC), coralligenous biocoenosis (C), and biocoenosis of semi-dark caves (SDC). In squares characterized as SDC, sponges covered 22-60 % of the substrate, while in those characterized as C (algal cover 70-92 %) they covered 0-15 %. Although many encrusting sponges were present, in the biocoenosis of semi-dark caves massive and erect forms dominated. This can be related to ample food availability throughout the passage. In the area covered by the coralligenous biocoenosis, maximum recorded light intensity at noon of a clear summer day was 1 Lum/m^2 while in the area covered by the biocoenosis of semi-dark caves it was 0.8 Lum/m^2. It was noted that transition between C and SDC is not gradual but abrupt. Since there is not a big difference in maximum light intensity between these two biocoenoses, it might be supposed that the total annual light energy received plays a key role in determining algal distribution which can directly influence the sponge distribution in the dark areas of the passage.

DENSITY MEASUREMENTS FOR BIODIVERSITY STUDIES

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Studies on the sponge *Polymastia janeirensis* (Boury-Esnault, 1987) were conducted at an average depth of 3 m on the southeast side of Praia Brava, Buzios, Rio de Janeiro, Brazil during March 4-6, 2001. The sponges measured an average of 17.5 cm x 23.9 cm (circular in shape) and their distribution was very slightly aggregated (Morisita Index: 1.13). Densities of *P. janeirensis* were as follows: actual count – 1.7/m^2; stratified random sampling – 5.8/m^2; point center quarter – 1.2/m^2; 3rd nearest neighbor – 1.6/m^2; Weinberg – 1.2/m^2; Strong-Eberhardt – 2.0/m^2; and Nishiyama medium – 1.1/m^2. The most accurate method of estimating densities was the 3rd Nearest Neighbor.

PALAEOENVIRONMENTAL AND PALAEOECOLOGICAL INTERPRETATION OF THE EARLY JURASSIC PORIFERA-BEARING FACIES (UMBRIA-MARCHE AREA, NORTHERN APENNINE, CENTRAL ITALY)

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The new finding of Lower to Middle Lias spongolithic facies and spicolithic levels is reported from the transitional lithotypes comprised between the top-most Calcare Massiccio Unit and lowermost Corniola Unit in the Umbria-Marche Appennine area.

The sponge facies are not continuously widespread and their thickness is extremely variable from each outcrop to the others. The analysed outcrops belong to the northern Monte Nerone palaeohigh, the Monte Catria, the Monte Cucco, the Monte Sasso di Pale and to the southern palaeohigh of the Monti Martani, the Monterivoso and the Castelsantangelo sul Nera area.

The more rich and varied spongolithic facies come from the condensed and reduced sections (types 4 and 5, Colacicchi *et al.*, 1988). The Demosponges, especially with the boring genus *Aka*, and the Calcarea dominate the assemblages, while Hexactinellids are less numerous. The sponge bodies are generally stacked and in some cases four generations at least are identifiable. Substrate lithotypes are variable from packstone to wackestone with micrite matrix containing spicules, radiolaria, foraminifers, gastropods, echinoid and rare ammonite fragments; the lithologies are depending on the sponge types.

In both lithologies of the Porifera bio construction, tuberolithic structures (tuberoid micrites) and laminated algae are present while calcareous algae are rare or absent.

The spongolithic facies shows clear evidences of reduced depth (0-15 m), with quiet and warm water and normal oceanic salinity, light and temperature.

It is possible to identify, on the basis of the assemblages found, three different lithofacies:

massive spongolithic facies - characterized by Demosponges and Calcarea and subordinate sponge boring (*Aka* genus);

tuberolithic-spicolithic facies - characterized by tuberoids, Calcarea and sponge boring (*Aka* genus);

spicolithic facies - characterized by common spicules into a micritic mud and smol Hexactinellids.

The sponge growth is referable to mound and meadow morphologies. These morphologies are correlated with the substrate types and the hydrodinamic energy; the mound is typical of firm ground substrate and moderate water energy, while the meadow is typical of soft ground substrate and very low water energy. The sponge bio construction extension is very restricted in time and space. Three hypotheses are proposed to justify the sharp growth break: 1- a variation of the physical-chemical parameters, 2- an increase of shale amount into the seawaters, 3- a disease induced by bacterial attack promoted by an increase in water temperature.

UNUSUAL STEROL COMPOSITION AND CLASSIFICATION OF THREE MARINE SPONGE FAMILIES

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In contrast to the terrestrial sterols, sponge sterols show complex variations in both the tetracyclic nucleus and the side chain. Over 100 unprecedented sterols have been found to date from marine sponges. Sponges, which occupy a low position in the evolutionary scale, are difficult to classify due to to the few available useful morphological characteristics. Thus, they often pose a difficult identification task for systematists.

We report the major results of our systematic comparative biochemical study on sterols from three sponge families, namely Suberitidae, Halichondriidae and Axinellidae. All Suberitidae species investigated to date contained 80-95 % of 5α-stanols, mainly cholestanol (60-74 %). Three *Collocalypta* species (Halichondriidae) contained four quite unusual 24-isopropyl and 24-isopropenyl Δ5 sterols accounting for 70-80 % of the total sterol mixture, mainly 24-isopropylcholesterol (41-59 %). The Saudi Red Sea sponge *Acanthella carteri* (Axinellidae) contained mostly very uncommon A-nor-sterols associated with common sterols at low level. A-nor-sterols have been found in sponges almost only from the family Axinellidae.

Several interesting exceptions to these results will be given and that pose the question of the taxonomic status of the corresponding sponge species. The sterol mixtures were analysed by gas chromatography coupled to mass spectrometry. Several sterols were isolated by HPLC and identified by NMR. These results, confirming previous data obtained in other laboratories, may provide useful chemotaxonomic criteria for the classification of these sponge families.

A CHEMICAL ECOLOGY OF SPONGES IN CULTURE

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The concept of culturing sponges in the sea for economic return of biologically active metabolites should find increasing favor as a reliable method by which to produce hard to synthesize compounds with medical applications.

There is a need to grow biomass fast and efficiently while maximizing the biosynthesis of target compounds. Most sponges do not maintain constant levels of biosynthesis of biologically active compounds. They are presumably metabolically expensive for the sponge and or its symbionts, to manufacture and appear to be biosynthesized on demand in response to cellular challenge.

To succeed in the culture of sponge species, specifically for production of one or two metabolites with desirable biological activities, there is a need to identify high yield stock and to understand what may influence variability in biosynthesis.

The chemical ecology of sponges in culture is contrasted with that of their "wild" donor populations, to explore how explants respond to culture conditions over a range of latitudes, depths and embayment systems. Experiments utilizing *Lissodendoryx* sp. n. which produces the Halichondrins are detailed. Biosynthesis of nine Halichondrin metabolites varies seasonally, with depth, across small distances within bays and over large spatial ranges. Total Halichondrin content is however relatively consistent in time and space, and follows similar seasonal patterns when wild populations at 100 m depth are compared to cultures at 10 m depth grown over 300 km away. This pattern appears to follow cycles of reproduction and seasonal biomass regression. Quantitatively, Halichondrin profiles are inversely related to sponge condition. Halichondrin yields can be enhanced by an order of magnitude during serial cloning, suggesting a defensive response to damage. Halichondrin profiles also vary in response to the type of fouling the sponge experiences, suggesting a differential biosynthetic response to different stimuli. Cell separation experiments suggest that the sponge, rather than symbionts, is indeed responsible for biosynthesis of these compounds. The sensitivity of the sponge to microenvironment as evidenced in variation of metabolite chemistry, suggests that locality for culture will be an important factor in successfully producing target compounds. There is evidence that cultured sponge may be manipulated to enhance yields of desirable compounds.

THE SPONGE INDIVIDUAL - AN INTEGRATOR OF LONG TERM BIOPHYSICAL ENVIRONMENTAL EVENTS

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The size and shape of sponges, and indeed that of many benthic sedentary invertebrates, reflects the prevailing biological and physical microenvironment. There have been a number of modeling studies which demonstrate this, but there is a need to "ground truth" these studies in the field.

We examine the patch dynamics of an encrusting community characterized by sponges on a variety of reef walls including caves and archways. In a natural experiment utilizing the dramatic subtidal cliff-face topography of New Zealand's Poor Knights Islands, we examine the patterns of patchiness of sponge, ascidian and bryozoan assemblages. Vertical reef walls are so prevalent that it is possible to sample replicate cave, archway and open wall systems all with the same alignment. By examining species assemblage and individual patch sizes of organisms along and within these reef features, and by correlating observed community dynamics with relative measures of light, exposure and wave shock, insight is gained into the driving forces organising community structure. This is in terms of the demography of a species across a "preferred" distribution range, and in terms of the species' ability to compete for space near the edges of its range, in a changing neighbourhood, as other species from other phyla increase in dominance. How species gain or loose advantage depends on how well they cope with physical disturbance, contrasted with how well they feed or photosynthesise compared to their neighbours. They integrate these environmental conditions and reflect these forces in their structure and in the integrity of the species assemblage. Shifts in these parameters can be sensitive indicators of shifts in environmental conditions.

THE HYDROZOA SYMBIOTIC WITH PORIFERA: A REVIEW

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Many species of benthic marine organisms use sponges as temporary or permanent substratum, refuge or nursery, due to their massive body permeated by a complex canal system, and to their current-inducing filter-feeding activity. Thus, a complex associate fauna thrives on and/or inside sponges, showing a wide range of relationships with their hosts.

In this paper all the cases described of sponge symbiotic relationships with Hydrozoans are reviewed, together with new unpublished observations. The main steps of these symbiotic relationships may be summarised as follows: (i) polyps are simple epibionts of sponges, their stolons running on the sponge surface; (ii) stolons grow inside the sponge tissue and polyps emerge from the sponge surface; (iii) stolons grow inside the sponge tissue and polyps emerge from the sponge surface but they may be retracted inside the sponge; (iv) stolons and branches develop deeply inside the sponge body and produce an accessory skeletal network for supporting the sponge growth.

SPONGE ECOLOGY OF MESSIOUA BANC (TUNISIA)

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In order to study the benthic ecology of the "Messioua banc" (South of Tunisia), a traditional Tunisian sponge fishing ground, four marine campaigns were conducted with the R/V "Hannibal" from October 2000 to August 2001. The objectives of three of these campaigns were field validation, focusing on the sampling of the sponge population and the Posidonia oceanica meadow. However the goal of the fourth one, with the use of a multi-beam sonar SEABAT 9001 (Reson Co.), was the mapping of the surveyed area bottoms and the scanning of the Posidonia meadow and its sponge population.

Sixteen stations were studied, allowing a total sampling area of 240 m² for the analysis of distribution, composition, abundance and structure of the sponge community.

A list of 27 species of Demospongiae, represented by 973 individuals, was considered. Species richness of the sponge population was low compared to sponge communities from other Tunisian areas. The abundance of species, ranging from 6 to 70 individuals, is higher in rocky-paved bottom stations and 44.62 % of the total abundance is constituted by individuals of Crambe crambe, Hamigera hamigera, Phorbas sp., Cliona viridis, C. celata, Ircinia fasciculata and Sarcotragus muscarum. The occurrence's frequency was equal or higher than 50% for a whole group of 25 species, while the lowest values were those of the two remaining species i.e. Aplysina aerophoba and Chondrosia reniformis. The structure of the sponge community (species richness, species diversity and evenness) shows that evenness values, high and constant along 15 stations, reach their lowest value in the deepest sampling station at 33 m depth. Therefore there is no species domination within the sponge community of Messioua banc. Meanwhile, species richness and diversity (Shannon-Weaver, Simpson) are higher in the Posidoniao ceanica meadow, but no clear indications exist on whether these indexes are linked with the meadow density. Furthermore, if compared to each other, Simpson's index of diversity shows a higher amplitude of variability for the sponge community of Messioua banc.

Commercial sponge species (Hippospongia communis, Spongia officinalis and S. nitens) were found at a depth lower than 29 m, on Posidonia oceanica rhizomes, but there is no correlation between their abundance and the density of the seagrass meadow. Furthermore their highest abundance value was found in a station where the bottom is a mosaic of paving dales and Posidonia patches.

OXFORDIAN SPONGES FROM THE NEUQUEN BASIN, SOUTHERN MENDOZA, ARGENTINA

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An Upper Jurassic association of siliceous sponges is reported from the Neuquén Basin, southern Mendoza Province, at the Río Potimalal section. This Late Jurassic carbonate complex of the Neuquén basin displays characteristic facies and biological associations. One of these facies consists of small siliceous sponge build-ups of moderate diversity. They are well developed at the Río Potimalal section, where wackestones and massive sponge-bearing micritic limestones represent the La Manga Formation (*plicatis* Zone). The sponges are fossilized in their original shape and exhibit calcareous preservation.

A preliminary approach to the association of fossil sponges has allowed us to determine that a high percentage of these specimens belong to the Class Hexactinellida, Subclass Hexasterophora, Order Hexactinosa (*Laocoetis* sp. *Cribrospongia* sp., *Ordinatus* sp., *Linonema calyx*) and to the Order Lyssakinosa (*Poligonatynm* sp.). The majority of the sponge specimens belong either to *Cribrospongia* or *Laocoetis* and so correspond to the Family Cribrospongiidae or Craticulariidae. The dominant growth forms of the Hexactinellids are tubular, cylindrical and cup shaped. Additional benthic faunal elements are bivalves, serpulid worms, bryozoans and echinoids.

The Oxfordian sponge-bearing rocks of the La Manga Formation were deposited in outer shelf to slope settings characterized by low energy. In addition, favourable conditions for siliceous sponge colonization and development existed, including very low sedimentation rate, available hard substrates and sufficient nutrient availability.

The sponge-bearing Upper Jurassic carbonates are interpreted as deposits of a transgressive to early high stand systems tract, similar to many other Oxfordian basins throughout the world, where sponge faunas have developed under similar conditions.

VARIATION IN CORALLINE SPONGE ARAGONITIC SKELETON PB PROFILES: PHYSIOLOGICAL AND/OR ENVIRONMENTAL INFLUENCES ?

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To assess the robustness of the skeletal record of coralline sponges to study climate variability in the Tropical Ocean, lead concentrations were measured along the growth axis of the aragonitic skeleton of three samples of *Ceratoporella nicholsoni*. Specimens were collected off Jamaica and the Bahamas and analysed using inductively coupled plasma-mass spectrometry on dissolved powders.

A lead increase between 1930 and 1970 (already demonstrated in scleractinian corals, ice cores or sediments and linked to the atmospheric lead pollution) is shown. However, absolute maximum lead concentrations vary between the three specimens (0.91 ppm, 1.25 ppm and 2.1 ppm), either due to different growth rates, variations in physiological processes during uptake or environmental influences. Therefore, the aragonitic skeleton may not truly represent seawater in terms of elemental composition. The relationships between elements laid down within the aragonitic skeleton of *C. nicholsoni* with respect to concentrations of elements present in seawater are investigated. The uptake pathway of dissolved trace elements into the living tissues, the subsequent incorporation of elements into the aragonitic skeleton, and the final relationship between precipitated elements in the skeleton and free elements in seawater are explored.

LIPID SPECIFICS OF THE HEXACTINELLIDA AND THE DEMOSPONGIAE - EVIDENCE FOR A CLOSE PHYLOGENETIC INTERRELATIONSHIP?

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(i) Hexactinellid sponges display a fossil record up to the late Precambrian (e.g. Brasier *et al.*, 1997) and are often considered as the most ancestral metazoans (Müller, 1997). However, their phylogenetic position within the phylum Porifera is still under debate. Lipid biomarkers from 23 species were studied for information on their phylogenetic properties, particularly their disputed relation to the two other sponge classes (Demospongiae, Calcarea) (Thiel *et al.*, 2002). The most prominent lipid compounds in the Hexactinellida comprise C_{28} to C_{32} polyenoic fatty acids. Their structures parallel the unique patterns found in demosponge membrane fatty acids ($\Delta^{5,9}$ long chain fatty acids, "demospongic acids") and strongly support a close phylogenetic association of the Demospongiae and the Hexactinellida. The lack of these features in calcareous sponges further contradicts the still common view that Calcarea and Demospongiae are more closely related to each other than either is to the Hexactinellida (Thiel *et al.*, 2002).

In addition, the steroid compositions of hexactinellid sponges were investigated (Blumenberg *et al.*, 2002). Most of the species contain cholest-5-en-3β-ol (cholesterol) and/or its saturated derivative 5α(H)-cholestan-3β-ol, along with their C-24 alkylated homologues. Where 5α(H)-stanols are present, they regularly co-occur with their 3-keto analogues, components which are very rare in nature (Guella *et al.*, 1988). The steroid concentrations generally decrease with increasing carbon numbers, similar to sterol distributions typically deposited in marine sediments (Gagosian *et al.*, 1982). These features argue against *de novo* sterol biosynthesis operating in hexactinellid sponges. Rather, we suggest a dietary uptake of Δ^5-stenols and their stereo selective transformation via 3-keto intermediates.

(ii) Studies on lipid biosynthesis are generally achieved by ^{14}C incorporation experiments (Raederstorff *et al.*, 1987) and are - due to the analytical complexity - very limited. In order to overcome these limitations, we proved gas chromatography isotope-ratio mass spectrometry (GC/C-IRMS) of specific lipid compounds as a tool in that field of experimental work. As a first result, $^{13}C/^{12}C$-ratios of brominated and non-brominated counterpart fatty acids in the demosponge *Phakellia ventilabrum* support the common view of bromination as the last step of phospholipid biosynthesis.

EVOLUTIONARY ECOLOGY OF THE ENDEMIC PORIFERAN FAMILY LUBOMIRSKIIDAE AND THE RECONSTRUCTION OF THE PALAEOECOLOGICAL DEVELOPMENT IN LAKE BAIKAL BASED ON RECENT SPONGE ASSOCIATIONS

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In Lake Baikal the endemic sponge family Lubomirskiidae are the most important part of the benthic community (biomass > 1 kg/m², Kozhow 1963). Therefore, their ecological relevance is particularly high and sponge spicules also contribute considerably to the fossil fauna within the sedimentary record. We here present especially the methods how we are currently working in order to find out more about the present distribution and the environmental conditions which might have led to the diversification of the Lubomirskiidae. Most of the field-work has been carried out by scientific SCUBA diving mainly in the southern basin of Lake Baikal:

-digital mapping of sponge associations in well-defined 30 x 30 cm plots by SCUBA diving and sampling;

-photo/video, digital evaluation of deeper areas. We evaluate mainly population density and growth morphology in dependence on substrate and light (e.g. green sponges have been observed down to 70 m water depth);

-photogrammetric measurement of *Lubomirskia baicalensis* to determine an annual growth rate and the development of the organism;

-transfer of selected sponges to different light- and depth-conditions to observe them and their reactions to the changed habitat.

Reproduction seasonality and the different types of symbionts are investigated by histological techniques.

Detailed documentation and classification of the types of skeletal architecture and spicule forms allow the differentiation of form groups within the Lubomirskiidae species published so far. Whether or not these form groups or some of them should be regarded as different species, depends on the morphological variation range of each of the known taxa and on the results of molecular systematic investigations in progress.

Recent results from sequences of the mitochondrial COI gene point towards the monophyly of the Lubomirskiidae (Itskovich *et al.* 2002, Müller *et al.* 2002). Our working group sequenced a larger rDNA cluster of ca. 1500 bases, including the 3-end of the 18S, the ITS I and II and the 5- end of the 28S. This analysis was performed for 16 species altogether, including 12 Lubomirskiidae, 3 Spongillidae and 1 Potamolepidae. Our diagram based on the 28S rDNA reveals that the Lubomirskiidae are paraphyletic in origin with part of them forming the sister group of the Spongillidae/Potamolepidae.

However, the form groups help in the classification of particularly the fossil sponge spicules found in sediments. These siliceous spicules are abundant and well preserved so the potential to use the distribution of spicules in the stratified sediments for palaeo-environmental reconstructions is obvious, especially in the sedimentary record of Lake Baikal where no calcitic fossil legacies exist. The variation of fossil spicule associations in time has proven to be a very significant palaeo-ecological indicator (Weinberg *et al.* 1999). The quantitative analysis of spicule abundance for each taxon or form group in closely distributed sampling layers of sediment cores (BDP '96 and '98, provided by Dr. T. Kawai) compared with the extant distribution of Baikal sponges helps illuminate the phylogenic and evolutionary development of the endemic Lubomirskiidae since the Miocene.

THE SYSTEMATIC POSITION OF *ALECTONA* (PORIFERA, DEMOSPONGIAE), A MEMBER OF THE ORDER ASTROPHORIDA

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The long standing problem of the affinities of the genus *Alectona* Carter, 1879 and some related excavating sponges has been investigated by using 18S and 28S rRNA sequences and by SEM study of the boring pattern. The sexual development of genera *Alectona* and *Thoosa* Hancock, 1849 displays unique features among Porifera, with a hoplitomella larva showing many peculiarities, including absence of flagella, long planktonic life and special larval spicules disappearing in the adult. The larval spicules of the hoplitomella of *Alectona* include an armor of discotriaenes, clearly indicative of tetraxonid affinity. This character, however, is not shared by *Thoosa*, in which the hoplitomella armor is made of discs derived from monaxonid spicules. Although the adult spicule complement, lacking tylostyles and possibly true megascleres, and including nodulose amphiasters similar to the amphiasters of the Clionaidae *Cliothosa* Topsent, 1905 is difficult to interpret, there is a general consensus for the classification of these sponges in the order Hadromerida, either in family Clionaidae or in a family of their own, Alectonidae or Thoosidae. However, they have been sometimes considered as "intermediary" between Astrophorida and Hadromerida, and Alander (1942) even classified both genera *Alectona* and *Thoosa* in tetractinellid sponges. Rützler (in press) has recently proposed for the Systema Porifera a revision of these excavating genera based on skeletal characters, in which *Alectona* and *Thoosa* are separated in two families, respectively Alectonidae and Clionaidae, whereas *Cliona levispira* (considered as a junior synonym of *Spirozyga heteroclita*) is considered as a new member of Alectonidae.

From our RNA sequences it clearly appears that *Alectona millari* is related to Astrophorida, hereby confirming that the larval discotriaenes are not a secondary acquisition. Although sequences could not be obtained yet from *Thoosa*, the presence of hoplitomella larva and the adult spicule complement both strongly indicate affinities with *Alectona*. This is confirmed by the presence in both genera of a peculiar ornamentation of the excavated pits, including radiating lines in complement to concentric rings that are absent in other excavating sponges. RNA sequences confirmed by SEM study of the boring pattern also unambiguously indicate that *Cliona levispira*, whose synonymy with *Spirozyga heteroclita* is questioned, is related to Clionaidae.

It is suggested to classify the family Thoosidae, restricted to *Alectona* and *Thoosa*, with the possible addition of the poorly known genus *Delectona* de Laubenfels, 1936 - whose discs may be the armor of hoplitomella larva- in the order Astrophorida. These sponges are interpreted as Astrophorida that have lost more or less completely true megascleres and tetraxonid spicules in their adult stage. Tetraxons are conserved in *Alectona* as larval spicules constituting a special armor, whereas in *Thoosa*, and possibly in *Delectona*, the armor has a monaxonid origin.

THE PRESENT STATE AND FUTURE PERSPECTIVES IN MOLECULAR NATURAL HISTORY OF SPONGES

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In this review we will discuss the recent contributions of genetics to our understanding of the biology and evolution of sponges.

Are sponges animals?

After several centuries of controversy, there is a consensus, now, that sponges are metazoans. Spongologists had demonstrated over a century ago the metazoan nature of sponges, but due to their simple organisation and their plasticity, not all biologists accepted this and, indeed, some textbooks still describe this issue as controversial.

Recently, phylogenetic reconstruction using Hsp70 sequences indicate that animals are closely related to choanoflagellates. Consequently, the old hypothesis of James-Clark (1866, 1868) about the homology of choanoflagellates and choanocytes is supported. These phylogenetic results suggest that choanoflagellate-like structures should not be considered a synapomorphy of the Porifera since they may be plesiomorphic for Metazoa.

Is the phylum Porifera monophyletic or paraphyletic?

The phylogenetic relationships among classes, orders or families of sponges still remain too confusing to answer this question. Three main clades are presently recognised, Hexactinellida, Demospongiae and Calcarea. Several recent papers, using proteinase kinase K and full length 18 S rDNA sequences, hypothesise that Calcarea may be the sister-group of Cnidaria and Ctenophora and consequently should be considered as a separate phylum. This hypothesis needs to be confirmed by other molecular markers. Hexactinellida could be either the sister-group of Demospongiae (spicules with axial filament secreted intracellularly) or the sister-group of all other Metazoa or, still, be placed in a basal position of a poriferan monophyletic clade. These two last hypotheses seem to be less supported.

Are the currently accepted Classes supported by molecular data?

Within the Hexactinellida too few sequences are available to allow any hypotheses to be drawn about their internal classification.

Within the Calcarea molecular data confirm the hypothesis of Bidder and Minchin of two monophyletic clades: Calcinea and Calcaronea. This particular point will be discussed by Michaël Manuel in his intervention.

Within the Demospongiae the phylogenetic hypothesis made by Lévi based on morphology and embryology have been under dispute for the last 20 years. Recent morphological and molecular data both support the monophyly of sub-class Homoscleromorpha. However, the distinction of the other two sub-classes of Demospongiae: Tetractinomorpha and Ceractinomorpha, based on an oviparous versus viviparous reproduction, has been rejected by all molecular phylogenies produced so far. Instead, the Demospongiae seem to be formed by five monophyletic clades unrelated to Lévi's previous classification.

Are there true cosmopolitan sponge species?

Until very recently, it was accepted by systematists that sponges had extremely high levels of phenotypic plasticity. That, together with the fact that many species were defined, sometimes, by single diagnostic characters, resulted in many species being reported worldwide, regardless of any existing knowledge on their larval dispersal. In the mid 80's, when molecular markers started to be used for sponge alpha systematics, it became clear that most of those "cosmopolitan" sponges were, in fact, groups of evolutionary distinct species. Thanks to those results, most sponge taxonomists now would be very reluctant to assign unidentified specimens from one ocean to species described in another.

Can sponge populations be homogeneous over large areas?

There have been few studies of sponge population genetics. Most sponge species studied to date have shown a rather small capability for long-range dispersal. This indicates that sponge larvae, both from viviparous and oviparous species, do not disperse very far. One exception was the viviparous *Chondrosia* sp. from the Western Atlantic, whose populations showed a remarkable homogeneity over 8000 km. Although rafting has been suggested as a possible means of dispersal in other species, its effectiveness for gene flow has never been tested through the use of molecular markers.

How important is asexual reproduction for the establishment and maintenance of sponge populations?

It is a well-known fact that sponges can reproduce asexually. However, it is yet not clear how much of a sponge population is made of clone-mates, i.e. what is the proportion of genetically unique (=genets) and genetically identical (=ramets) individuals in sponge populations. Graft acceptance/rejection experiments indicate that asexual reproduction can be highly important in sponges. However, since the number of genes involved in graft rejection and the mechanism of historecognition are not known, it is unclear how accurate grafting experiments are for estimating the extent of asexual reproduction in sponge populations. Allozyme studies indicate that sponges collected 2 m apart can be clone mates, but, on that scale, ramets do not account for more than 10 % of the individuals studied. Nonetheless, there are no published studies, to date, where carefully mapped sponge individuals were compared, on different scales, using molecular markers. Obvious candidates for such studies are allozymes and microsatellites, and anonymous polymorphic nuclear markers such as RAPDs and AFLPs.

Do true allogenic chimeras exist in sponges?

Some reports (mostly anecdotal) indicate that larval fusion can occur in sponges. However, even in the cases where fusion was demonstrated (like in *Crambe crambe*), it was not clear whether the fused individuals were genetically different. Allogenic chimeras have been described in bryozoans and colonial ascidians, but in those organisms individual polyps could be identified and attributed to different genotypes. However, unlike what would happen to any other colonial organisms, allogenic fusion in sponges could result in a complete cell mixing between the contributing genotypes. This would result, in allozyme and microsatellite studies, in high heterozygote excesses, which have not been reported to date. Yet, even if they were found, such excesses would only give an indirect evidence of larval fusion. Recent molecular techniques, like *in situ* PCR, make it, now, possible to determine the fate of the individual cells in a sponge chimera.

AN EXCEPTIONALLY PRESERVED LYSSACINOSAN SPONGE FAUNA (PORIFERA, HEXACTINELLIDA) FROM THE UPPER CRETACEOUS (CONIAC) OF BORNHOLM

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The island of Bornholm in the Baltic Sea is a horst within the Fennoscandian Border Zone, which represents the marginal area between the stable Precambrian Baltic Shield and the subsiding late Palaeozoic-Mesozoic Danish sub basin. The northern part of Bornholm consists of Precambrian basement. The southern and western parts of the island consist of Palaeozoic and Mesozoic rocks in the form of down-faulted blocks. The lyssacinosan Hexactinellida occur only at the type locality of the Arnager Limestone Formation (Coniac), which is exposed in a small stripe on the south coast immediately to the West of the town of Arnager. The preserved two-dimensional sponge fauna is largely comprised of lyssacinosan Hexactinellida, which consist predominantly of Rossellidae. The preservation of these non-rigid sponges is normally very poor, because of the lack of a rigid skeleton. Without a rigid skeleton the sponge disintegrates as soon as the soft parts decay and, therefore, only isolated spicules can be found. The good preservation of these non-rigid and fragile Arnager sponges is the result of a fast sediment covering and a bacteria-induced pyritization of the spicules, which took place already during the decay of the just covered sponges. The palaeoenvironment's characteristics were probably a relatively low level of sedimentation and lack of turbulence, which are preconditions for the settlement of the described Hexactinellida. This state of environment was altered by rapid sedimentation events caused by episodical mudflows. Difficulties in the classification of the lyssacinosan Hexactinellida -both in general and in this particular case- result from the use of different systems in the classification of recent and fossil lyssacinosan sponges. The most important tool for the classification of recent lyssacinosan (and other) Hexactinellida are the microscleres. This type of spicules, however, is normally not preserved in the fossil record, and in the unusual case of such preservation, the microscleres are found only isolated in the sediment. Due to the general absence of microscleres, the megascleres and the external habitus are the most important criteria for classification of fossil sponges. Therefore, the comparison of the lyssacinosan sponges from the Arnager limestone with recent representatives by analysing the skeletal architecture is attempted. This includes the occurrence, distribution, sizes and pattern of megascleres, particulary the choanosomalia, hypodermalia, lateralia and basalia. The assessment and analysis of their general form, fixation and external wall structures (internal/external openings, small elevations) are equally important. However, the two-dimensional preservation of the Arnager sponges and the pyritization of the spicules add to the difficulties of comparison and classification.

AN EXCEPTIONAL SPONGE FAUNA (HEXACTINELLIDA) FROM THE SEPTARIENTON (RUPELIUM, OLIGOCENE) OF BAD FREIENWALDE (NE-GERMANY): PALAEOECOLOGIC AND TAPHONOMIC IMPLICATIONS

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Exceptionally well-preserved hexactinellid sponges were found in the Septarienton (Rupelium, Oligocene) of a play pit near Bad Freienwalde (NE-Germany). The fauna is of moderate diversity, but with high individual numbers. It consists of amphidiscophoran, as well as hexasterophoran species. *Asconema oligocaena* n. sp. (Hexasterophora, Lyssacinosa), *Aphrocallistes* sp. (Hexasterophora, Hexactinosa) and *Hyalonema* sp. (Amphidiscophora). *Asconema* and *Aphrocallistes* show a high plasticity in body form, which seems to be linked with environmental parameters. The three-dimensional, pyritic preservation suggests specific embedding and preservation conditions, the fast embedding is assumed to have been caused by mudflows; second, early diagenetic processes in the anoxic microenvironment are considered responsible for the special kind of pyritic preservation. The palaeoenvironment of the sponges is reconstructed as a shallow shelf -possibly in the distal range of a delta. Sedimentological observations and the low diversity of the comparably small sponges indicate a slightly restricted, maybe temporarily dysoxic, environment. This was characterized by relatively low levels of sedimentation and turbulence -disturbed only by mudflows- which is a precondition for the settlement and body-preservation of lyssacionosan hexactinellids. The occurrence of Hexactinellida in an environment shallower than it is usually the case, has several reasons. First, relatively cool water offers good living conditions for hexactinellid sponges. Second, the soft sediment with small hardgrounds (e.g. shelly material) offers good settling conditions for sponges adjusted to soft grounds. Third, the relatively low diversity of the sessile benthos caused only little ecological pressure for the slow growing sponges.

MAINTENANCE AND GROWTH OF SPONGES IN AQUARIUMS: FUNDAMENTALS FOR *IN VITRO* CULTIVATION APPROACHES FAR FROM THE SEA

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During the last years, research efforts on cultivation of marine sponges have increased noticeably. There are at least three main reasons:

1. Sponges have been recognised as a rich source of natural products of potential pharmaceutical value. The bioactivity of the isolated substances includes antiviral, antitumor, antimicrobial or in general cytotoxic properties and is, therefore, of considerable biotechnological interest;

2. For the development of new methods for *in vitro* cultivation of sponge cells viable sponge material is essential. Especially, laboratories far from the sea are strongly dependent on this supply;

3. The biomass for bioassays in the laboratory range might be obtained via wild harvests in a responsible extent. Enormous quantities of sponges, however, for pre-clinical tests will be required. Wild harvests to such an extent will not only have negative effects on the ecology of the sampling area, it also will be a massive offend against the precept of sustainable use of natural resources given by the Agenda 21 of the Rio Conference in 1992 and by the Convention of Biological Diversity.

In our laboratory, therefore, research has focused on the development of the cultivation of functional sponges from the Mediterranean Sea in aquariums under controlled conditions.

However, before bringing sponges into an aquarium, knowledge of their living conditions is required. From this closer look to the ecology we got the values of e.g. salinity, temperature, light conditions and the substrate sponges growing on. To avoid any damages to sponges we optimised our sampling and transportation methods as well as the feeding strategies for the maintenance of the sponges in the aquarium.

All these data allowed us to install an aquarium using artificial seawater with the control of temperature, salinity, light, currents and soluble organic matter.

So far we tested 22 species from the Mediterranean Sea. Most of them (14 species) can be maintained in the aquarium for several months or even years and for five species growth was observed.

From our results we conclude the absolute need of detailed ecological knowledge of the habitat conditions for the species designated for cultivation as well as the ability to simulate these parameters in an aquarium or in a bioreactor system respectively.

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OVERVIEW ON THE SPONGE FAUNA OF THE LIMSKJ CANAL, CROATIA, NORTHERN ADRIATIC SEA

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The Limskj Canal is an 11 Km long fjord near Rovinj, in the Istrian peninsula. Its position is peculiar since it lies along the E-W axis, with the entrance at the West side, so that the two parallel coastlines of the fjord face one South and the other North. The fjord is in a carstic area and presents evident erosive phenomena. The bottom of the fjord is mainly detritic with only few relatively large rocks, although a *trottoir* is present along the S side of the canal. From 1979 the upper half of Limskj Canal, where floating cages fish farms integrated with mollusc cultivation are present is a protected area. The benthic community is mostly represented by different species of Porifera, Cnidaria (especially the scleractinian *Cladocora caespitosa*), colonial and solitary ascidians, bryozoans, mollusks and polychaetes. The observed high sedimentation rate together with the input of fresh water, due to the rainfalls and to the presence of underground small rivers, render the canal an unstable environment, especially in its inner part. We studied the sponge community by means of SCUBA diving surveys, underwater photography and 30 m line transects. Mean densities of the most abundant species have been also calculated. Almost along the whole canal we encountered a *Chondrilla nucula* facies. This sponge species is dominant in the shallow water (between 0 and about 6 m), forming large patches that can cover more than 80 % of the substrate. *C. nucula* is often associated with *Aplysina aerophoba* and different species of *Ircinia*, also abundant in the canal. On the hard calcareous substrate, in shallow waters, boring sponges are also well represented and the bio erosive phenomenon is evident. Differences on the sponge distribution have been observed between the initial part of the canal and the end, between the northern and the southern side.

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ANTI-PREDATORY DEFENSE STRATEGIES OF RED SEA SPONGES VERSUS CARIBBEAN SPONGES: PHYSICAL DEFENSE

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In addition to the commonly used chemical defense mechanism against predation, sessile organisms such as terrestrial plants, soft corals and seaweeds are known to have a physical defense mechanism comprising structural elements made of lignin, $CaCO_3$, silica, etc. Most sponges have siliceous spicules that play a key role as skeletal elements. To date, there has been no evidence to show that these spicules also play a role in defense against predation. It is known that low nutritional value of a prey may make it less susceptible to predation. The siliceous spicules found in sponges are indigestible to predators and so sponges that produce large amounts of these spicules may be less susceptible to predation due to their low nutritional value. In the present research we tested the physical defense of six Red Sea sponge species and six Caribbean sponge species, against predation by the generalist Red Sea wrasse *Thalassoma klunzingeri*. The physical defense of the six species collected in the Red Sea was also tested against predation by the Caribbean wrasse *T. bifasciatum*. The spicules of four out of the six Red Sea sponges deterred predation by *T. klunzingeri*. Two out of the six Caribbean sponges were found to deter predation by *T. klunzingeri*. In assays conducted in the Bahamas on the Caribbean wrasse *T. bifasciatum*, only one Red sea sponge species, *Suberites clavatus*, was found to be physically defended by its spicules. A positive correlation was found between the size of the spicules and their ability to deter predation by *T. klunzingeri*. Only spicules larger than ~250 µm deterred predation. On the other hand, *T. bifasciatum* seemed to be deterred based on reduced nutritional quality resulting from high concentration of spicules in a sponge, irrespective of their size. Crude extract of the Red Sea sponge *Grayella cyatophora* deterred predation by both predators tested, *T. klunzingeri* and *T. bifasciatum*, as well as by the natural assembly of reef fishes in Eilat, as tested in field assays. The combination of *G. cyatophora* spicules and crude extract deterred predation to a greater extent than that observed for each defense mechanism separately. This finding most probably indicates an additive mechanism of defense used by this sponge species.

EXCAVATING MICRO-PATTERNS AS DIAGNOSTIC CHARACTER IN BORING SPONGES

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Eroding sponges produce a series of connected holes and galleries into the calcareous substratum where they live. The cavities are excavated by chemical substances secreted by special cells that are able to remove mechanically the substratum in the form of characteristic, small fragments (chips) that are expelled through the oscula. The boring activity results therefore in typical scars (pits) on the wall of the excavations.

Until now the pattern (shape and size) of holes and cavities have been used as tools to identify a single genus or species of boring sponges while no diagnostic relevance has been attributed to the pitting pattern, the marks left by boring cells on the calcareous substratum.

Aim of this work is to study the differences in the pitting pattern of several species belonging to different genera and families excavating in the same calcareous substrata. In specimens from the Mediterranean Sea, Philippines and Indonesia, we have compared, by SEM analysis, the microstructure of the pits. Several examined cases evidence, sometimes at genus level, a variable micro topography on the pit surface.

These differences in the micro etchings, related to the mechanism of perforation of the etching cells, may have some important applications in ecological and palaeontological analysis of the macro boring communities to distinguish boring sponges groups.

A FACILITATION EXAMPLE INVOLVING SPONGES: GRAZING ON FLESHY SEAWEEDS BY SEA URCHINS ENHANCES *CLIONA VIRIDIS* ABUNDANCE

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Facilitative interaction and indirect competition are believed to be important structuring forces in marine environments despite experimental support has rarely obtained. Here we present the results of a correlation study and a field experiment that examine competitive and facilitative interactions involving sponges, seaweeds and sea urchins in a shallow seaweed-dominated community of the western Mediterranean Sea. For the correlation study, we quantified coverage of fleshy algae, encrusting calcareous algae, and the excavating sponges *Cliona viridis* and *C. celata* in 30 randomly selected quadrats of 3600 cm^2 each. Fleshy algae coverage was recorded and then algae were removed to allow accurate quantification of the subjacent calcareous algae and excavating sponges. The relationships among variables were assessed by regression analysis. The significant associations resulting from the correlation study were tested for cause-effect relationships by means of a manipulative experiment. The experimental design included three treatments, which consisted in quadrats deprived of fleshy algae (intensive grazing effect), quadrats only deprived of sea urchins (fleshy algae effect) and quadrats with 4 sea urchins per quadrat (natural sea urchin effect) (N=10 per treatment). We monitored the absence and presence or sea urchins in the respective treatments and scraped the "intensive grazing" treatment weekly. Treatments were photographed at the beginning and at the end of the experiment (after ca 7 months). Coverage was estimated from pictures by image analysis. Data were analysed by one-way ANOVA.

We found that the abundance of the excavating sponge *C. viridis* was positively correlated to sea urchins abundance and negatively correlated to seaweeds coverage. In contrast, *C. vastifica* was negatively related to *C. viridis*, what suggests competition between both sponges. *C. vastifica* prevailed when erect seaweeds covered it whereas *C. viridis* dominated in seaweeds free zones, probably due to the presence of photosynthetic microsymbionts (i.e. zooxanthellae). *C. viridis* grew faster when sea urchin and artificial grazing removed seaweeds. We interpret this outcome as a result of increasing light availability in the absence of fleshy seaweeds, which favours the primary production of the symbiotic zooxanthellae.

THE SEASONAL RELATIONSHIPS BETWEEN ANTARTIC SPONGES AND DIATOMS

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Several sponges host large populations of autotrophic symbionts: cyanobacteria and zooxanthellae in marine species and zoochlorellae in fresh-water species. Frustules of diatoms have been episodically described inside sponge tissues but such microalgae, alive and reproducing, have been widely recorded only in several sponge species from the Antarctic Ocean. In some cases (e. g. *Mycale acerata* and *Scolymastra joubini*) the relationship is species-specific. Diatom assemblages inside sponges are sometimes so abundant to cause a wide necrosis of tissue.

In this work we describe the dynamic of diatom populations, inside the body of some common Antarctic sponges, from November 2001 to February 2002, at Terra Nova Bay (Ross Sea). During November and December, when the pack covers the sea surface, diatoms inside sponges are almost absent; ther number drastically increases in January, concomitantly with the ice melting and the phytoplancton bloom in the water column. In this period the cell number reaches several tens of millions per g of sponge wet weight. At the end of February the amount of diatoms starts to decrease. Qualitative analysis indicates that only few groups of diatoms are able to penetrate inside sponge tissue.

These data confirm the diatom relationships with Antarctic sponges; put in evidence the seasonality of the phenomenon and suggest some degree of specificity in the selection of diatoms by sponges.

LAGOON SPONGES FROM CARRIE BOW CAY: RELATIONS BETWEEN THEIR FREE-LIVING STYLE AND SEDIMENT INCORPORATION

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Sponges typically live on hard substrata and cannot escape from sediment raining but, thanks to the ability to quickly mobilise their cells, they can easily either remove or selectively take up settled particles. On the other hand psammobiontic sponges must, at the same time, face the clogging by sediments of their aquiferous system and ensuring a stable anchoring in the soft substrata. The latter function is essential especially in shallow waters, which are subject to strong water movement at least in certain periods of the year. In this work we suggest that the incorporation of foreign matter in sponges, a widely diffused but poorly known phenomenon, is an adaptive strategy for sponges living in shallow tropical lagoons. We studied this behaviour in sponges living in the lagoon between Carrie Bow Cay and Twin Cays (Belize). The *Thalassia testudinum* sea grass beds adjacent to Twin Cays lie on a shallow shelf that increases gradually in depth from the shoreline to approximately 1.2-1.5 m, before dropping more steeply towards the lagoon bottom (approximately 7 m depth) about 300 m offshore. The lagoon sediments consist primarily of *Halimeda* sand mixed with fine clay.

In this habitat, we have recorded 18 sponge species showing two different ways of anchoring. A first group anchors by vertical growth, with significant penetration into the unconsolidated substrate. A second group of species rolls on the substratum until strong sediment incorporation produces a gravitational stabilisation of the specimens. During the rolling phase sponges show generally irregular shapes while, when stabilised, they rearrange their body developing a more definite morph. The selectivity of these species towards incorporated sediments has been studied.

The importance of sediment incorporation in sponges is discussed in the light of their ability to colonise both hard and soft bottoms.

MODERN SPONGE REEFS ON THE WESTERN CANADIAN SHELF – AN OVERVIEW

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Hexactinellid sponges of the Subclass Hexactinosa construct extensive reefs on the western Canadian continental shelf. The reefs consist of four discrete complexes of mud mounds or bioherms, and ridges and biostromes up to 21 m in height discontinuously covering 700 km² in area in water depths of 165-240 m. A low diversity assemblage of three species of Hexactinosa through sediment trapping and framework construction builds these bio constructions. The process of framework construction involves the attachment by living sponges to macerated skeletons of dead hexactinosan sponges (see abstract Krautter *et al.*). Other sponge taxa found on the reefs include four species of rossellid sponges, which, while occasionally abundant, do not play a role in reef framework construction. Distribution of the sponge reefs is readily mapped by using a variety of remote acoustic methods including high-resolution seismic, side scan sonar, hull mounted profiling and multibeam bathymetry. These techniques permit rapid recognition of the sponge bio constructions but do not resolve living sponge distribution on the reef surfaces, which must be observed directly by submersible, or remote operated vehicle.

The reefs mantle a low angle, relict glaciated seafloor where sedimentation is negligible and iceberg furrows, created 13-14 kaBP, are exposed at the seabed. The long-term stability of geological and environmental conditions, combined with moderate seabed currents and nutrient rich bottom waters provide the conditions in which the reefs have flourished and expanded for the past 9000 years. Delivery of suspended sediments by bottom currents provides the matrix sediments that encase and protect the siliceous skeletons from dissolution. Association of certain species of fish and invertebrates with the reefs, and differences in biota relative to the adjacent seafloor areas, has been noted.

The ecological relationships of other species with the sponge bio constructions have not been examined in detail.

TAXONOMY OF BRAZILIAN MICROCIONIDAE (POECILOSCLERIDA, DEMOSPONGIAE)

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Over 500 microcionids have already been described, 90 % of which still considered as valid species. There are ca. 150 spp known from Australia, 30 from the Caribbean, 20 from the Antarctic and 10 from Brazil. Our study originated from a faunistic survey conducted in SE and S Brazil during 1996-2001, where 5-10 microcionids were collected. *Clathria* (*Microciona*) *campechiae* Hooper was the most abundant species, observed in 39 out of 59 stations visited in a semi-quantitative assessment of sponge abundance conducted in the São Sebastião Channel area during 1998-1999. The species is easily recognisable by its vermilion-red live colour and its finely encrusting habit (less than 1 mm thick). Sometimes it grows larger than 400 cm². The species had already been recorded from the State of Rio de Janeiro, as *Rhaphidophlus minutus* van Soest. Additionally, we recognised *Artemisina* sp., *Clathria* (*C.*) sp., *Clathria* (*Isociella*) sp. (all from the State of São Paulo) and *Clathria* (*M.*) sp. (from the States of Rio de Janeiro and Santa Catarina), the latter three –which are rare- are likely new species. Another three species which are under study, may increase the number of new species found in the area. Other microcionids known from Brazil are: *C.* (*C.*) *cahpso* Boury-Esnault; *C.* (*C.*) *prolifera* (Ellis & Solander); *C.* (*Thalysias*) *basiarenacea* (Boury-Esnault); *C.* (*T.*) aff. *procera* (Ridley), as *Rhaphidophlus gracilis* (Ridley); *C.* (*M.*) aff. *calla* (De Laubenfels), as *C. calla* (De Laubenfels); and *Artemisina* aff. *melana* van Soest.

ULTRASTRUCTURE OF *MYXILLA ROSACEA* (LIEBERKÜHN, 1859) (DEMOSPONGIAE, POECILOSCLERIDA)

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Marine sponges are important subjects for biomedical studies, as sources of natural products with potential human therapeutic value. In the past few years, a variety of compounds with antibiotic, anti-viral and anti-cancer activities have been isolated from sponges, including a sulphated polysaccharide with anti-HIV activity isolated from *Myxilla rosacea*. Most studies were limited to the identification of the products, lacking an evaluation of their location and function, essential for understanding their biological role and for future biotechnological applications. In this work we present a first preliminary ultrastructural study of *M. rosacea*, in order to obtain data important for understanding sponge physiology, for correlation of chemistry and structure and for taxonomic purposes.

Specimens of *M. rosacea* were collected by scuba diving at the Arrábida coast (Portugal). Immediately after collection, specimens were cut into small pieces and fixed. For light microscopy studies, formaldehyde and Bouin fixed samples were dehydrated, paraffin embedded, sectioned (3-5 mm) and stained with haematoxylin-eosin, periodic acid Schiff (PAS) and Masson's trichrome. For electron microscopy studies, fixed samples were dehydrated, embedded in epoxy resins, sectioned and stained according to standard techniques. Semi-thin sections were stained with toluidine blue. The skeleton of *M. rosacea* is constituted by spicules embedded in a well-developed spongin coat (PAS+, staining green by trichrome) and by localised bundles of collagen fibres in close association with collencytes. A loose extra cellular matrix, compartmentalised by thin cells with long cytoplasm extensions is found between the skeleton elements. It is constituted by granular, PAS-, metachromatic (toluidine blue) material, that stains green by trichrome. The chemical nature of this matrix is unknown. The concentration of symbionts is remarkably low and their localization is restricted to spongin coat or collagen fibres, being absent from the loose matrix. These features are unusual, departing from the more common pattern of a mesohyle matrix packed with a network of collagen fibrils and symbionts that was observed in all other studied species of the same area.

Choanocytes are relatively small cells, with spherical and anucleolate nuclei (3-15 µm) and vacuoles of variable number, size and content. Large amounts of cell coat material (glycocalyx), surrounding the flagellum and connecting the microvilli of the collar were observed. Glycocalyx was also found connecting neighbouring collars.

Archeocytes were detected in the loose matrix. Other mesohyl cells, containing anucleolate nuclei, well-developed Golgi apparatus, endoplasmic reticulum and numerous glycogen-like granules were observed.

TAXONOMY AND DISTRIBUTION OF DEMOSPONGES FROM COASTAL BASINS OF THE WESTERN MEDITERRANEAN SEA

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Several studies have shown that lagoon environments of the Mediterranean Sea can host a rich and diversified sponge fauna. This work focuses on demosponges from some of the most representative Western Mediterranean coastal basins, with the aim of describing their taxonomic composition, spatial and temporal distribution.

Literature data and unpublished observations relative to the following basins have been considered: Faro, Fusaro, Ganzirri, Lesina, Marsala, Porto Cesareo, Tindari and Venezia, located along Italian coasts; Godulla and Karavasta (Albania); Thau (France).

In all, 76 sponge species have been recognized, 57 of which are occasional, occurring in less than 20 % of the studied sites. Seventeen species are present in about 30 % of the basins, where they can form populations richer than those inhabiting deeper marine environments. Most of these species (*e.g. Cliona celata, Geodia cydonium, Irtinia variabilis, Tethya aurantium* and *T. citrina*) are confined to waters with salinity values higher than 30 ‰ (annual mean), whereas *Halichondria bowerbanki* and *Tedania anhelans* can tolerate salinity values of about 17 ‰ and 24 ‰, respectively. Only 2 species (*Hymeniacidon sanguinea* and *Halichondria panicea*) occur in more than 50 % of the basins. They tolerate salinity values of about 24 and 21 ‰, respectively.

No sponges have been found in waters with salinity values lower than 17 ‰ (annual mean) or exceeding 50 ‰ during the dry season.

Ten of the 11 coastal basins studied host demosponges, even if wide differences in species richness and abundance occur. Lagoons with wide communication with the sea generally host a rich and diversified sponge fauna (*e.g.* Marsala, Porto Cesareo, Venezia) while in coastal ponds (*e.g.* Lesina, Tindari) the sponge fauna is limited to a single or few species, often with high abundance values.

In general, the comparison between present data and literature records shows that sponge assemblages from the studied basins are quite persistent.

At Marsala and Porto Cesareo species composition is almost unchanged during the last decades and a high percentage (65 %) of the species collected from the Venice Lagoon during 2001 had already been found there in 1960.

THE SPONGE GENOME: STARTING TO GET SOME CLUES

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Despite their crucial position in animal evolution, information on sponge genomes in the postgenomic era is scanty at best. In GeneBank release 129 (April 2002), the Porifera section contained 153 complete protein-coding sequences, of which only six were genomic sequences. *Suberites domuncula*, *Geodia cydonium* and *Ephydatia fluviatilis* represented 84 % of those sequences.

The first two species were selected for our study. The absorbance profile and modal buoyant density of genomic DNA were determined by analytical ultra centrifugation in CaCl density gradient. Furthermore, genome compartments, characterised by different G+C content, were physically separated by density gradient centrifugation. These compartments were characterised further by determining the distribution of a number of genes within and across them.

During the course of this study, a major difficulty was the presence of associated organisms, among which prokaryotes were dominating. We thus performed prokaryote-specific SSU rDNA sequencing and phylogenetic analyses in order to characterise these "contaminating" organisms.

Altogether, these results allow us to gain some insight into the genome organisation as well as the biology of sponges.

GLYCOLIPIDS FROM SPONGES

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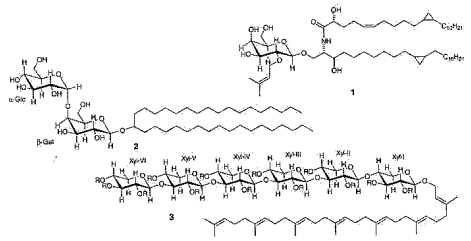
In recent years, Porifera have shown to be one of the richest source of new glycolipids (Fattorusso & Mangoni, 1997). Many glycolipids from marine sponges, even though belonging to the wide class of glycosphingolipids, have unusual structure, such as the immunostimulating α-galactosyl-glycosphingolipids from *Agelas* species (Costantino *et al.*, 1996) or the immunosuppressive plakoside A (Fig. 1) and B from *Plakortis simplex* (Costantino *et al.*, 1997). In addition, marine sponges contain a number of atypical glycolipids, without counterpart among glycolipids from other phyla. For example, simplexides (Fig. 2) are glycoside of a very-long-chain secondary alcohol (Costantino *et al.*, 1999), whereas plaxyloside (Fig. 3) (Costantino *et al.*, 2001) possesses a linear polyisoprenoid aglycon and a carbohydrate chain composed of six linearly arranged xylopyranose units.

The systematic study of glycolipids from Porifera is far from being terminated, and many new compounds are currently under investigation. This communication will deal with our most recent results in this field.



EARLY STEPS IN THE EVOLUTION OF MULTICELLULARITY: DEEP STRUCTURAL AND FUNCTIONAL HOMOLOGIES AMONG THE HOMEOBOX GENES IN SPONGES AND IN HIGHER METAZOANS

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Metazoans are now considered to be monophyletic, sponges being the most ancient and primitive group of multicellular animals. Their sister group among the protozoans are choanoflagellates. These postulates imply that molecular mechanisms underlying the functional cell integration in multicellular organisms have been created, at least in part, during the evolutionary step from choanoflagellates to sponges. In higher organisms, cell fate, proliferation and differentiation are controlled in large part by the homeobox genes. Here we have studied EmH-3 sponge homeobox gene that has not yet been attributed to any homeobox family. Comparative sequence analyses suggested that it is close to the Hox11 gene belonging, together with prox2, EfH-1 and SpoxTa1 sponge genes, to the Tlx homeobox gene family. These genes are highly expressed in proliferating progenitor cells and down regulated during the cell differentiation. This attribution was further supported by the conserved Eh1 repressor sequence at the aminoterminal region, and the ordered presence of putative nuclear factor-binding regions in the EmH-3 promoter. We assayed the capacity of the EmH-3 promoter to respond to molecular controls in human K562 erythrolukemia cells that expresses constitutively an endogenous Tlx gene, which is downregulated upon differentiation-induction with sodium butyrate. EmH-3 promoter constructs with luciferase gene reporter transfected into K562 cells showed the same behavior. We propose that the sponge and mammalian Hox11 genes are homologous both in their structure and in the functional regulation of their promoters, representing a deep homology in controls of cell proliferation, commitment and differentiation fate, required for multicellular grade of organization.

CARNIVOROUS DEEP-SEA SPONGES FROM THE DIVA I EXPEDITION IN THE ANGOLA BASIN (SOUTH ATLANTIC)

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DIVA I is the first in a series of expeditions dedicated to the study of benthic diversity in the deep-sea of the Atlantic Ocean. On RV "Meteor" a team of 28 scientists and technicians collected samples along a transect of about 700 km in the Angola Basin (July 2000). The transect lies west of the Walfish Ridge, a submarine mountain range. More than 40 specialists are cooperating to identify deep-sea organisms of all taxonomic groups, from bacteria to the large macrofauna.

Samples were collected with different kinds of dredges (Agassiz trawl, epibenthic sledge, multicorer, box corer) to get all size-classes of benthic organisms.

The Mega-Epifauna was collected with a modified Agassiz-Trawl. The seabed was trawled for 2 to 3.5 hours at 2 knots, which equals a trawling distance of approximately 7500 – 13000 m and a trawled area of at least 22500 – 39000 m².

The trawl was used, as a rule, 2 times at every station. Because of time losses during sampling, time had to be saved at some stations. In these cases the trawl was left on the bottom for 3.5 hours whenever possible. The dominant taxa were fish, es echinoderms, bivalves, actinians, and sponges. Other animal groups were present at times, but not regularly.

The collection of Porifera from the Diva I expedition is composed of 50 specimens or in some cases pieces of specimens collected between 5200 and 5450 m deep. Many sponges were already broken when they reached the surface, because they are very delicate and for this reason at the beginning we always considered each piece as a different sample. We identified 7 species in total, belonging to 2 Classes (Demospongiae and Hexactinellida) and 3 Orders. This work is related only to carnivorous sponges of which we collected 15 specimens belonging to 3 species. Since Vacelet and Boury-Esnault (1995) discovered carnivorous sponges from a cave wear Marseille, 3 genera *Asbestopluma*, *Cladorhiza* and *Chondrocladia* are considered to present this peculiar behaviour. All our specimens belong to the latter. They are erect, with a short straight stalk with divergent rooting processes that sometimes appear arranged around conical processes in a confused spiral manner. As microscleres they have anchorate isochelae with several teeth.

A NEW MARINE BIOLOGICAL STATION IN THE BEGINNING OF XXI CENTURY

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The Estación de Bioloxía Mariña da Graña is an institution of the University of Santiago de Compostela, founded in September 2001, with the purpose of propitiating, fomenting and facilitating research and teaching in marine biology, and the advice and diffusion of topics related to the fauna and marine flora of Galicia. It is located in the Ría of Ferrol (Galicia, Spain) and has two buildings: the Casa do Hórreo and the Casa da Estrela placed scarcely 10 m from the sea.

The Casa do Hórreo has a surface area of 400 m^2. It is dedicated to the research with the purpose of providing the scientists with facilities and means necessary for the development of works and study projects of the marine environment. It possesses a pump room with a seawater tank of 15 m^3, a diving room with a compressor to refill tanks, wardrobes, a generator, freezers, aquariums, a humid laboratory, two research laboratories, a cold chamber, a small library, a computer and image analysis room, and administrative sections. In the station the investigators will develop their work, in the same way as those of other universities and national or foreign centers that request the use of the facilities.

The Casa da Estrela, with a surface of 500 m^2, is dedicated to educational ends, including residence, with the purpose of facilitating the realization of educational activities for the students, as well as the establishment of relationships of scientific exchange with other universities and scientific institutions of Spain and of other countries. It possesses an educational laboratory for 24 students, histology cabinet, a classroom-seminar-room, a living room, a kitchen, and a residential area with two rooms for four lecturers, six rooms for 24 students, and different warehouses for the service of the station. All the laboratories are equipped with aquariums with circulating seawater and the necessary scientific material for the development of their functions. It has a craft of 5,25 m in length with an outboard motor, and a fibreglass boat of 12 m in length.

CELLULAR DYNAMICS IN ALLOGENEIC REACTIONS OF *HYMENIACIDON HELIOPHILA* (HALICHONDRIDA, HALICHONDRIIDAE)

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Allograft reactions of the sponge *Hymeniacidon heliophila* Parker, 1910 have been studied and quantified at cellular level *in vitro*. Changes in the cellular subpopulations of diamorphs belonging to different individuals and placed in direct contact were recorded. The initial contact was followed by partial fusion of the diamorphs and ended by their isolation, although without a marked collagen barrier. Using cytospins, cellular subpopulations were morphologically distinguished based on overall shape, nuclear and cytoplasmic characteristics. In the beginning of the cultures up to seventeen cell types could be recognized. Within five days, following reaggregation and diamorph formation, populations of eight of these types suffered marked reduction, while nine were either maintained or reduced at a slower pace. When submitted to allogeneic contact, the cellular dynamics of four of the remaining nine types were altered. Contact and adhesion with inert materials (glass and polystyrene) produced no relevant changes at cellular level. Surprisingly, the percentage of the archeocyte population is reduced, probably depleted by differentiation to other, differentiated cell populations. Cellular responses to allogeneic contact were characterized by a transient increase in spherulous cells, a larger participation of presumed collagen-secreting cells, and by a remarkable expansion of the collencyte population. The collencyte population remains altered after the isolation of the individuals by a collagen barrier, and could represent a mechanism of short-term immune memory.

EXPRESSION OF DEVELOPMENTAL GENES IN SPONGE LARVAE: EVIDENCE FOR A SHARED REGULATORY ARCHITECTURE IN ALL METAZOANS

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While the adult body plan of demosponges bears little resemblance to other metazoans, the parenchymella larva has clear similarities to other animal larvae. Following cleavage, the sponge embryo undergoes extensive cellular rearrangements that results in a bilayered larva with differentiated cells patterned along anteroposterior and centrolateral axes. We have assessed the regulatory and biosynthetic capacity of the larva of the tropical sponge *Reniera* sp. by isolating and characterising members of a range transcription factor gene families and analysing 196 expressed sequence tags (ESTs). A diversity of transcription factor genes, including members of *POU, LIM-HD, Pax, Bar, Prox2, forkhead, brachyury, Sox, Ets, b-Zip* and *nuclear receptor* gene families are expressed in the larvae, and during sponge embryogenesis and metamorphosis. Most of these genes appear to belong to metazoan-specific families, suggesting that a major evolutionary change in genome organization and complexity occurred around the time of the appearance of the first metazoans. A total of 91 ESTs produce significant matches when compared with GenBank. The 63 different proteins identified in this screen include range of "housekeeping" proteins, components of signalling pathways and transcription factors. The phylogenetic distribution of these proteins indicates a majority of these genes originated before the evolution of animals. From this survey of sponge larval gene expression, we infer that many of the fundamental components of the highly conserved regulatory program used in bilaterian development were present in the very first multicellular animals.

DIVERSITY AND ANTIBIOTIC ACTIVITY IN BACTERIA FROM TEMPERATE AUSTRALIAN MARINE SPONGES

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The occurrence of permanent sponge-bacterial symbioses in five temperate marine sponges from Western Australia (*Ircinia* sp., *Chondrilla australiensis*, *Echinodictyum* sp., *Tethya* sp. and *Coelosphaera* sp.) was investigated using classical culturing techniques, facilitating both bacterial characterisation and subsequent screening for the presence of antimicrobial activity. Based on biochemical tests, most the 136 bacterial isolates obtained from the sponges and surrounding water column on several occasions were tentatively identified as members of the Vibrionaceae. Fatty acid methyl ester analysis indicated that the isolates represented 32 distinct species groupings, four of which contained distinctive subgroups. Few bacteria appeared to have a permanent association with the sponges although some were found only associated with sponge tissues. Some (10) were found only in the water or were present in the water and one or more sponges (14) on one occasion. Twenty-three were cultured only from sponge tissue, 11 of which were detected in only one sponge on one occasion and 12 were found in more than one sponge. For only four bacteria was there evidence of a permanent relationship with their sponge hosts, being present in host tissues on every sampling occasion. One of these isolates occurred in three of the sponges studied (*C. australiensis*, *Tethya* sp. and *Coelosphaera* sp.). Crude extracts of *Coelosphaera* sp. were not inhibitory against *Staphylococcus aureus*, but a bacterium from this sponge produced a heat labile, non-dialysable inhibitor of *S. aureus*. Crude extracts of the other three sponges inhibited *S. aureus*, but culture supernatants of the bacteria from these sponges did not. These results are discussed in terms of their contribution to understanding marine and sponge microbial diversity and the potential for discovery of new pharmacologically active chemicals.

DISTRIBUTION AND PHYLOGENETIC AFFINITIES OF NITRIFYING MICROBES ASSOCIATED TO EPIBIONT SPONGES OF NUTRIENT LIMITED *RHIZOPHORA* MANGLE STANDS

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Sponges are recognized as one of the most diverse and abundant colonizers of red mangrove roots in various Caribbean mangrove systems. The involvement of three Caribbean sponges species in mediating the microbial process of nitrification have been recently documented through metabolic and molecular studies. This study attempts to further evaluate the extent of the presence and the distribution of this important type of animal-microbial association among common epibionts from a nutrient-limited mangrove community in Belize.

The community structures of proteobacterial beta subgroup of ammonia-oxidizing bacteria (AOB) among six common sponges species (*Haliclona implexiformis*, *Geodia papyracea*, *Spongia* sp., *Tedania ignis*, *Lissodendoryx isodictyalis*, *Biemna tubulifera*) and a water sample were compared by PCR amplification of 16S ribosomal DNA (rDNA). A series of nested PCR, implying two steps of increased biased amplification, allowed the detection of AOB in a DNA mixture extracted from internal sponge tissues. AOB 16S genes were detected from the all sponge species and water samples. To evaluate the diversity of AOB found a denaturing gel electrophoresis analysis (DDGE) was performed on amplification product of the variable region of the 16S r gene (V3, positions 338-519). This method proves to be of extreme usefulness for an initial fingerprinting of AOB strains associated to internal sponge tissues. Band migration in the DGGE analysis allowed the distinction of at least 4 different bands (each corresponding to AOB strains) in the water and 5 among the sponges. Only one type of strain was exclusively present in the sponges, and not in the water. Furthermore this strain was detected in five of the six mangrove species studied. Sequences from the DGGE bands were related either to the *Nitrosomonas*, or the *Nitrospira* clusters of AOB of the Beta subclass of Proteobacteria. Considerable differences (3-8 %) among band sequences suggest a larger diversity of AOB that the one shown by the DGGE analyses. This study demonstrates the widespread occurrence of AOB among marine tropical sponges, and the unique composition of AOB between different sponge species.

BIOASSAY GUIDED FRACTIONATION OF THE CRUDE EXTRACT OF A MARINE SPONGE: HIV-I INHIBITION

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A large variety of natural products have been described as anti-HIV agents. In the primary AIDS antiviral screen performed by the National Cancer Institute of USA an high percentage of crude aqueous extracts of marine sponges shown some activity. Here we present the HIV bioassay guided fractionation of the crude aqueous extract of a marine sponge from the Portuguese coast.

The screening for HIV inhibition compounds from sponge specimens, which were collected at the Berlengas Natural Reserve, Portugal, showed that the samples with highest anti-HIV activities were the aqueous extracts from *Cliona celata* and *Erylus discophorus*. Accordingly, a crude aqueous extract of a specimen of *E. discophorus*, which presents HIV-I inhibition with moderate cytotoxicity against a Jurkart lymphoblastic cell line (clone E6) was selected. The fractionation consisted in ethanol precipitation, amminonium sulphate precipitation and several chromatographic steps.

The in vitro HIV inhibitory activity of each fraction was evaluated by the degree of antigen p24 production by HIV-I NL4-3 infected lymphocyte cell lines. This procedure was performed with pre-determined tittered viral samples. Duplicates with the same sample dilution were performed in the absence of viral infection, to determine the cellular viability in the presence of the sponge fractions.

Each fraction was monitored by SDS-PAGE with silver nitrate stain and the content in proteins (Lowry method), anionic polysaccharides (toluidine blue as a metachromatic reagent) and total carbohydrates (orcinol-sulphuric acid assay) was determined.

EFFECT OF FOOD TYPE AND CONCENTRATION ON THE CULTURE RESPONSE OF TWO TROPICAL SPONGES

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Aquaculture of marine organisms is one approach to the sustainable supply of bioproducts such as pharmaceuticals. A number of factors need to be optimized for successful *in vitro* culture of sponges, including determining suitable feeding regimes. In this study, we examined the effect of food type and concentration on the culture response of two tropical sponges from contrasting habitats. *Halichondria* sp. is found in turbid waters where the natural concentration (NC) of particles 1-10 µm in size exceeds 2000000 cells per ml. *Axinella corrugata* (George & Wilson, 1919), in contrast, occupies coral reefs where the natural food concentration is an order of magnitude less, approximately 200000 cells per ml. For *Halichondria* sp., explant growth increased as food concentration increased (from 1/5NC, 1NC, to 5NC). However, explants fed at the highest concentration (5NC) had poor survival. For *A. corrugata*, explants fed at 3NC grew well, but explants fed at 5NC shrunk in size. These findings suggest that very high cell concentration may overload the filtration system of sponges, resulting in poor explant growth or survival. Food type was also important, with growth significantly greater for *Halichondria* sp. explants fed a mixed diet consisting of bacteria, microalgae and yeast, than explants fed a diet of bacteria only. For both species, some explants grew by 20 % of initial weight in two weeks. These studies indicate that the optimal feeding regime for *in vitro* sponge culture is species specific.

NEW NITROGENEOUS EUDESMANE-TYPE COMPOUNDS ISOLATED FROM THE CARIBBEAN SPONGE *AXINYSSA AMBROSIA*

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Fractionation of an acetone-methanol (1:1) extract of the Caribbean marine sponge *Axinyssa ambrosia* yielded three new sesquiterpene compounds whose structures were established by spectroscopic methods as (4*R**, 5*R**, 7*S**,10 *R**)-eudesman-4-yl amine hydrochloride, axinyssamine hydrochloride, (4*R**, 5*R**, 7*S**, 10*R**)-eudesman-4-yl isocyanide, and (4*R**, 5*R**, 7*S**, 10*R**)-eudesman-4-yl formamide. Compound exhibited significant cytotoxic activity against cancer cells and was also active in a lethality test using polyps of the coral *Madracis mirabilis*.

GENETIC VARIATION IN POPULATIONS OF THE SPONGE *CRAMBE CRAMBE* (POECILOSCLERIDA) ASSESSED USING POLYMORPHIC MICROSATELLITE MARKERS AND MTDNA SEQUENCE DATA

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We have developed novel microsatellite markers for the sponge *Crambe crambe* from an enriched genomic library. Six polymorphic loci were successfully amplified. The number of alleles per locus ranged from 4 to 19. We used these markers to analyse genetic differentiation in 8 populations spanning most of the known geographic range of the species (from Madeira to Corsica, ~ 3000 Km). The results were compared with sequence data of the mtDNA COI gene from the same individuals.

No linkage disequilibrium was detected among the selected loci, and most of them were under Hardy-Weinberg equilibrium depending on the population analyzed. Reduced number of alleles and heterozygosity were detected in some populations (i.e. Gran Canaria) indicating a recent founder effect. The high FIS values found might be the result of a Wahlund effect as a consequence of isolation by distance at a microgeographical scale. Significant inter-population differences in allele frequencies were found among populations, resulting in high Fst values. Mitochondrial sequences, on the other hand, were highly conserved, with only one nucleotide change out of 534 positions, indicating a lower variability in this gene than reported for most Metazoa. However, the frequencies of the two haplotypes differed between close populations, suggesting a restricted gene flow.

The results confirm the restricted dispersal abilities usually assigned to sponge larvae, and indicate the potential usefulness of microsatellite markers in sponges. Reasons for the contrasting results obtained with the two types of molecular markers are discussed.

OXYGEN ISOTOPE ANALYSIS OF BIOGENIC SILICA IN SPONGE SKELETONS AS A POSSIBLE TOOL FOR PALAEOCLIMATOLOGY

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The high content of sponge spicules in the sediments of some freshwater lakes and polar oceanic regions potentially allows this source of biogenic silica to be used for palaeoclimatic reconstructions. By analogy to calcareous microfossils, the oxygen isotope composition of biogenic tests may provide some of the best constraints for this objective. The problems for measurements of oxygen isotope compositions of biogenic silica are based on its nature of occurrence. Amorphous hydrated silica contains up to 13 weight-% free-bonded water, which may overprint the original oxygen isotopic composition of silica. To overcome these analytical problems, we used a method that employs a 30 W CO_2-laser that spontaneously heats and melts the biogenic silica in a vacuum, followed by fluorination of the residual melt bead. Initial rapid heating avoids exchange between released water vapours and the Si-bonded oxygen, as the vapours are condensed in a trap cooled with liquid nitrogen. The melted sample beads are then transferred to a new Pt sample holder for oxygen extraction using the CO_2-laser and ultrapure F_2 as reagent. Use of a distinct sample holder for melting eliminates cross-contamination that may occur through vaporization during melting and condensation of the vapours on the sample holders of a common sample chamber. The oxygen extracted is then analysed by gas-source mass spectrometry. In this way reproducible oxygen isotopic compositions for biogenic silica of sponge skeletons can be obtained. The application of the method is currently being tested using measurements on recent sponge spicules, which also serves as a calibration for fossil material. Isotopic-chemical characterization of poriferan species in freshwater systems with high contents of sponge spicules in sediments (e. g. in the Lake Baikal, Lake Biwa and some tropical lakes) will be discussed. For the marine realm, we will present data from high-latitude environments in the Weddell Sea, NE-Pacific and the White Sea. Current results indicate that demosponges reflect the oxygen isotope composition of the surrounding waters according to known biogenic silica-water fractionations. For different species from Lake Baikal, such as *Lubomirskia baicalensis, Baikalospongia bacillifera* and *Swartschewskia papyracea*, oxygen isotope compositions of 28.5 ± 0.4 per mil (relative to VSMOW) have been measured. The Caribbean shallow-water demosponges from St. Crux have values of 35.4 ± 0.4 per mil, independent of the species assemblages. In contrast, values of hexactinellids from the coast of British Columbia, such as *Aphrocallistes vastus* and *Heterochone calyx*, differ in the range of 34.0 and 38.0/ml. These values may reflect possible vital effects, which lead to variations in silica-water isotopic fractionation for different sponge species. Experiments using sponge spicules from cultured specimens of *Spongilla lacustris* and *Ephydatia mülleri* are in progress to place further constraints on vital effects. Our investigations should provide a realistic perspective for the use of sponge spicules as proxies for palaeoclimatic changes.

PHOTOTAXIS IN SPONGE LARVAE: A COMPARATIVE STUDY

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Recent work has shown that larvae of the tropical demosponge *Reniera* sp. are capable of instantaneous responses to abrupt changes in light intensity, a behaviour that allows them to settle in dark areas under the coral rubble on the reef flat at Heron Is. GBR (Leys and Degnan, 2001 Biol. Bull. 201: 323-338). To determine how widespread this kind of phototactic behaviour is among sponge larvae, ontogenetic changes in the photoresponse of larvae from two temperate demosponges and a calcareous sponge were studied by analyzing the swimming paths of individual larvae in response to diffuse white light. Larvae from *Sycpha* sp. swam strongly away from a diffuse light source for 3 days until settlement and metamorphosis; *Haliclona* cf. *permollis* (Bowerbank, 1866) larvae swam weakly away from light for 48 hours; and larvae from *Halichondria panicea* (Pallas, 1766) were benthic until settlement and metamorphosis, and showed no responsiveness to gradients of light intensity. Scanning electron microscopy revealed very different patterns of ciliation in all larvae. In Haliclona, swimming ability and sensitivity to light is presumed to be generated by longer cilia that arise from a ring pigmented cells at the posterior pole, as has been demonstrated in *Reniera* sp. larvae. *Halichondria* larvae lack pigmented cells and the ring of long cilia at the posterior pole. How *Sycpha* larvae, which lack both the long posterior cilia and ring of pigment-filled cells at the posterior pole, are such strong swimmers and exhibit such marked negative phototaxis is the subject of further investigation. These results 1) demonstrate that sponge larvae are capable of responding rapidly to environmental stimuli, like other metazoan larvae; 2) show that a coordinated behavioural response to stimuli is possible even in the absence of neurons or junctions that would allow electrical signalling between cells; and 3) suggest that photodetection and photoeffector systems may be significantly different within the Porifera.

CLONING AND CHARACTERIZATION OF CYCLIN DEPENDENT KINASE (CDK) GENES FROM THE MARINE SPONGE, *AXINELLA CORRUGATA* (GEORGE & WILSON, 1919)

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Cyclin dependent kinases (CDKs) constitute central factors of the cell cycle control machinery. Cdk genes encoding these proteins appear to be highly conserved throughout eukaryotic evolution and have been identified in a number of diverse phyla. Here we present the isolation of two partial cDNAs by RT-PCR (reverse transcription polymerase chain reaction), Ac-cdk1 and Ac-cdk2, from the marine sponge, *Axinella corrugata* (Demospongiae, Axinellida, Axinellidae) which encode two distinct homologues of the CDK protein family. Molecular phylogenetic analysis places these two novel cdk sequences descendent to fungal and protist cdk sequences yet basal to previously reported invertebrate and vertebrate cdk sequences, consistent with metazoan hierarchy. In addition, base composition and codon usage analyses of both sponge cdk genes conform to profiles of previously characterized sponge sequences in DNA databases. Implications of the new sponge cdk sequences for the molecular evolution of the cdk multigene family are discussed.

COMPARATIVE EMBRYOLOGY OF SPONGES AND ITS APPLICATION FOR SPONGE PHYLOGENY

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The objectives of the present work were the delimitation of developmental types in sponges, the determination of specificity of developmental type for poriferan macrogroups, and the assessment of the possibility to apply comparative embryological method for reconstructing phylogenetic relations of Porifera. Twenty-seven species from 7 orders of Demospongiae were studied and a revision of the data on embryonic development of all Porifera, available from literature, was made. Delimitation of developmental types is based on the analysis of a complex of embryological characters: 1 - egg type; 2 - cleavage pattern; 3 - blastula type; 4 - character of larva morphogenesis; 5 - period of anterior-posterior polarity formation; 6 - larval type; 7 - ultrastructural features of the larva; 8 - characteristics of metamorphosis. As a result, 7 types of development are suggested: I. Type of development of Hexactinellida; II. Type of development of Calcinea; III. Type of development of Calcaronea; IV. Type of development of Homoscleromorpha; V. Type of development of Halisarcida; VI. Type of development of Spirophorida (direct development); VII. Parenchymula type of development. The latter includes three subtypes: the first, characteristic of Dendroceratida, Dictyoceratida, Halichondrida and Hadromerida; the second, characteristic of Poecilosclerida; the third, characteristic of Haplosclerida. Viviparity and oviparity are not purely embryological characters, but rather belong to the sphere of reproductive ecology. Oviparity has originated several times independently in different groups of Demospongiae and has neither taxonomic, nor phylogenetic significance.

The presence of several developmental types within Demospongiae suggests that this class could be paraphyletic. This suggestion emphasizes the necessity of re-evaluation of the phylogenetic relationships between poriferan clades and Eumetazoa. The type of development of Homoscleromorpha deserves special attention, as it possesses a number of apomorphic characters. This supports the monophyly of Homoscleromorpha and their relative proximity to Eumetazoa. The results of the present investigation demonstrate that comparative embryological methods can be successfully used in modern spongology, alongside with morphological and molecular biological methods.

THE SYSTEMATICS OF HALICHONDRID DEMOSPONGES - A MULTIPLE GENE APPROACH

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In the past decades the important role of Halichondrids in demosponge systematics became evident when analyses of morphological characters yielded evidence for the paraphyletic nature of the two major recognized demosponge subclasses Tetractinomorpha and Ceractinomorpha. Van Soest (1987, 1991) and Hooper (1990) showed in their subsequent cladistic analyses a pivotal role of the Halichondrida s.l., including the formerly recognized orders Axinellida (Tetractinomorpha) and Halichondrida s.s. (Ceractinomorpha).

However, systematics of the redefined taxon Halichondrida *sensu* van Soest *et al.* (1990) remains mostly unresolved. Halichondrid monophyly as well as the assignment of the four major families (Halichondriidae, Axinellidae, Desmoxyidae and Dictyonellidae) has to be tested in phylogenetic analyses as no single synapomorphic character unites the four families and several analyses gave evidence for halichondrid para- or polyphyly (e.g. McCormack & Kelly, in press). The lack of complex morphological characters such as microscleres in most groups prevents a robust phylogenetic reconstruction based on skeletal features. In recent years molecular systematics have become established in sponge classification, but the resulting phylogenies are almost entirely based on single gene trees. Previous molecular analyses contained only a subset of halichondrid taxa (e.g. Alvarez *et al.*, 2000; McCormack & Kelly in press; Erpenbeck *et al.*, in press).

In our approach to resolve the systematics of a representative set of halichondrid demosponges we employ three different gene fragments that are assumed to evolve independently: a mitochondrial protein (Cox1), a cytoplasmatic rRNA gene (28S-rDNA) and a genomic protein. With the resulting phylogenetic hypothesis we aim not only to resolve the existing controversies but also to provide insights in halichondrid skeleton evolution and in the value of chemical data in sponge systematics.

FROM EXPERIMENT TO ENTERPRISE - SPONGE AQUACULTURE VENTURES FOR REGIONAL AUSTRALIAN COMMUNITIES

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The last 15 years have seen a resurgence of interest in sponge culture for a variety of historical (e.g. bath sponges) and novel (e.g. as a supply of fine chemicals) applications. While sponge aquaculture itself is not a new notion, ventures that demonstrate long term environmental, financial and operational sustainability are few and far between. An absence of a balance in the nexus between target species, their commercial application, environmental sustainability of supply, quality control and other market parameters resulted in the collapse of the historical sponge culture industry in Europe and America, and the marginal nature of the global wild harvest industry that succeeded it. More recently, environmental regulatory schemes and issues relating to access to biogenetic resources for discovery of new applications have played a higher profile. Achieving a balance between all of these practical and policy aspects is a prerequisite to realising successful sponge aquaculture enterprise in the future.

This paper will present two case studies from research undertaken at the Australian Institute of Marine Science, to illustrate the process of experimentally establishing the potential of new sponge aquaculture species, and the transformation from experimental to commercial viability. The first focuses on a potential bath sponge venture for the indigenous inhabitants of the Palm Islands, just north of Townsville off the tropical Queensland coast. This group of islands does not have existing aquaculture infrastructure. They lie within the Great Barrier Reef World Heritage Area and so are subject to rigorous environmental and other management scrutiny and regulation. Additionally, use of this region is subject to complex indigenous ownership issues. The second case study is of a potential fine chemical aquaculture venture in the temperate Cockburn Sound, south of Perth in Western Australia. In this case, environmental regulatory control of operations is less stringent, there are no indigenous ownership issues, and the project has been able to utilise existing bivalve aquaculture infrastructure. However, the focus on utilising the biochemical capacity of native Western Australian biota has triggered another set of policy issues regarding access to biological diversity for "bioprospecting", and benefit sharing.

The foundation of both case studies is the fundamental biology and ecology of the target species. Thus, presentation of each case will commence with a synopsis of the results of assessment of natural distribution, abundance, reproduction, and population dynamics as well as optimal methods of culture, in-culture growth and survivorship, and the potential for deleterious environmental impacts. However, beyond the experimental demonstration of the environmental viability of commercial production, the case histories diverge in their application of market forces, environmental regulation, and the effect of other regulatory and policy issues.

EXPERIMENTALLY INDUCED MORPHOLOGICAL CHANGES OF *PETROSIA FICIFORMIS* IN AQUARIUM

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The reparative and regenerative behaviour of *Petrosia ficiformis* (Poiret, 1789) was studied in aquarium on clones, obtained by fragmentation. Sponge samples were collected in June 2001 at depths between 10-15 m on rocky bottoms at Paraggi-Portofino (Ligurian Sea), reduced in fragments of about 30 cm^3 and cultured in constant conditions of temperature, light, current and feeding in the Mediterranean System tanks of the Genova Aquarium. After a period of four months in artificial conditions, we observed the growth of peculiar white short finger-like projections from the cutting surfaces. We compared, both by light and electron microscopy, the tissues and the skeletal organisation of finger-like projections versus the tissues of wild specimens. Our observations highlighted that spicular orientation in the skeletal architecture of *P. ficiformis* from the aquarium was extremely irregular and not organised; on the contrary sea living specimens showed the typical skeleton architecture of this Mediterranean Haplosclerid.

THE BORING SPONGOFAUNA OF THE MARINE PARK OF BUNAKEN (NORTH SULAWESI, INDONESIA)

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Indonesia's coral reefs are among the richest and diverse in the world. The Indonesian Archipelago is in fact an area with an extremely high biodiversity due to geographic reasons (it acts as a link between the Indian and the Pacific Ocean), and to its complex geologic history. Since 1999 the Biodiversity Project allows to study the spongofauna of the marine Park of Bunaken (North Sulawesi). In this work our attention focuses on boring sponges. They may act as small scale disturbance that maintains the high level of biodiversity and, together with physical agents, deeply contributes to the structuring of coral reefs, controlling the rate of reef accretion and the redistribution of reefal sediments.

The most recent work on Indonesian sponge is the review of van Soest (1989) who estimated about 830 sponge species. Anyway very little is known about boring sponges in Indonesia, though they are a ubiquitous component of the reef community.

During our first campaign the greatest effort was dedicated to survey the boring sponge species richness. Our first data confirm the extremely high biodiversity of the area. Until now it was possible to record, only around Bunaken Island, 29 species of boring sponges. Among these 14 species are already known for the Indo-Pacific area, while 8 species of *Cliona*, 5 of *Aka*, 1 of *Cervicornia* and 1 of *Spirastrella* are probably new for science.

GLYCOSAMINOGLYCAN DIVERSITY IN SPONGE EXTRACELLULAR MATRIX

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It is known that sulphated polysaccharides from sponges perform anti-inflammatory and antiviral activities, whereas other compounds are involved in cell-cell recognition. The composition in sulphated polysaccharides from the sponge extracellular matrix (ECM) is almost unknown, with few exceptions. Aim of this paper is to report on a screening on sponge ECM glycosaminoglycan (GAG) diversity in some Mediterranean and Caribbean sponges. Sulphated polysaccharides were extracted by proteolytic treatment from specimens preserved in absolute ethanol and subjected to both quantitative and qualitative analyses by standard methods. Most samples displayed a notable amount in sulphated polysaccharides, with an extremely wide range in their content at different taxonomic levels. From the qualitative point of view, we detected that sponge GAGs do not fit the standards used for vertebrates, therefore they are not suitable substrata for specific enzymatic reactions able to degrade GAGs from vertebrates. These findings strongly suggest a lower sulphation degree associated with a different sugar composition, substitution and sequential arrangement compared with classical GAGs. The astonishing diversity of sulphated polysaccharides could be related to a wide adaptive radiation in the structural and functional organization of sponge ECM. Some taxa display sulphated polysaccharides with peculiar electrophoretic patterns, however this trait appears to be not exclusive of each taxon and therefore not diagnostic for a taxonomic discrimination. Sponge GAGs need, however, a deeper structural characterisation in order to test their potential role as molecular markers.

MARINE SPONGES OF THE DAMPIER ARCHIPELAGO: THEIR DISTRIBUTION AND ABUNDANCE

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Marine sponges of the Dampier Archipelago, North Western Australia are poorly known (14 species described in the literature from this region to date). This lack of knowledge prompted their inclusion in a major study of the marine biodiversity of the Archipelago undertaken by the Western Australian Museum from 1998 to 2000. Results of a quantitative component to the study, undertaken in subtidal localities, are presented here.

Non-hierarchical classification of 42 sites, based on quantitative transect data, yielded 11 groups of sites. Groups were defined because sites had similar assemblages of species; however for some groups there were relatively few species common to all sites, revealing a high degree of spatial variability in sponge assemblages. Station groups are discussed in relation to habitat and species assemblages.

The analyses also revealed correlations between species richness (number of species), abundance (number of individual sponges) and depth. More sponges, and more species of sponges were found in deeper sites. The highest number of species at a single station was 43, comprised of 179 individuals in 25 m^2. At the other extreme 6 individuals of 1 species were found in a silty soft bottom habitat in an area of 25 m^2.

ANALYSIS OF TRAWL SURVEY AND OBSERVER REPORTS OF SPONGE BY-CATCH IN THENORTHWEST ATLANTIC: WHAT ARE WE LOSING?

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The Northwest Atlantic has typically been seen as low in sponge diversity and abundances as compared to other areas of the world. There have been relatively few studies on the sponges of this area, and most are limited to species lists. The effects of fishing on benthic habitats has become a concern as fisheries around the world collapse. Analysis of trawl survey records and observer reports show that a significant biomass of marine sponges are removed each year, and as fishing efforts move into deeper waters, catches have increased. A population of glass sponge, *Vazella pourtalesi* (Schmidt, 1870) is reported for the first time in northern waters. The implications of the effects of fishing on Northwest Atlantic sponge communities area are discussed.

TRAWLING EFFECTS ON SPONGE VOLUME AND DIVERSITY IN THE GULF OF MAINE, NORTHWESTERN ATLANTIC

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Fishing gear can remove epifaunal species from the sea floor resulting in decreased habitat complexity and species diversity. Marine sponges are common constituents of benthic communities, especially on rock and cobble substrates. I investigated the effects of reduction of fishing effort on sponge populations on Stellwagen Bank, Northwest Atlantic. A closed area was implemented in 1998 in response to the decline of the groundfish stocks. Total sponge volume was higher inside the closed area than outside, while sponge diversity (assessed as Shannon-Wiener, H') was less inside the closed area than outside. Diversity of associated fauna is also assessed for both areas, and several associations with sponges are described for the first time. A reduction in trawling effort can lead to the recolonization of the sponge community, and this offers increased habitat for other invertebrate species.

METABOLITES FROM INDIAN OCEAN AXINELLIDAE: *AXINELLA* CF. *BIDDERI* AND *A. WELTNERI*

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Marine sponges are known to be a rich source of bioactive metabolites and the identification of some original structures may have pharmacological interests. In this view, we studied the ethanolic extract of the marine sponge *Axinella* cfr. *bidderi* from the Yemeni Socotra Island in the Indian Ocean, which showed antiproliferative activity in the initial sea urchin egg bioassay. The crude extract was subjected to solvent partitioning and the cytotoxic activity was found to be concentrated in the resulting heptanic extract. Chromatographic treatments allowed the purification of several triterpenoids with some original structures in the series of the sodwanones and the yardenones, as well as some steroids. The structures were established using spectroscopic analyses and the cytotoxicity of these compounds were evaluated against human lung carcinoma cells line L16. The similarity of the metabolites from *A.* cfr. *bidderi* and from *A. weltneri* is discussed.

BUDDING IN THE TWO MEDITERRANEAN *TETHYA* SPECIES: MORPHOLOGICAL AND ECOLOGICAL ASPECTS

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The budding sponges *Tethya aurantium* and *T. citrina* have been studied in some Mediterranean lagoons (Marsala, Porto Cesareo and Venice) from 1997 to 2001. Bud formation takes place differently according to the species and the environmental conditions of the habitat where they grow. In Marsala Lagoon specimens of *T. aurantium* living on the rhizomes of *Posidonia oceanica* produce a remarkable number of buds whereas no budding specimens have been found on hard substrates. On the contrary, specimens of *T. citrina* bearing some buds have been occasionally observed in the same area. This feature contrasts with the production of a large amount of buds by *T. citrina* in Venice Lagoon. In both species bud differentiation is rapid and occurs in autumn (October-November), probably in response to the sharp drop of water temperature. In *T. aurantium* buds tend to remain adherent to the sponge body for two-three months before the detachment; in *T. citrina* bud adhesion lasts only a few weeks. The sponge incorporates non-released buds into its tissue, thereby enhancing its volume.

Histological and ultrastructural investigations (SEM, TEM) highlighted some differences between the two species regarding both shape and organization of buds. In *T. aurantium* the budding process becomes evident through the differentiation of round protrusions, with a wide basal region, gradually bulging from the sponge surface. In *T. citrina* buds are connected to the mother sponge by a stalk that develops a distal swelling. While buds of *T. aurantium* mainly consist of a thick collagen matrix including scattered spherulous cells, sclerocytes and lophocytes, those of *T. citrina* show a more complex structure. Indeed, collagen matrix is less abundant in the latter and the cell component is more conspicuous, resulting from numerous wandering cells with long and thin cytoplasmic extensions, lophocytes, cells with electron-dense inclusions of various sizes, sclerocytes, spherulous cells and archeocytes. Cells with inclusions are the principal cell type and tend to arrange in parallel rows. On the whole, owing to the lack of canals and choanocyte chambers, bud organization resembles more the parental cortex than the choanosomal region. As in the mother sponge, the skeleton of buds consists of microasters, gathered along the outermost surface, sparse megasters, spicular bundles irradiating towards the periphery and emerging from the sponge surface.

Differences in bud organization seems to reflect the different time requested for maturation, which takes place more rapidly in *T. citrina* than in *T. aurantium*. It is worth stressing that newly detached buds do not show canals and choanocyte chambers, a feature in keeping with the delayed differentiation of the aquiferous system.

CURCUPHENOL, ANTIFUNGAL METABOLITE FROM THE MARINE SPONGE *DIDISCUS OXEATA*

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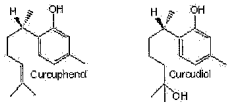
As part of our general interest in the isolation and characterization of bioactive metabolites from sponges, the sesquiterpenoids (+)-curcuphenol and (+)-curcudiol were isolated from the antifungal extract of the Curaçao marine sponge *Didiscus oxeata*.

Curcuphenol and curcudiol were already isolated from the marine sponges *D. oxeata, D. flavus, Myrmekioderma dendy* and *Arenochalina* sp.. Both sesquiterpenes were reported previously as ichthyotoxic (*Labistes reticulates*) and antifouling (barnacle *Balanus* amphirite) metabolites. (+)-Curcuphenol also exhibited antiyeast activity against *Candida albicans* and citotoxicity against P-388 murine leukaemia, A-549 lung, HCT-8 colon, and MDAMB mammary cancer cell lines.



The antifungal activity of the sesquiterpenoids curcuphenol and curcudiol isolated from the Caribbean sponge *D. oxeata*, were evaluated against the fungi *Absidia ramosa, Aspergillus niger, Botrytis cinerea, Cladosporium cucumerinum, Fusarium oxysporum, Penicillium expansum, Rhyzopus oryzae*, and *Trichoderma harzianum* at a concentration of 200 µg/disc. Curcuphenol was also tested, at same concentration, against the fungi *Fusarium solani, Nodulosporium* sp., *Phytophthora* sp., *Trichoderma* sp., *T. koningii, T. lignorum* and *T. virgatum* and against the bacteria *Staphylococcus aureus, Streptococcus faecalis* and *Salmonella enteritidis*. While Curcuphenol inhibited the growth of all the first group of fungi tested as well as *Trichoderma koningii* and the bacterium *Staphylococcus aureus*, curcudiol only partial inhibited the growth of *Absidia ramosa*.

HIGH ANTARCTIC CARBON AND SILICON CYCLING - HOW MUCH DO SPONGES CONTRIBUTE?

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Antarctica is a sponge kingdom. In many benthic communities on the Antarctic shelf sponges contribute more than 90% of the biomass — not only in terms of wet mass but also in terms of carbon (e. g. 45.3 gC m^{-2} in the depth horizon of 100-700 m). Despite this large standing stock and the known strong influence on the structure of the system, our knowledge about the contribution of the sponge to carbon and silicon flow patterns within the system have long been impeded by their extremely slow growth. With the expanding interest in sponges primarily from a bioactive-products-point-of-view we urgently need to improve our knowledge about basic population dynamic parameters for an impact assessment in case of commercial exploitation.

Growth of most Antarctic sponges is too slow to be measured directly. Furthermore traditionally used methods such as isotope analysis, analysis of growth marks, or interpretation of length-frequency-distribution histograms are not applicable for Antarctic sponges. In this study an indirect approach was chosen to assess growth rates: mass specific oxygen consumption rates which vary with body mass were used as a proxy for metabolic rate. The fraction of available energy that is used for growth ($P_s/\Sigma R$) also varies with body mass. Based on experimentally established relationships between both parameters and body mass, I developed a modelling routine (AMIGO: Advanced Model of Invertebrate Growth from Oxygen consumption data) to calculate growth rates from oxygen consumption rates of differently sized individuals. The demosponges *Stylocordyla borealis*, *Cinachyra antarctica* and the hexactinellids Rossellidae spp. were part of this study.

From modeling results it can be concluded that average Antarctic sponges show extremely low productivity values ($P_s/B=0.007$). It is worth noting, however that the lollypop sponge *S. borealis*, which is a known relatively early settler in iceberg scour marks, substantially exceeds the overall low productivity values (*S. borealis*: $P_s/B=0.106$). Sponges thus do not contribute substantially to overall carbon flow patterns on the eastern Weddell Sea shelf. Results for silicon cycling differ markedly from those for carbon cycling. Opal production (biogenic amorphous silica) varies between 0.01 g SiO_2 m^{-2} y^{-1} for *C. antarctica*, 1.2 g m^{-2} y^{-1} for *S. borealis*, and 9.6 g SiO_2 m^{-2} y^{-1} for Rossellidae spp. (all for patches of average abundance). Thus sponge opal production can exceed opal depositions of phytoplankton (0.003 g SiO_2 m^{-2} y^{-1}) to the seafloor in the same area by several orders of magnitude. Sponges — where present — can be the predominant pathway from dissolved silicic acid to opal.

Modeling results furthermore indicate that Antarctic sponges are among the oldest living creatures on this planet: largest Rossellidae spp. individuals in the Weddell Sea are 1515 years old at a body mass of 1680 gC.

GLYCOLIPIDS FROM MARINE SPONGES: MONOGLYCOSYLCERAMIDES AND ALKYLDIGLYCOSYLGLYCEROLS: ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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Glycolipids from marine sponges are known to possess immunomodulating and antitumoral activity. We report the isolation and characterization of two types of sponge glycolipids. Glycosphingolipids were isolated from the sponge *Axinyssa djiferi* (Senegal) and from a Mediterranean sponge *Aaptos papillata*. Alkyldiglycosylglycerols were isolated from the sponges *Trikentrion loeve* (Senegal) and *Myrmekioderma dendyi* (Vanuatu).

The glycosyl ceramide from *A. djiferi* corresponded to a mixture (axidjiferosides) including the same ß-galactopyranose with C_{22}-C_{26} α-hydroxy fatty acids and C_{14}-C_{22} sphingoid bases (with an unusual double bond between C-6 and C-7). Axidjiferosides showed an activity against *Plasmodium falciparum* (CI_{50}=0.45 µg/ml). The major components of the glycolipid mixture were separated by HPLC as peracetates and will be studied chemically and biologically. *A. papillata* contained two families of glycosphingolipids possessing hydroxylated or non hydroxylated fatty acyl chains.

A *O*-alkyl-*O*-glycosyl glycerol, already reported in *T. loeve* was isolated in order to perform pharmacological screening. This unusual glycolipid already known includes a glycerol unit, two xylopyranoses, and a C_{24} alkenyl ether chain and it was associated with compounds differing by chain length and unsaturation pattern. *M. dendyi* contained as major glycolipids two alkyldiglycosylglycerols including xylopyranose and *N*-acetylglucosamine, a glycerol backbone and alkyl long-chains with a terminal primary alcohol group. Similar glycolipid isolated from another *Myrmekioderma* species exhibited an antitumor activity.

Glycolipids were separated from other lipids by column chromatography on silica gel, and purified by HPLC. Structural studies were performed by high resolution FAB-MS, electrospray ionisation MS, and NMR. Acid methanolysis of glycosphingolipids afforded the three parts of the molecule : sugar as a methyl oside, fatty acyl chains as methyl esters, and sphingoid bases. All the mixture of derivatives have been analysed by GC/MS. Biochemical and pharmacological studies on our glycolipids are in progress.

BACTERIAL DIVERSITY IN THE BREADCRUMB SPONGE *HALICHONDRIA PANICEA* (PALLAS)

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Associations of sponges and micro organisms are widely distributed. Unfortunately studies describing the structure, diversity or dynamic of the associated bacterial population are remarkably few. Hence, because of the limited dataset, it is still uncertain if there are specific and stable associations of sponges and bacteria. Since molecular techniques require a lot of work, in most of the studies only few samples are analyzed and it remains questionable if representative samples were taken. The present study aimed to investigate the structure, the dynamic and the diversity of bacterial populations associated with the bread crumb sponge *Halichondria panicea* sampled nearby Helgoland Island (North Sea) using molecular tools. Key techniques applied were DGGE using different primer sets, cloning and sequencing of 16S-rDNA of bacterial isolates and clones. Regarding the DGGE results, the bacterial community structure varied between sampling locations, sampling dates and between the aquiferous system and the tissue. Even samples from single specimens sometimes displayed heterogeneous band patterns. Also the total number of detectable OTUs (Operational Taxonomic Unit) differed between the samples. In contrast, parenchymula larvae from one *H. panicea* specimen displayed identical band patterns consisting of one major band and one faint band, which were not comparable with the pattern of the parent animal. The result of a cloning experiment (16S-rDNA) of one sponge with low bacterial diversity (DGGE) displayed also low diversity in respect to the sequenced clones. Of 71 clones obtained, 45 identical clones were affiliated to a sequence matching the genus *Rhodobacter* (91% similarity with *Rhodobacter capsulatus* [D1627.1]). In total eight clone types were detected. A newly developed PCR-DGGE specific for marine alpha-proteobacteria also displayed very low diversity with sometimes only one OTU. We think *H. panicea* harbours a specific alpha-proteobacterial population but seasonal or microgeographical bacterial co-populations occur, sometimes even dominating the whole bacterial population.

POLYCHAETE INFAUNA ASSOCIATED TO *IRCINIA STROBILINA*

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A census of invertebrates associated to *Ircinia strobilina* (Lamarck, 1816) was performed in specimens collected at Carrie Bow Island (Belize) on July 2001. The invertebrate community is notably heterogeneous and mainly represented by priapulids, polychaetes, copepods, isopods, amphipods, decapods, chitons, fissurellids, bivalvs and ophiuroids. Among polychaetes were found representatives of different families such as Syllidae, Polynoidae, Eunicidae, Nereididae. Syllidae, the dominant taxon, are represented by the numerically dominant *Haplosyllis spongicola* (Grube, 1855), *Branchyosyllis oculata* Ehlers, 1887, and *Trypanosyllis* cfr. *zebra* (Grube, 1860). In addition, some species probably new for science, whose characterization is presently in progress, were detected. To investigate the sponge-worm relationships different approaches were performed on *H. spongicola* by SEM, spectrophotometric and histological analyses. Our data suggest that diversity of polychaete fauna associated to sponges is underestimated due to the difficulty of identification of worms at the specific level. This last fits the case of *H. spongicola* considered till now a single cosmopolitan ubiquitous species, but probably an extremely diversified species complex. Finally we hypothesize a possible species-specific relationship in sponge-worm bi-systems.

CELL REACTIVITY AGAINST SILICA: THE WIDESPREAD ROLE OF ASCORBIC ACID

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Field observations and laboratory experiments performed on the common Demosponge *Chondrosia reniformis* indicate that this species selects and incorporates exclusively siliceous materials, in particular quartz particles and opaline sponge spicules, even though, in the particulate matter, available in the environment, carbonatic particles are widespread and often dominant.

The opaline spicules remain unaltered within the sponge tissue, after the incorporation, whilst the engulfed quartz particles are quickly etched. The etching activity on quartz particles detected in *C. reniformis* is operated by means of an intense production of ascorbic acid. This production may be morphologically put in evidence by the presence of calcium oxalate crystals partially emerging from the sponge surface.

In this context, an unexpected behaviour of ascorbic acid has been pointed out in the change of quartz surface features, leading to an increased radical production in the water environment and a consequent dissolution of quartz. On the other hand this activity is not performed on amorphous opaline silica and this fact may clarify the different interactions of quartz-spicules with the cells of the sponge surface and the differences in the dissolution of the two kinds of incorporated siliceous materials.

This behaviour reminds some intriguing analogies that arise from studies on silica-cell interaction both in animal and plants. In particular, it is well known the different toxicity of silica powder versus mammalian lungs, in which chronic exposure to crystalline silica determines the developing of silicosis, while amorphous silica is generally non-toxic. Recent results obtained from some of us evidenced an increased cytoxicity of quartz powder pre-treated with ascorbic acid, compared to untreated quartz. Moreover, the different chemical behaviour of crystalline or amorphous silica versus ascorbic acid could explain some other interesting data on diatoms growth described by some of us. Diatoms cultured in presence of crystalline silica, in fact, showed an increased growth rate respect to the diatom cultured with amorphous silica. This particular behaviour suggests an active role of ascorbic acid, abundant in diatoms, in facilitating the silica uptake by cell wall.

CHEMICAL VARIABILITY AND ECOLOGICAL ACTIVITY OF COMPOUNDS FROM THE CARIBBEAN SPONGE *PLAKORTIS* SP.

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On many Caribbean reefs, sponges form a major component of the exposed coral reef fauna. Many sponges are able to persist in these exposed habitats by virtue of their chemical defences against diverse predators. Sponges of the genus *Plakortis* contain a diversity of biologically active secondary metabolites, and extracts of these sponges are deterrent to Caribbean reef fishes. We collected individuals of the sponge *Plakortis* sp. from various sites and depths along the north coast of Jamaica, in the Bahamas, and on the barrier reef in Belize. In addition, we compared the chemical ecology of the sponge *Plakortis* sp. from both reef and cave habitats near Lee Stocking Island, Bahamas. Reef and cave habitats differ considerably in both physical and biological parameters, including light, water motion, and microbial and predator communities. Chromatographic fingerprints indicated variation in the chemical profiles of these sponges with depth, site and location. These data were compared to molecular and morphological characteristics of the samples. Three marker compounds were further used to quantify differences between populations. We are presently testing the hypothesis that variability in chemical constituents of this sponge is the result of differential predation regimes; spongivorous fishes are virtually absent from Jamaican reefs and Bahamian caves, but are abundant on Bahamian and Belizean reefs. Reef sponges and their extracts were more deterrent to fish than were cave sponges. Whereas marine caves are free of predatory fishes, they do act as microbial sinks that concentrate a microbial community on the cave substratum, although the associated sponge fauna is relatively free of microbial overgrowth. By comparison, reef sponge surfaces harbour significantly more microbes than do their cave counterparts. Extracts of cave sponges exhibited greater antimicrobial activity against cultured marine microbes than did reef sponge extracts, and phytagel discs embedded with cave sponge extracts were significantly less susceptible to fouling than were those containing reef sponge extracts. There appears to be a trade off between predator deterrent and antimicrobial chemical defences in the reef vs cave habitats.

IDENTITY OF CYANOBACTERIAL SYMBIONT OF *XESTOSPONGIA MUTA*

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The common barrel-shaped Caribbean sponge *Xestospongia muta* harbours large numbers of an *Aphanocapsa*-like unicellular rod-shaped cyanobacteria (2.5 by 1.2 µm). From fluorescent microscopical observations, they were found to occur in the mesohyl of the peripheral tissue of the sponge. The cyanobacteria were observed individually or in clumps and some were dividing. No especially close association with sponge cells was observed, although commonly cyanocytes containing large numbers of the cyanobacteria up to 24 cyanobacterial cells were found. Cell suspensions of *X. muta* were obtained by cutting parts of the sponges in small cubes that were squeezed through a 100 µm nylon mesh in cold calcium/magnesium-free artificial seawater (CMF, pH 7.4) containing 10 mM EDTA. The suspensions were subsequently filtered through a 50 µm nylon mesh to remove cell aggregates and debris. Differential centrifugation in Percoll gradient of the cell suspension from internal and peripheral parts of the sponge yielded different cell fractions, including a band with mainly cyanobacteria from the peripheral part. Extracted DNA of this purified fraction of cyanobacteria was amplified, cloned and part of the 16SrRNA region was sequenced. According to the phylogenetic analysis performed in BLAST the sequences show a 95% similarity with *Prochlorococcus* and *Synechococcus* species. According to its absorption spectrum the cyanobacterium contains phycourobilin and phycourythrobilin which are not found in *Prochlorococcus*, but do occur in some *Synechococcus* species. From TEM observations, it appeared that the *X. muta* cyanobacteria have a spiral thylakoid, consisting of ± 5 turns, which is likewise not reported in *Prochlorococcus*, but present in *Synechococcus* species. Symbiont-specific oligonucleotides for *in situ* hybridisation were designed from the variable regions of the SSU rRNA gene. Four target sites were selected by checking different sites of the gene on the CHECK PROBE (RDP) and BLAST (GenBank) database. We were unable to obtain a positive FISH signal with these probes on the cyanobacteria isolated from *X. muta*. Because of this failure we cannot state with absolute certainty that we have isolated the DNA from the prolific cyanobacteria observed in the periphery of *X. muta*. However, we did obtain FISH signals with general cyanobacterial probes (CYA(c) 762 and CYA(c) 664), so the most likely reason for FISH to fail with the specific probe is that target sites are scarce and inaccessible for probes in this area of the 16S rRNA.

In order to detect if the *Synechococcus*/*Aphanocapsa*-like symbiont of *X. muta* is involved in the production of the bioactive compounds characteristic for this species (bromo-acetylenic acids, among which XMA11 was dominant), a "light-dark" experiment was performed *in situ*. 12 similarly sized individuals of the sponge were transported to a flat part of the reef at 20 m. Two specimens acted as controls, 6 were put under a canopy allowing only reflected light to reach the sponges, 6 were kept in the light. At three time intervals over a period of 7 weeks two individuals from both lots were taken for subsampling and extraction. If the cyanobacteria were involved in compound production and were affected by the loss of light, then a clear decrease over time was assumed to occur. Subsamples were analyzed for loss of cyanobacterial cells by counting tissue samples under fluorescent light microscopy, and for loss of compound by quantification of the major derivative XMA11 in standardized subsamples of the sponge individuals. Cell counts were down in individuals shielded from the light compared to those kept in the light. XMA11 on average increased rather than diminished. The combined data indicate that the *Aphanocapsa*-like cyanobacteria are probably not involved in its production or storage.

POLYMORPHISM IN FREE-SWIMMING LARVAE OF *HALISARCA DUJARDINI*

(DEMOSPONGIAE, HALISARCIDA)

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Polymorphism is a characteristic feature of Porifera. Most sponges have a stage of free-swimming larva in their life cycle. The phenomenon of polymorphism characterizes this stage as well. It is expressed both in the characteristics of permanent morphological structures and in the emergence of new characters in a part of conspecific larvae. Some characters develop in the larvae during their free life period, whereas some develop in embryogenesis. However, polymorphism usually appears during the period of embryonal development, which allows us to estimate the degree of stability and the level of regulation of embryonal morphogeneses. Demospongiae larvae provide the most extensive material for such studies.

We investigated the larvae of *Halisarca dujardini* (Halisarcida) with the use of SEM, TEM and light microscopy. The larvae are characterized by variability of different morphological structures. The causes of this variability are diverse. We have delimitated a number of characters emerging during embryogenesis, determined the developmental stage when they emerge and possible morphogenetical processes causing their variability. The larvae with different morphological characters may develop in the same mother organism. Therefore we termed this phenomenon "individual larval variability".

The size of the larvae varies: diameter 120-152 µm, length of anterior-posterior axis 112-136 µm. Anterior-posterior polarity is morphologically expressed in the structure of the external layer of flagellated cells. The number of cells in the layer at the posterior pole and the anterior hemisphere of the larva varies, and so does their size. Thus, the curvature of the poles and, consequently, the shape of the larvae are slightly different.

The larval cells belong to three types: flagellated, amoeboid and granular mother cells. The external layer of flagellated cells borders the internal cavity. The number of amoeboid cells in the cavity varies significantly. Larval morphotypes of *H. dujardini* are delimited on the basis of the differences in the internal cavity structure. If it is filled by a conglomerate of amoeboid cells, the larvae are called parenchymulae. In synchronous culture, larvae with only single amoeboid cells in the cavity are sometimes observed. We called these larvae coeloblastulae. In some larvae, an inner chamber (rarely two) is present, formed by flagellated cells, whose ultrastructure is identical to that of the external flagellated cells of the anterior larval hemisphere. The number of the cells forming the chamber, their shape, and, correspondingly, the size of the chamber vary. We called these larvae disphaerulae (Ereskovsky, Gonobobleva, 2000).

Internal cavity formation in *H. dujardini* larvae takes place after the third cleavage division, its anterior-posterior axis is morphologically expressed at the stage of ca. 600-1000 cells. The formation of internal cells starts in parenchymula consisting of 100-120 cells, and the flagellated chamber of disphaerula is formed at the final stages of embryonal development.

Various endogenous and exogenous factors may determine the formation of polymorphic structures in the course of embryogenesis. Revealing the function of larval structures in Porifera and the ways of their transformation in morphogenesis remains a very important task.

SPONGE ASSOCIATED BACTERIA FROM BOREAL SPONGES

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The microbial part of the BMBF BOSMAN project (Boreal Sponges; Sources of Marine Natural Products) investigates the diversity of bacteria associated with sponges from a boreal deep water system. A variety of sponges which belong to the subclasses Tetractinomorpha, Ceractinomorpha and Homoscleromorpha collected from the Sula Reef as well as from the shelf of the Norwegian coast were analysed for their bacterial content. Diverse approaches for analysis were chosen: in addition to the direct enrichment, cultivation and isolation of the sponge associated bacteria continuous culture systems with low nutrient conditions were set up to enrich for oligotrophic microorganisms. The information obtained from sequencing and phylogenetic affiliation of the isolates was compared to data obtained by molecular biological methods e.g. cloning. To date more than 500 sponge associated bacteria were isolated and phylogenetically characterized. A cloning approach is in progress from sponges of the families Geodiidae and Axinellidae.

THE HIGH PROTEIN DIET OF THE TROPICAL MARINE "SPONGE"

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The sponge, *Haliclona cymaeformis*, has a unique symbiotic relationship with the red alga *Ceratodictyon spongiosum* in that the association is extracellular and contains more alga than sponge. *H. cymaeformis* can meet its nutritional needs through a combination of heterotrophy and photoautotrophy. Previously, we have shown that the algal symbiont contributes a significant component to the overall carbon budget of the association while nitrogen is supplied by heterotrophy. Here we report the potential heterotrophic contribution of the sponge to the association's nutrition. We conducted feeding clearance studies during the day and at night over two seasons (winter and summer) at One Tree Island (23°30' S, 152°06' E), on the southern Great Barrier Reef. Abundance of ultraplankton (heterotrophic bacteria, *Prochlorococcus* sp., *Synechococcus*-type cyanobacteria and protozoans) was monitored over a 30 minute time series and quantified using flow cytometry. Ultraplankton concentrations in the lagoon were extremely low, less than 10^5 cells ml^{-1}. It appears that only protozoans were at high enough concentrations to be retained by the sponge. Removal rates for protozoans were significantly higher in summer than in winter. During the summer, retention of protozoans occurred only during the night, whereas during the winter removal rates were not different between day and night. Protozoans are an excellent source of nitrogen. During winter *H. cymaeformis* retained 0.2 mg C day^{-1} g wet $weight^{-1}$ and 0.3 mg N day^{-1} g wet $weight^{-1}$. During summer the C:N ratio of the retained material is also 0.67, but only about half as much C and N are retained in total, as the sponge retains particles only at night. It has been suggested that translocated carbon is preferentially used in invertebrate-algal symbioses, but it may be that the diet of mixotrophs are higher in nitrogen than carbon. Like deep sea mixotrophic organisms, *H. cymaeformis* seems to be using heterotrophy to acquire new nitrogen to support growth.

NEW AND BIOLOGICALLY ACTIVE IMIDAZOLE ALKALOIDS FROM TWO SPONGES OF THE GENUS *LEUCETTA*

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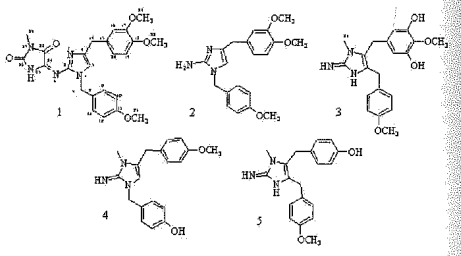

Chemical investigation of two sponges *Leucetta chagosensis* and *Leucetta* cfr. *chagosensis*, collected from the Great Barrier Reef and the Fiji Islands, respectively, has led to the isolation of three new imidazole alkaloids (1-3), along with the known compounds isonaamine B (4) and naamine A (5). The structures of the new compounds (1-3) were elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). The structures of the known compounds 4 and 5 were determined by comparison of their ^{1}H and ^{13}C NMR spectroscopic data with published values. Compounds 1 and 2 were found to be cytotoxic towards several tumour cell lines (GI_{50} values ranged from 1.3 to 7.0 μg/mL).

MOLECULAR STUDIES ON INTRASPECIFIC GENETIC POLYMORPHISMS OF *HYMENIACIDON HELIOPHILA* (PORIFERA)

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The phylum Porifera shows considerable morphological variability. *Hymeniacidon heliophila*, Parker, 1910 is reported for the Atlantic Ocean from the North Carolina coast (USA) to the Caribbean Sea, where it is widely distributed. This species is also reported for the Southeastern and South Brazilian coast (Rio de Janeiro, São Paulo and Santa Catarina States). In Brazil, no taxonomic description has been published for specimens from these areas. However we have observed extensive morphological variation depending on the environment where the sponge lives: long papillas in sandy bottoms and short ones in rocky shores under impact of waves. We looked for molecular characters in order to check if different morphotypes belong to the same species. The polymerase chain reaction based single-strand conformation polymorphism method (PCR-SSCP) was used to detect sequence variation in the first and second internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA genes. The PCR-SSCP method allowed us to quickly detect different migration patterns of fragments, which reflect DNA sequence variability. Furthermore, this method is simple, of low cost, fast and non-radioactive. Another advantage is the presence of the ribosomal DNA transcription unit in multiple copies in the genome, which allows us to amplify the ITS sequences even from specimens conserved in Museum collections (dried or in alcohol). Our results demonstrate that is possible to distinguish different individuals and populations by the migration patterns of DNA fragments. Although several alleles can be distinguished in each population, the genetic variability is much higher inside a single population than between distinct populations. This feature is not uncommon in marine invertebrates and maybe due to evolutionary processes. To access the number and type of mutations, experiments of dideoxynucleotide sequencing of the ITS1+5.8S+ITS2 fragment are being carried on.

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LOCAL DESCRIPTIVE BIOGEOGRAPHY - SPONGE DISTRIBUTION AT THE SÃO SEBASTIÃO CHANNEL AREA

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An increase in collecting effort conducted in the last decades, summed up to the initial taxonomic screening of large Brazilian oceanographic collections, has contributed not only to double the number of species known, but also to highlight the essentially fragmentary aspect of sponge biodiversity inventory along the Brazilian Coast. Faunas being poorly known even on the vicinity of many major coastal cities, where most universities are located. A detailed inventory of the fauna of the São Sebastião Channel and its vicinities (ca. 23°49' S – 45°25' W, São Paulo State, Brazil) has been conducted during the years 1996 to 1998, aiming at producing a species list, but also at mapping sponge distributions in order to select highest density localities for natural products targeted surveys. Collecting has been conducted by scuba diving, snorkelling or wading at low tide. In total, 79 stations spread over some 80 km of rocky coasts were visited; 58 of which within a predefined protocol consisting of only 30 min observation. These observation periods were homogeneously distributed in depth, as well as over different microhabitats (e.g. surface, wall and under surface of small, medium and large boulders). This biodiversity inventory improved the list of marine sponges known from the area from 34 in 1995 to over 120 in 1996. Many of these are still in need of detailed morphological study in order to be fully identified. A small component has been described recently, either as new species (14), or as new records for the area (3). From the ca. 120 species found, 68 are confidently assigned to species, and were recognisable *in situ*. From these, the affinities of the shallow water sponge fauna of the area can be summarised as follows: 57 % Tropical western Atlantic, 24 % are provisional endemics to the study area, 10 % are provisional endemics to the Paulista Biogeographic Province (ca. 20-35° S), and 9 % possess largely discontinuous distributions and are thus considered dubious identifications. The depth range analysed varied from intertidal to 21m. Richness, from 3 to 24 species. Abundance, measured as number of stations of occurrence, from 1 to 40. Richest stations had only between 25 and 50 % of the species found through the predefined protocol (14 stations, with 17-24 species). Under the quartets definition for the recognition of rare

entities, the great majority of stations was poor (76 %, 44/58 stations, 1-16 species) with less than 25% of the species, and from these, a few (5 %, 3 stations, 1-6 species) are considered very poor, as they possess less than 10% of the species. Two of these are obviously highly impacted places. Very common species (10/68) were all those occurring in 50 to 75% of stations (29-40, e.g. *Dragmacidon reticulatus* and *Scopalina ruetzleri*), common ones (13/68) occurred in 25 to 50% of stations (15-24, e.g. *Amphimedon viridis* and *Mycale magnirhaphidifera*), rare (11/68) in less than 25 % (7-14, e.g. *Mycale laxissima* and *Polymastia janeirensis*), and very rare (34/68) in less than 10 % (1-6, e.g. *Biemna* sp. and *Callyspongia* sp.). *Scopalina ruetzleri* confirmed its status as one of the most abundant sponges in the Tropical western Atlantic.

MICROBIAL DIVERSITY OF MARINE SPONGES

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Sponges (Porifera) form one of the deepest radiations of the Metazoa whose origins date back to the Precambrian times. Sponges contain large amounts of bacteria that are embedded within the animal matrix and that can amount to 40% of the biovolume. This population consists mostly of extracellular bacteria that are enclosed within the mesohyl matrix and that are physically separated from the seawater by contiguous host membranes. Because sponge-bacteria interactions are presumably evolutionarily ancient, widely distributed, and in some cases specific to their host, it is generally believed that symbiotic interactions exist between sponges and microorganisms.

With the availability of molecular tools for community analyses in microbial ecology, the area of sponge microbiology has gained new momentum. It is now possible to obtain phylogenetic information on complex microbial consortia, including those that have so far eluded cultivation efforts. In order to provide insights into the microbial diversity of marine sponges, we performed a comprehensive diversity survey based on 190 sponge-derived 16S rDNA sequences. The sponges *Aplysina aerophoba* and *Theonella swinhoei* were chosen for library construction of bacterial 16S rDNA because they are taxonomically distantly related and populate non-overlapping geographic regions. In both sponges, a uniform microbial community was discovered whose phylogenetic signature is distinctly different from that of marine plankton or marine sediments. Altogether 14 monophyletic, sponge-specific sequence clusters were identified that belong to at least seven different bacterial divisions. By definition, the sequences of each cluster are more closely related to each other than to a sequence from non-sponge sources. These monophyletic clusters comprise 70 % of all publicly available, sponge-derived 16S rDNA sequences reflecting the generality of the observed phenomenon. This shared microbial fraction represents the "smallest common denominator" of the sponges investigated in this study. Bacteria that are exclusively found in certain host species or that occur only transiently would have been missed. Fluorescence *in situ* hybridization (FISH) confirmed the existence of the respective microorganisms in sponge tissues. A picture emerges where sponges can be viewed as highly concentrated reservoirs of so far uncultured, elusive and possibly evolutionarily ancient marine microorganisms.

GEODIA BARRETTI (GEODIIDAE, DEMOSPONGIAE): AN ANOXIC ECOSYSTEM?

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Geodia barretti is common in Norwegian fjords and Arctic oceans below 40 m depth. This massive, globular sponge can grow up to 50 cm in diameter and is known to contain secondary metabolites which are biologically active. The dense choanosome of *G. barretti* contains about 2,45 x 10^{11} bacteria per g wet weight. Community structure of associated bacteria was examined by 16S rRNA sequencing of cloned DNA fragments, and by fluorescence *in situ* hybridization (FISH) with specific oligonucleotide probes on histological sections. Sulfate reducing bacteria (SRB) were found to be evenly distributed in the mesohyl. About 2 x 10^{10} SRB/cm^3 tissue were counted, comprising approximately 6,8 % of the bacterial community. Sulfate reduction rates were measured with $^{35}SO_4^{2-}$ and showed very strong spatial variability ranging from 1-1000 nmol cm^{-3} d^{-1} within the same sponge. The measured sulfate reduction rates depend on the incubation time indicating that sulfate reduction is directly coupled to sulfide re-oxidation. Profiles measured with oxygen sensitive microelectrodes showed steep gradients and anoxic zones in the mesohyl of actively pumping *G. barretti*. Anaerobic bacterial communities may play a key role in fermentation processes, as well as in the production of secondary metabolites. Our results show that this sponge encompasses a complex chemical environment with numerous micro-niches and rapid turnover rates. In the future, more attention should be paid to chemical gradients and anaerobic processes in sponge tissues, which may lead to new ideas about sponge biology and evolution.

SYSTEMA PORIFERA. A GUIDE TO THE CLASSIFICATION OF SPONGES .... THE END OF A BEGINNING

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The *Systema Porifera* collaboration (36 authors from 16 countries) produced a two volume treatise revising and defining the supraspecific classification of sponges and spongiomorphs (Kluwer Academic/Plenum Publishers). The *Systema* recognises approximately 680 genera of living sponges and 1000 genera of fossil sponges from many thousands of nominal taxa, and places these taxa in a unified higher systematics scheme (including over forty new higher taxa proposed). Most genera were revised from their type material (where available), reinterpretation of the vast sponge literature, and incorporation of other biological evidence where available. The *Systema*, therefore, has an important theoretical basis, being: the most comprehensive taxonomic revision of sponges at genus level and above; addressing the many long-outstanding nomenclatural problems (and thus stabilising the nomenclature); and providing a sound baseline to focus detailed research questions on sponges in the future. It also has a strong practical focus as a tool for sponge identification: providing concise definitions, diagnoses, keys and illustrations of all the valid (i.e., reinterpreted) genera of extant sponges, and some key fossil sponge genera, unified into a single classification of Porifera); and serving as a manual to achieve more accurate faunal inventories that will be of benefit to biodiversity and biogeographic analyses etc., and thus marine conservation and planning. In this paper we critically analyse the strengths (achievements) and weaknesses (remaining challenges) of the *Systema Porifera* project, and highlight some areas where research might be productively directed in the future, including questions of the monophyly of Porifera itself.

SYSTEMATIC STATUS OF *HALICHONDRIA JAPONICA* (KADOTA) (DEMOSPONGIAE, HALICHONDRIDA) FROM JAPAN

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Halichondria japonica (Kadota) of the family Halichondriidae is commonly found in the intertidal rocky shore around Honshu, Shikoku and Kyushu in Japan, and also in southern Korea. These sponges thickly and broadly encrust the lower part of rocks. The color in life is bright to dark orange depending on habitat. In this study, the systematic status of *H. japonica* was reevaluated based on the external general morphology, spicule character and arrangement, and sequence of genomic DNA.

This species had been originally referred to the genus *Reniera* Nardo, 1847 (Chalinidae, Haplosclerida) by the original author in 1922, and later transferred to the genus *Halichondria* Fleming, 1828 in "Coloured Illustrations of Seashore Animals of Japan" (Utinomi, 1962; Hoikusha Publishing Co., Ltd., Osaka, Japan) without discussion. We guess this transference is due to disagreement with diagnostic characters of *Reniera* having reticulate skeleton of oxeote spicule. *Halichondria* is characterized by having mainly oxeote spicules, a thin membrane and tangential spicule bundles in the ectosome, and tracts of spicules in the endosome. However, it is apparent that H. japonica having only stylote spicules instead of oxeote spicules differs generically from the *Halichondria* species.

The type specimen of *Halichondria japonica* might be lost; therefore specimens examined were mostly collected from the type locality, Aburatsubo in Sagami Bay, Pacific coast of central Japan. After observing external morphology, part of each sample was fixed in 70 % ethanol for morphological study, another part was fixed in 99 % ethanol for molecular analysis. A small fragment of each specimen was boiled in nitric acid to isolate spicules and make spicule preparation. To observe spicule arrangement, some parts of each specimen were dried or embedded in Tissue prep, and preparations of vertical and tangential sections were made by hand section.

The following morphological characters were observed. Oscula are opened on each apex of conical or cylindrical processes that are irregularly scattered on the surface. The spiculation consists of only styles that are straight or slightly curved, 120-350 μm long, and 3-10 μm wide. The ectosome consists of a thin membrane containing a few rows of spicules. In the surface, styles are arranged in a loose reticulation. In endosome a few or several rows of styles form tracts which are arranged perpendicular to the ectosomal membrane.

Considering the characters observed above, it is definitely said that *H. japonica* has *Halichondria*-like arrangement of spicules and less well-developed ectosomal membrane. However, this species differs from the diagnostic characters of *Halichondria* in having only stylote spicule. These characters agree with those of *Hymeniacidon* Bowerbank, 1861 (Halichondrida, Halichondriidae) which is closely related to *Halichondria*, having no oxeote spicules, and retaining styles. Morphological characters suggest that *Halichondria japonica* should be referred to the genus *Hymeniacidon*.

We extracted the total genomic DNA from several specimens of *Halichondria*

japonica by using CTAB. ITS2 (approximately 180-215 bps) of rDNA was amplified. Each PCR product was sequenced and compared with those of *Halichondria panicea* (Pallas, 1766), *Hymeniacidon heliophila* (Parker, 1910) and *Axinella damicornis* (Esper, 1794) obtained from the GenBank.

The sequence of ITS2 region of *Halichondria japonica* was completely coincident with that of *Hymeniacidon heliophila*, and 65 % and 30 % with those of *Halichondria panicea* and *Axinella damicornis*, respectively. These results also suggest the systematic status of *H. japonica* is in the genus *Hymeniacidon*.

INSIGHTS IN THE CHEMISTRY OF MARINE SPONGES

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Marine organisms have long been recognised as likely to contain many potential new drugs because of the environmental conditions that are unique to their habitat. Due to the increased number of sponge secondary metabolites of economical value, the interest in sponge studies has increased dramatically over the last years (Faulkner, 2001).

Although secondary metabolites represent a very important part of the sponge chemistry they are not the only interesting feature of these organisms. Recently attention was driven to some other biological polymers such as proteins and polysaccharides that have been recognized as biologically active in heterologous systems.

Chemical and biochemical studies on sponges from the Portuguese coast are extremely scarce. The presence of considerable amounts of halogens in sponges prompted us to investigate the presence of halogenating enzymes and some other oxido-redutases which can be related to the use of sponges as bioindicators.

Inspired by the discovery of two proteins that inhibit the HIV action (O'Keefe, 1997; O'Keefe,1998) we found that extracts from a marine sponge of our coast have a strong anti-HIV activity.

Our attention was also focused on sulphated polysaccharides, as a source of HIV inhibition, and isolation and characterization of polysaccharides from Portuguese sponges was also performed.

PSAMMOBIONTIC CLIONAIDAE (DEMOSPONGIAE: HADROMERIDA) IN LAGOON OF THE RYUKYU ISLANDS SOUTHWESTERN JAPAN.

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In the Ryukyu Islands (Southwestern Japan), there are well-developed coral reefs, where the lagoon bottoms are composed of calcareous debris such as coral sands, molluscan shells, etc. The field survey of clionaid sponge fauna in the Ryukyu Islands revealed peculiar species in the lagoon. The largest specimen collected was columnar, 13 cm long and 4 cm in diameter, and about 80 % of the body was vertically buried in sand. The color in life was dark brown for the epibenthic part and cream for the infaunal part. Some oscular openings are located on top of the epibenthic part, and its ostia are scattered the entire surface of the body. Infaunal part incorporates various calcareous materials such as coral rubbles, molluscan shells, foraminiferan shells and spines of echinoderms. Surfaces of the incorporated calcareous materials show many conspicuous polygonal subcircular pits of 39-63 μm in diameters. These are typical erosion traces by excavating sponges, suggesting bioeroding activity of the specimen. Tylostyles as megascleres have two size classes. Larger tylostyles have elongate heads, which are sometimes reduced; tips are sharply pointed; their average dimensions of shaft length-shaft width-head width are 464-14.7-13.4 μm. Smaller tylostyles are usually straight, with elongated heads and sharp pointed tips; their average dimensions of shaft length-shaft width-head width are 276-9.7-10.0 μm. Spirasters as microscleres usually have two or three bends, ornamented with clusters of small spines mostly at the convex side of the shaft. Clusters of spines are particular in both ends. Smaller spirasters with straight shaft are modified to amphiasters. Average dimensions of shaft length-shaft width-spine length are 14-1.8-1.8 μm. In the epibenthic part, ectosomal brushes of tylostyles are arranged perpendicular to the surface, subectosomal tylostyles form dense tracts. In the infaunal part, tylostyles are also arranged perpendicular to the surface, but less conspicuous. Spirasters are mainly confined to the peripheral region of the ectosome. The component and arrangement of the spicules mentioned above suggest systematic position of this species in the family Clionaidae D'Orbigny, 1851.

Excavating into solid calcareous substrate and living in the cavities are common for most of the other clionaid species. But an early excavating stage has not been found for this species. The other clionaid species that lacks an excavating stage is *Cervicornia cuspidifera* (Lamarck, 1815) inhabiting sandy bottoms of the Caribbean Sea. The endopsammic habit of this species is similar to that of *C. cuspidifera*. The epibenthic part of *C. cuspidifera* is special inhalant fistule and the exhalent stolons are ending underground (Rützler & Hooper, 2000). However this species has oscular openings on top of the epibenthic part.

Based on their columnar shape, spicule arrangement and single or multiple oscular openings on top of the epibenthic part, we allocate the specimens from the Ryukyu Islands to the genus *Spheciospongia*, Marshall, 1892.

MULTIPLE VARIATIONS OF THE FATE OF THE FLAGELLATED CELLS DURING THE METAMORPHOSIS OF THE PARENCHIMELLA LARVAE IN FRESHWATER AND MARINE DEMOSPONGES

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Studies on the ultrastructure of the flagellated cells during free-swimming stage and during metamorphosis were made for the larvae of the marine *Halichondria panicea* and three freshwater sponges: *Spongilla lacustris, Ephydatia muelleri* and *Eunapius fragilis*. It was found that most flagellated cells of the larvae take part in formation of the young sponge body, and only a small part of them endures the phagocytosis. After the settlement, some flagellated cells migrate towards the inside of the larva, taking amoeboid form; their flagella remain in the cytoplasm for a long time. Later, these cells may transform in multiple ways. For example, they may take part in formation of the choanocyte chambers of the young sponge. The cells remaining at the surface *in situ* transform into exopinacocytes, which may keep their flagella and characteristic morphology in the dermal membrane of the functioning sponge. The process of transformation of the larva flagellar cells into exopinacocytes is accompanied by the loss of flagellar axoneme. The multiple variations of the surface flagellar during larval metamorphosis were observed either in a single larva and in different larvae descended from a single sponge.

The obtained data are discussed regarding the questionable application of the terms.

NOTES ON IRCINIDAE FROM KOREA

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Sponges of the order Dictyoceratida are poorly known in Korea. Only 11 species of the family Ircinidae and two species of the family Spongiidae were reported from many island and coastal areas of the East Sea, Yellow Sea and South Sea of Korea. Sponges belonging to the family Ircinidae are characterised by an anastomosing skeletal network of primary and secondary spongin fibres and fine filaments with terminal knobs. A new massive sponge, probably belonging to the genus *Sarcotragus*, has been collected from Jejudo Island. The primary fibres are slightly fasciculated and divide into two or three branches, which support conules and protrude out of the surface. Primary fibres usually lack any coring material. The simple secondary fibres are clear of debris. Besides primary and secondary fibres the skeleton is supplemented by dense aggregations of fine filaments. The studied species seems closely related to *Sarcotragus arbuscula* (Lendenfeld, 1889) as to growth form and simple fibre network. In Lendenfeld's species the distinction of the fibres into main and connecting fibres is difficult, whereas in the Korean species primary and secondary fibres are easily distinguished. Filaments are thinner than those of *S. arbuscula* and it is remarkable that two or three filaments may coil each other forming a thicker filament which shows a peculiar surface aspect. To date, within the genus *Sarcotragus*, only very fine filaments have been described. However, the present study has proved that the filament thickness in some *Sarcotragus* species largely depends on the species. It was found that the thickness of filaments in some *Sarcotragus* species was even similar to that of sponges belonging to other genera within the family Ircinidae. Consequently, further proof is required in order to define the thickness of filament of *Sarcotragus* filaments as always thin. This study shows that there is a possibility of a wide variety in the filament thickness within the genus *Sarcotragus*.

FIRST REPORT ON THE SPONGES FROM THE ANTARCTIC DEEP-SEA EXPEDITION ANDEEP II (NORTHERN WEDDELL SEA AND SLOPE OF SOUTH SANDWICH TRENCH)

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During the Antarctic deep-sea expedition ANT XIX/4, 28.02-1.04.2002, the sea bottom in the northern Weddell Sea and the slope East off South Sandwich Islands were intensively explored by video and different collecting gears. Eleven out of 12 stations, where the agassiz-trawl was operating, were successful in terms of sponges.

A significant divergence seems to exist between the western and the eastern Weddell Sea in the sense that sponge abundance and species diversity are generally higher closer to the Antarctic Peninsula and decrease eastwards. This is true for both the Demospongiae and the Hexactinellida, and the only three calcarean specimens obtained were also collected in the western part of the Weddell Sea. The tendency is independent of water depth, but it might be related with a decreasing supply of suspended nutrition towards the East, or maybe with the chemistry of the mostly basaltic substrates from the volcanic Sandwich Islands.

The upper limit of the true deep-sea sponge fauna in the Weddell Sea is very deep: at 2000 m many shelf species are present and around 3000 m there seems to be a general impoverishment in the numbers of both species and individuals, before the true deep-sea sponge association sets, somewhere between 3000 and 4000 m. The deep-sea poriferan community mainly consisted of cladorhizid Demospongiae and some specialized Hexactinellida species, such as *Bathydorus* and *Caulophacus*. For the first time, calcarean sponges are reported from the Antarctic deep sea: two specimens were collected from ~ 1120 m and one from ~ 4065 m depth.

Because of the patchy occurrence of the Antarctic sponge associations, species turnover between the investigated stations is high and thus the necessary biogeographic comparison with other deep-sea sponge faunas on the basis of our limited data are very difficult. We are now only beginning to understand the nature of these peculiar specialized deep-sea poriferan communities, and the controlling ecological parameters are largely unknown. Further investigations of the deep-sea benthic life are necessary.

LEUCAMIDE A: A NEW CYTOTOXIC HEPTAPEPTIDE FROM THE AUSTRALIAN SPONGE *LEUCETTA MICRORAPHIS*

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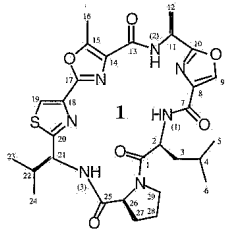
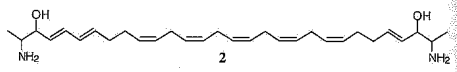


1

Leucamide A (**1**), a bioactive cyclic heptapeptide containing a unique mixed 4, 2-bisheterocycle tandem pair consisting of a methyloxazole and thiazole subunit was isolated together with the known compound BRS1 (**2**), from the dichloromethane extract of the Australian marine sponge *Leucetta microraphis*.



2

The planar structure of leucamide A (**1**) was elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). Its absolute stereochemistry was established by chemical degradation, derivatisation and chiral GC-MS analysis. A conformational analysis of (**1**) was made using MMFF. Leucamide A (**1**) was found to be moderately cytotoxic towards several tumour cell lines.

A NEW FOSSIL LITHISTID SPONGE FROM SOUTHERN NEW ZEALAND: PALAEOECOLOGY AND EVOLUTIONARY LINKS

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Numerous remarkably well-preserved lithistid sponges, recovered from the late Eocene - early Oligocene Ototara Limestone at Kakanui in the South Island of New Zealand, represent the first sponge body fossils to be described from the New Zealand Coenozoic. The sponges are scattered throughout a 1-3 m thick volcaniclastic limestone horizon immediately overlying the Kakanui Mineral Breccia. The sponge fossils are now solid calcite, the former siliceous skeleton having been replaced by calcite during diagenesis. The fossils are morphologically indistinguishable from a new species of living *Pleroma* ("Order" Lithistida: Family Pleromidae) from deepwater seamounts and banks off north-eastern New Zealand. The present day limited distribution of this new species of *Pleroma* to silica-rich deeper waters is in marked contrast to the relatively shallow warm water volcanic environments occupied during the Palaeogene. This restriction, and that of related lithistid sponges to silica-rich deeper waters off northern New Zealand, is paralleled in other demosponges and several non-siliceous invertebrate groups such as barnacles, bryozoans, and crinoids.

THE FAUNA OF CALCAREOUS SPONGES (CALCAREA) OF THE RUSSIAN PART OF THE SEA OF JAPAN

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Until present time the fauna of calcareous sponges of the Russian part of the Sea of Japan remains weakly studied. Only two papers containing information about calcareous sponges in this region have been published. One paper by Burton (1935) recorded seven species of Calcarea for the Sea of Japan. The other by Koltun (1955) reported one species for shallow waters of the Sea of Japan.

This work is based on the collection of Calcarea of the Sea of Japan of the Zoological Institute of the Russian Academy of Sciences. By studying this collection and analysis of the literature, the species list of the Russian part of the Sea of Japan is presented here.

Subclass Calcinea Bidder, 1898
Order Clathrinida Hartman, 1958
Family Clathrinidae Minchin, 1900
1. *Clathrina canariensis* (Miducho-Maclay, 1868)
2. *Clathrina clathrus* (Schmidt, 1864)
3. *Clathrina coriacea* (Montagu, 1812)

Family Leucettidae Borojevic, 1968
4. *Leucetta poculiformis* (Hozawa, 1918)

Subclass Calcaronea Bidder, 1898
Order Leucosolenida Hartman, 1958
Family Leucosoleniidae Minchin, 1900
5. *Leucosolenia sp.*

Family Sycettidae Dendy, 1892

6. *Sycon compactum* Lambe, 1893
7. *Sycon protectum* Lambe, 1896

Family Grantiidae Dendy, 1892
8. *Grantia nipponica* Hozawa, 1918

9. *Grantia uchidai* Hozawa & Tanita, 1941
10. *Leucandra pancispina* Hozawa, 1929
11. *Leucandra pyriformis* (Lambe, 1893)

Family Heteropiidae Dendy, 1892
12. *Grantessa nemurensis* Hozawa, 1929
13. *Heteropia mediocarticulata* Hozawa, 1918
14. *Vosmaeropsis japonica* Hozawa, 1929

Order Baeriida Borojevic, Boury-Esnault & Vacelet, 2000
Family Baeriidae Borojevic, Boury-Esnault & Vacelet, 2000
15. *Leucopsila stilifera* (Schmidt, 1870)

To summarize, fifteen species of Calcarea are listed for the Russian part of the Sea of Japan. Among them, five: *Leucetta poculiformis* (Hozawa, 1918); *Sycon protectum* Lambe, 1896; *Grantia uchidai* Hozawa & Tanita, 1941; *Heteropia mediocarticulata* Hozawa, 1918; *Leucopsila stilifera* (Schmidt, 1870) are new records for the fauna of the Sea of Japan.

BROMOTYROSINE DERIVATIVES FROM THE MARINE SPONGE *SUBEREA* AFF. *PRAETENSA*

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All sponge genera of the order Verongida (class Demospongiae, subclass Ceractinomorpha) which have so far been examined chemically contain secondary metabolites derived from bromo- or chlorotyrosine in which the side chain has been converted into a variety of nitrogenous groups while the aromatic ring has been retained or has undergone rearrangement or reduction. A subclass of these metabolites consist of compounds such as the fistularins in which one or two modified tyrosine moieties are attached to a chain consisting of variously modified 3,5-dibromo-4-(γ-amino propoxy)-phenylethylamines.

The first and so far only bromotyrosine derivatives isolated from a non-verongid sponge were the agelorins A and B found together with 11-epifistularin B in *Agelas oroides*. Recently we have reported isolation from a Gulf of Thailand collection of *Suberea* aff. *praetensa* (Demospongiae, Ceractinomorpha, Verongida, family Aplysinellidae) of the agelorins A and B , the new 11,17-dideoxyagelorins A and B and the related, fistularins-3 as well as clionasterol. Further investigation of the extracts of *Suberea* aff. *praetensa*, collected at the same localist but in different periods led to the isolation of cavernicolin 1, cavernicolin 2, 5-chlorocavernicolin, 5-bromocavernicolin, 3,5-dibromo-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide, 5,5-dibromo-4-hydroxyphenylacetamide as well as *bis*-2-oxazolidone derivative. The *in vitro* effect of these compounds on the growth of five human cancer cell lines: MCF-7 (breast), NCI-H460 (lung), SF-268 (CNS), TK-10 (zenal) and UACC-62 (melanoma) has been evaluated. The results showed that while the acetate of 3,5-dibromo-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide gave a modest inhibitory effect (GI_{50} >20 μM), the acetate of 5-chlorocavernicolin was found to be a potent inhibitor of the growth of MCF-7, SF-268 and UACC-62 cancer cell lines (GI_{50}<10 μM).

GENETIC CHARACTERISTICS OF *HALICHONDRIA* SPP. POPULATIONS IN SOUTHCENTRAL ALASKA USING MICROSATELLITE LOCI AND ITS SEQUENCE DATA

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Eight microsatellite loci were isolated from genomic DNA of *Halichondria* cf. *panicea* collected in Kachemak Bay, Alaska. After primer development and PCR optimization for the eight loci were conducted, four intertidal populations of *H.* cf. *panicea*, two from semi-exposed hard substrate habitats and two from protected soft-sediment sites, were analyzed for genetic population structure and gene flow. The results showed a striking difference between the individuals at different habitat types, raising taxonomic issues. Analyses of portions of the internal transcribed-spacer sequences (ITS-1 and ITS-2) located between the ribosomal subunits coding regions revealed three primary genotypes within the four sample populations. A single genotype (A) was almost exclusively found at exposed hard-substrate sites, while two genotypes (B and C) were primarily found in sheltered, soft-sediment habitats. Ongoing morphological, ecological and genetic analyses should result in identification of the sponge species and/or subspecies colonizing various coastal habitats of south-central Alaska.

MULTIPLE ECOLOGICAL FUNCTIONS OF SPONGE SECONDARY METABOLITES

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In our recent studies on marine sponges we have found two examples of secondary metabolites with multiple ecological functions. One example is from tropical waters, where bromopyrrole alkaloids fulfil at least two ecological roles for sponges of the genus *Agelas* (feeding deterrency against fish and inhibition of bacterial attachment; for details see abstract of Assmann *et al.*). In another example from artic waters, the isolated major metabolites possessed two ecological functions (feeding deterrency against amphipod and starfish, and antibacterial activity; for details see abstract of Volk *et al.*). The latter sponge was identified as *Haliclona viscosa*. Since the structure elucidation of the secondary metabolites of *Haliclona viscosa* was challenging, some details will be presented. Despite the large number of protons, the structure elucidation of the two new compounds has been difficult. More than 50 % of the protons are located in one signal, revealing a major problem of structure elucidation by NMR spectroscopy. The structures were finally solved by a combined approach of NMR spectroscopy, MS spectrometry (including MS/MS) and chemical synthesis. The compounds consist of two *N*-alkyl, 3-alkyltetrahydropyridin rings which are connected by C_9/C_{10} and C_{11} alkyl chains.

Structurally diverse polycyclic alkaloids with two heterocyclic nitrogens and the absence of aliphatic methyl groups have been obtained from several sponges of the order Haplosclerida; alkaloids containing *N*-alkyl, 3-alkyl pyridine or piperidine motifs are frequently isolated from marine sponges of the genera *Haliclona*, *Xestospongia* and *Amphimedon*. The main problem in the structure elucidation of the two new compounds isolated from *Haliclona viscosa* was that the two heterocycles were connected by saturated alkyl chains.

HEXACTINOSAN SPONGES AS REEF BUILDERS IN EARTH HISTORY

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Today reef-building organisms are mostly characterized by tropical frame-building scleractinian corals, which commonly occupy the term "reef" and are used *pars pro toto* for reef-building organisms. In Earth history however, the variety of reef-building organisms was much more diverse than it is today. These groups included, among others, brachiopods (Permian), rudists (Cretaceous), bryozoans (Carboniferous, Permian) as well as calcareous (Permian, Triassic) and siliceous sponges (Cambrian through Recent). Siliceous sponges are a non-systematic artificial group of sponges with a mostly rigid siliceous skeleton. This group consist of the monophyletic class Hexactinellida and the polyphyletic "Lithistida" which belong to different groups within the class Demospongiae.

Hexactinosan sponges are important reef-building organisms in Earth history as they are able to create a three-dimensional reef framework comparable to those produced by scleractinian corals (see abstract Krautter *et al.*).

Although Hexactinosa appear in the Late Devonian, hexactinosan sponge reefs first occur in the Late Triassic. During the Jurassic, these reef facies expand in time and space, reaching an acme in the Late Jurassic, when hexactinosan sponge reefs were an important subset of the widespread siliceous sponge facies found across the northern margin of the Tethys Ocean and adjacent (proto) Atlantic Ocean. After Jurassic time a decline in the distribution of hexactinosan sponge reefs is notable.

After the Tertiary, no such sponge reefs were known, fossil or extant, until the late 1980's, when large hexactinosan sponge reefs were discovered on the Canadian Pacific shelf off British Columbia. These are the only known, living hexactinosan sponge reef on Earth (see abstract Conway *et al.*).

HEXACTINOSAN SPONGES: LARVAL ATTACHMENT MECHANISM AND RELATED REEF FRAMEBUILDING PROCESSES

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All modern and most fossil hexactinosan sponges require hard substrate upon which to settle. On the sea floor, cobbles, boulders and outcrops form a suitable substrate for the initial settlement of Hexactinosa. After death and maceration of hexactinosan individuals, their skeletons play an important role as a hard substrate for other hexactinosan sponges. Sponge larvae suspended in bottom currents, strand and attach to mazerated skeletons and develop into juvenile sponges using the fibres of the substrate skeleton for a solid substrate. Attachment is by means of tendril-like spicules wrapping around the substrate skeleton. The tendrils form a dense reticulate meshwork, which culminates in the formation of an enclosing envelope or layer around the substrate spicule. The tendrils are long and cover a relatively large area of the developing basal plate.

With increasing size the sponges develop root-like outgrowths as a secondary method to optimize skeletal stability and provide support to the increasing mass of the growing sponge. These outgrowths consist of very dense skeletal meshwork and fasten the sponge closely to the substrate or substrate sponge skeleton.

In addition to larval attachment and root-like outgrowths, a third mechanism contributes to sponge reef framework development. *Heterochone* and *Aphrocallistes* are able to grow together by means of their exothecal outgrowths and build a rigid framework. This skeletal "welding" together can happen between different species and also conspecifically but in all cases the "substrate sponge" must be dead and macerated first.

Working in combination these processes result in the creation of a three dimensional sponge reef framework comparable to those of scleractinian corals.

THE STUDY OF SECRETORY MATERNAL CELL FUNCTIONS IN THE SPONGE *HALISARCA DUJARDINI* (DEMOSPONGIAE, HALISARCIDA)

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The morphologic feature of Demospongiae is an availability of different secretory cells in their body. In White Sea sponge *Halisarca dujardini* are located spherulous, globular and granular secretory cells. But only spherulous cells penetrate into developing embryos and are saved within larvae right up to the first stages of metamorphosis (Ereskovsky & Gonobobleva, 2000). For understanding the role of these cells in larvae development it is necessary to study chemical structure of granules. By histochemical staining of *H. dujardini* tissues we have discovered that granules of spherulous or maternal cells maintain cationic peptides and (or) proteins. It is known that these proteins are molecular factors of anti-infectious resistance against bacteria, fungi and others in different groups of animals. The functional test of saltless cationic peptides extract from *H. dujardini* revealed an antimicrobial activity against *E. coli* and *Listeria monocytogenes*. This total saltless extract was separated into 40 fractions by HPLC. Then each fraction was tested against *E. coli* and *L. monocytogenes*. Antimicrobial activity was shown in fractions 11-15, 17, 21-25 and 37 against gram-negative *E. coli*. The individual fractions had shown antimicrobial activity against gram-positive *L. monocytogenes*. And the zone of lysis around hole where was the solute peptide fraction located was larger than in test with *E. coli*. This means that for *L. monocytogenes* killing a lower protein concentration is needed.

Activity against *L. monocytogenes* was shown in fractions 8-11, 14, 15, 22, 25. The strongest effect in this case was that of fraction 14. The results of electrophoresis in denaturing conditions with SDS show that all of the fractions are not homogenous and they contain peptides with molecular weights 16,6-23 kD.

In virtue of this work one can suggest that spherulous cells maintain cationic peptides with molecular weights 16-23 kD, which may play a defensive role in the larvae development and on the first metamorphosis stages.

RECONSTRUCTION OF PALAEOCLIMATE WITH THE HELP OF CORALLINE SPONGE DATA SETS

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We have studied three carbon and oxygen isotope records of a coralline sponge from the Caribbean.

The sponge of the species *Ceratoporella nicholsoni* was sampled during Meteor cruise No. 35 in 1996 by the submersible Jago. It was collected on the deeper fore slopes of Pedro Bank, a semi-drowned carbonate platform south of Jamaica, in 124 m depth.

Two sponge records date back to 1659 A.D. ±60 years according to several U/Th dating measurements. These measurements were done because there is, as yet, little understanding of density bands in coralline sponges. It is unknown whether the bands represent annual growth. On the basis of the U/Th dating, this specimen has been found to have grown, on average, 270 µm per year.

Unlike corals from shallow waters, this sponge species is azooxanthellate, which means it has not been influenced by primary production of symbiotic algae. Hence, it takes up carbon into its skeleton in equilibrium with the ambient seawater.

Three transects in the same sponge have been sampled. While two transects cover the whole time range, the third transect covers only approximately 4 years.

The resolution of the first one is lower than annual due to sampling with a dental drill. The results are not useful for frequency analysis since the discrete sampling method leads to aliasing and spurious signals.

The second transect is roughly semi-annual due to 200 µm drilling steps. There is no danger of aliasing because the line was continuously drilled, but the chronology is uncertain.

In the third transect there is no clear seasonality visible, but it shows a higher than monthly resolution due to 10µm drilling steps. Reasons for little seasonality could be that stable oxygen isotope ratios are not entirely suitable as a temperature proxy. There also is weak seasonality (according to the Levitus data) in 124 m depth (around 1°C). The size of the pseudocalices and orientation of the section plane make separate sampling of single pseudocalices almost impossible.

In general, the age control with U/Th is too inaccurate.

We conclude that Sr/Ca ratios should be measured as well as a sort of calibration. Our new sampling method with a microtome seems quite refined but should be improved by a micro sampler to reduce the danger of contamination.

A better age control has to be achieved.

SPONGES OF MUMBAI (BOMBAY) COAST

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Mumbai (Bombay) the island city situated on the west coast of India, located in between latitude 18°54' to 19°04' N, and longitude 72°47' to 72°56' E is one of the heavily populated cities in India. Mumbai is encircled by a shore area of 100 Km. The costae area of Mumbai was rich in faunal and floral diversity but with the increasing pressure of urbanization and industrialization this biota has dwindled. During present investigation a survey was carried out for recording sponges in coastal waters of Mumbai. *Tetilla dactyloidea* and *Tethya lyncurium* are the sponge species which are most deuse ialong the Mumbai coast. Ecological studies carried out on the identified sponges are discussed in detail.

CULTURE AND MOLECULAR IDENTIFICATION OF PROKARYOTES FROM MICROBIAL COMMUNITIES IN THE DEMOSPONGES *PSEUDOCERATINA CLAVATA, AXINYSSA* SP. AND *RHABDASTRELLA GLOBOSTELLATA* FROM THE GREAT BARRIER REEF

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We investigated the diversity of the microbial communities in three sponge species from the Great Barrier Reef - *Pseudoceratina clavata*, *Axinyssa* sp. and *Rhabdastrella globostellata* (formerly known as *Jaspis stellifera*). Sponge microbial symbionts were cultured under laboratory conditions and characterized morphologically. 16S rRNA genes were amplified by PCR using both Archaea- and Bacteria- specific primers, and resulting partial or near-complete sequences analyzed phylogenetically and with bioinformatics. The majority of Gram-negative bacteria belonged to the α subclass of the proteobacteria division within the Bacteria e.g. most closely related to *Roseibium hamelinense*, *Paracoccus* sp., and the *Crassostrea virginica* symbiont; but Gram-negative representatives of other divisions of bacteria were also isolated such as one closest to *Muricauda ruestringensis* belonging to the Cytophaga-Flavobacterium-Bacteroides division. We also isolated strains closest to *Bacillus firmus* belonging to the low % G+C Gram-positive Bacteria and to *Arthrobacter* sp., and *Micrococcus* sp. belonging to the high % G+C Gram-positive Bacteria. Thus taxonomic diversity in terms of presence of representatives of several distinct divisions of Bacteria is quite high in these sponge communities even in terms of culturable organisms. The proteobacteria and low % G+C Gram-positive divisions were present in all three sponge species. Some of the sponge bacterial isolates showed the production of inhibitory compounds which inhibited the growth of lawns of reference organisms (*Escherichia coli* and *Staphylococcus epidermidis*).

DISTINCTION OF *APLYSINA* SPECIES ON THE BASIS OF ITS SEQUENCE VARIATION REVEALED BY PCR-LINKED SSCP

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The genus *Aplysina* Nardo, 1834 (Verongida) includes several species from which secondary metabolites with biological activity were reported. The PCR-linked SSCP method has been successively used by our group to separate species of sponges. In this study we used total genomic DNA extracted out of samples of *Aplysina fulva*, *A. caissara*, *A. cauliformis*, *A.* aff. *cauliformis* and of an *Aplysina* sp. yet not described, from the collection of Museu Nacional do Rio de Janeiro (MNRJ). The region of the first internal transcribed spacer (ITS1) in the ribosomal DNA (rDNA) gene was amplified by Polymerase Chain Reaction (PCR). The PCR products were subjected to electrophoresis using the Single Stranded Conformation Polymorphism (SSCP) technique. The different migration patterns yield from the distinct conformations of the single DNA strand, reflect accumulated mutations in each sample and can be used to infer phylogenetic relationships between close related taxa and as molecular markers on population genetic studies. Our results allowed the diagnosis of *Aplysina* species through the combined PCR-SSCP method, showing the viability of this technique in the distinction of sponges species.

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GUITARRIDAE WITHOUT PLACOCHELAE DERIVED MICROSCLERES (MYCALINA, POECILOSCLERIDA) IS IT POSSIBLE?

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The Family Guitarridae Dendy, 1924 is characterized by the presence of placochelae or plachochelae-derived microscleres often together with smooth or spiny isochelae or sigmoid microscleres. Megascleres are monactinal or diactinal spicules with many intermediate forms. Exotyles can be present. An ongoing faunistic survey along southeastern Brazil revealed the first known guitarrid devoid of placochelae-derived microscleres, *viz* (bi)placochelae, coelodisks or tetrapocilla. The sponge is fluffy, black (mostly), yellow (rarely) or yellow with black spots (often), with a spicular component of (subtylo)styles and peculiar spiny isochelae, only characterizable under SEM. The latter are considerably irregular, frequently malformed, but indisputably related to the small spiny isochelae of *Guitarra* and *Tetrapocillon*, many morphotypes of which were described already. The acanthoisochelae in the Brazilian sponge are rudimentary, but heavily spined. Spines are long, delicate, irregular, frequently sinuous. The shaft is thin and the lateral alae at both extremities malformed. The frontal alae are nearly missing and almost touch each other. The absence of placochelae or placochelae-derived microscleres hampers an objective assignment of this species to a particular genus. However, it differs from them in the presence of acanthoisochelae with long and delicate spines which are not clustered in clumps as is the case of *Guitarra indica* Dendy, 1916 and *Guitarra fimbriata* Carter, 1874.

SPONGE LARVAE: THE BLUEPRINT OF A METAZOAN

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Development and metamorphosis of the haplosclerid demosponge *Reniera* sp. were studied to determine how gastrulation occurs, and whether the primary germ layers become inverted at metamorphosis. Embryogenesis occurs via unequal cleavage to form a solid blastula of two sizes of cells. Cellular migration of ciliated micromeres to the periphery of the larva results in a bi-layered embryo and is interpreted as gastrulation. Polarity is determined by the migration of pigment-containing micromeres to one pole; this pole later becomes the posterior pole of the swimming larva.

The sponge larva is a highly differentiated organism that is responsive to environmental stimuli. Sharp changes in light intensity cause an abrupt straightening and bending of long cilia that arise from the pigment-containing cells at the posterior pole. Video microscopy shows that the response allows the larvae to steer away from bright light to darker areas, such as under coral rubble, the habitat of the adult sponge on the reef flat at Heron Island, Great Barrier Reef.

Larvae settle on their anterior pole and metamorphose into a juvenile sponge in 5-7 days. Fluorescent labelling of the larva's monociliated epithelial cells with the cell lineage marker CMFDA demonstrates that these cells resorb their cilia, migrate inwards, and transdifferentiate into the choanocytes and into other cells of the juvenile sponge. The cellular changes that occur at metamorphosis reflect the reorganisation and dedifferentiation of already differentiated cells, rather than inversion of cell layers. This study demonstrates that sponge development includes recognisable hallmarks of metazoan embryogenesis – formation of a blastula by cleavage and of a highly differentiated 2-layered larva by gastrulation – and provides a means to relate the ontogeny of the sponge body plan to other animals.

A LOW-COST AND QUICK METHOD TO DISTINGUISH PORIFERA SPECIES ON THE BASIS OF ITS SEQUENCE VARIATION REVEALED BY PCR-SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP)

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The lack of effective morphological markers together with limited data on aspects of reproduction, life history, ecology and cell biology make the taxonomy of Porifera a complicated task. In a large, ongoing effort, to inventory Brazilian marine sponge diversity, some taxa proved particularly problematic for classical morphological identification. We have applied the polymerase chain reaction-based single-strand conformation polymorphism method (PCR-SSCP) to detect sequence variation in the first and second internal transcribed spacers (ITS-1, ITS-2) of ribosomal DNA (rDNA). These polymorphic DNA sequences have been used as additional characters to separate species of the following sponge genera: *Amphimedon* Duchassaing & Michelotti, 1864 (Haplosclerida); *Aplysina* Nardo, 1834 (Verongida); *Cliona* Grant, 1826 (Hadromerida), and *Xestospongia* de Laubenfels, 1932 (Haplosclerida). The PCR-SSCP method allowed us to quickly detect different migration patterns of fragments which reflect DNA sequence variability, both for individuals of different species and for individuals of the same population. We concluded that the PCR-SSCP method is: 1) useful to resolve differences at the species and subgenera levels; 2) simple, of low cost, fast, and non-radioactive; 3) it needs low amounts of tissue, as the rDNA transcription unit is present in the genome in multiple copies; 4) it is excellent as a fast screening method for species identification, and might thus be a useful tool to discriminate sibling sponge species prior to sequence analysis.

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SPONGE FAUNA ASSOCIATED WITH WHITE CORALS FROM THE WESTERN IONIAN SEA

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Taxonomic composition and distribution of the sponge fauna associated with a white coral bank were studied. The bank was located about 25 miles south of Cape S. Maria di Leuca (Ionian Sea), at depths ranging from 400 to 1100 meters.

Coral samples were collected during 2001 from ten stations located at a distance of about 1 km one from the other, using a collecting device (a wood cross with attached net called "ingegno") drawn by a boat. The samples consisted both of alive and dead colonies of *Lophelia pertusa* (L.) and *Madrepora oculata* L. (Cnidaria, Anthozoa) associated to form a thick calcareous texture.

Sponges were found in eight of the ten stations, mainly settled on dead colonies of both coral species. Species richness sharply decreased according to depth, while covering values were constantly low and not related to depth.

Twenty-nine species of sponges were recorded: twenty-seven Demospongiae, one Hexactinellida, one Calcarea. Nineteen of them represent new records for the Ionian Sea. *Poecillastra compressa* (Bowerbank), *Latrunculia insignis* Topsent and *Desmacella inornata* (Bowerbank) are the most common species. The sponge assemblage mainly consists of small or encrusting specimens. The few massive species, belong to Tetractinomorpha. Only two boring species (*Cliona levispira* Topsent and *Cliona* sp.) are present. The sponge assemblage here described shows a notable affinity with those reported by literature for deep hard substrata of the north western Mediterranean region: more than 40 % of the species found are reported for deep-coral biocoenosis, and about 20 % are reported for deep rocks. The remaining are species with a wide vertical range.

MORPHOLOGICAL CHARACTERIZATION OF *HALICHONDRIA AURANTIACA* (SCHMIDT, 1864) (DEMOSPONGIAE, HALICHONDRIDA) FROM THE BERLENGA NATURAL RESERVE

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Halichondria aurantiaca (Schmidt, 1864) was described from the Mediterranean Sea. It was thought to be a Mediterranean endemism until its recent finding in the NE Atlantic (Portugal) by some of us. It is a rare species whose knowledge comes from a few papers. In this work a morphological characterization of two specimens found in Portuguese waters, including ultrastructural data and a comparison with *Halichondria panicea* (Pallas, 1766), as well as some ecological data, are presented.

Samples of *H. aurantiaca* were collected in the subtidal zone of the Berlenga Natural Reserve on the ceiling of a semi-dark cave (6 m), using SCUBA equipment. Samples of *H. panicea* were collected in the intertidal zone of Praia de Ribeira d'Ilhas (Portugal). Sponges were processed for identification following standard methods. For electron microscopy, specimens were primarily fixed in glutaraldehyde in sea water buffered with sodium cacodilate and treated with 5 % hydrofluoric acid for removal of spicules. Further fixations in osmium tetroxide and uranyl acetate were followed by epon-araldite embedding. Thin sections were contrasted with uranyl acetate and lead citrate and observed in a Jeol 100S electron microscope.

Two massive orange specimens were studied. Skeletal architecture is the typical one of *Halichondria* Fleming, 1828. Oscules, together with superficial aquiferous channels, form a star pattern characteristic of *H. aurantiaca.* Comparison with Mediterranean specimens involving both morphological and ecological aspects was performed. An ultrastructural study of one of the specimens is presented. Comparison with specimens of *H. panicea* revealed an overall similarity of cell types and sponge organization. Two distinctive features of *H. aurantiaca* were the accumulation of very elongated symbiotic bacteria near the canals and choanocyte chambers, and a relatively high density of spherulous cells with large dense granules associated with spicule aggregates, these features were not found in *H. panicea.*

FIRST RECORD OF *STRYPHNUS MUCRONATUS* (SCHMIDT, 1868) (DEMOSPONGIAE, ASTROPHORIDA) IN THE NE ATLANTIC

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The genus *Stryphnus* Sollas, 1888 is represented in the NE Atlantic by two species, *S. fortis* (Vosmaer, 1885) and *S. ponderosus* (Bowerbank, 1866). A third one, *S. mucronatus* (Schmidt, 1868), is known from the Mediterranean Sea, where it seems to be a common species.

In a sponge survey carried out in June 2000 in the Berlenga Natural Reserve (Portugal) we found *S. mucronatus*. This is the first record of the species outside the Mediterranean Sea. In this communication we present a morphological characterization of the Portuguese specimens.

Two massive black, hard and hispid specimens were found in a superficial cave (7 m), at Ilhéu Maldito (39° 25' N, 9° 30' E) on vertical rocky surfaces, under attenuated or rather reduced light conditions. Specimens were processed for light microscopy following standard methods. For electron microscopy, specimens were primarily fixed in glutaraldehyde in sea water buffered with sodium cacodilate and treated with 5 % hidrofluoric acid for removal of spicules. Further fixations in osmium tetroxide and uranyl acetate were followed by epon-araldite embedding. Thin sections were contrasted with uranyl acetate and lead citrate and observed in a jeol 100S electron microscope. Sponges are similar to Mediterranean specimens in what concerns their morphology and skeletal organization. A detailed sicule description was performed.

Electron microscopy: The mesohyle is composed of a reticulum of very thin collagen fibers and is packed with numerous symbionts, sometimes associated with cellular elements. Spherulous cells containing large granules with a microgranular substructure are scattered in the mesohyle, possibly representing the pigmentar cells. Choanocytes suffered severe damage in the studied specimens in spite of the use of a fixative that provided fair preservation of other species in the same area, suggesting that they may be unusually sensitive.

S. mucronatus and *S. ponderosus* are sympatric species. They can be distinguished by their dichotriaenes and by colour. In *S. mucronatus* the protoclads are longer than the deuteroclads and its black colour is a rather constant feature that does not seem to depend on the light intensity.

CHARACTERIZATION OF GENETIC MARKERS FOR *IN VITRO* CELL LINE IDENTIFICATION OF THE MARINE SPONGE, *AXINELLA CORRUGATA*

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The marine sponge *Axinella corrugata* is being developed as a model organism for *in vitro* marine invertebrate research. Molecular genetics methods such as DNA fingerprinting (amplified fragment length polymorphism or AFLP), SSCP (single stranded conformation polymorphism) and single locus DNA sequence analyses were applied to this model to meet the primary objective of identifying positive *A. corrugata* specific molecular markers that will aid in verifying cell identity *in vitro*, and distinguish sponge cells from potential microbial contaminants. The extent of intra- and interspecific variation in these markers from geographically distinct samples of *A. corrugata* and closely related sponge taxa was also assessed. Two novel nuclear loci along with intervening transcribed spacer (ITS) regions of nuclear rRNA were characterized, although the latter appeared to better meet primary marker criteria, such as taxonomic specificity and high frequency of detection (via PCR) from different individuals (n > 40) and cell cultures. Phylogenetic and phylogeographic analyses of ITS DNA sequences helped clarify taxonomy and also suggested species boundaries between and among Western Atlantic and Eastern Atlantic/Indian Ocean *A. corrugata* and Axinellidae samples. Patterns of genetic variation have important implications for the systematic, evolution, and chemical ecology of *A. corrugata* and related axinellids and are discussed.

NEW ASPECTS ON THE BIOLOGY OF THE EXCAVATING SPONGE COMPLEX *CLIONA CARIBBAEA-C. LANGAE-C. APRICA*

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Excavating sponges burrow into calcium carbonate skeletons and substrata. Geomorphology of coral reefs, oyster banks and other limestone substrata can be modified substantially from their bioeroding activity. In the Caribbean Sea there is a complex of dark brown, zooxanthellate sponge species (variably reported as *Cliona caribbaea*, *C. langae* or *C. aprica*) which encrust the surface of calcium carbonate substrata, being able to both excavate and advance laterally against live tissues of many species of reef-building corals. These sponges penetrate the skeleton immediately below the coral tissues, weakening their support. Coral tissues may then slough off or be bitten by corallivorous fish, allowing further advance by the sponges. To complement the study on the biology of two species of the complex and their interaction with some reef organisms, 190 individuals were marked and followed for one year from 2001 to 2002 in two coral reef areas of Colombia in the Southern Caribbean. Multiple complementary observations of the sponges, their substrata and their neighbors were also made. Steel nails were driven in the sponge boundaries and the lateral advance and vertical descent of the sponge tissues were measured after six and twelve months. Fragments of sponges, substrata and neighbors were collected and fixed. In the laboratory, fragments were cut with a circular diamond saw to be observed directly under magnification, or to be embedded in resin, cut, mounted on slides, and ground and polished, to be observed under transmitted light. It was found that the most frequent growth stage for *C. langae* was the beta stage (fully encrusting). *C. aprica* occurred only in the α stage (papillae in various stages of fusion) in reefs of the continental coast of Colombia, while in oceanic reefs of the SW Caribbean the β stage (encrusting with scattered bits of free substratum) was predominant. In both species, a γ stage not previously described was found. In this case, once the sponges have encrusted the entire exposed surface of their substratum, not being able to further advance laterally, their tissues thicken and frequently their surfaces are colonized by epibiotic zoanthids. Contrary to other excavating sponges which reach the gamma stage, these species do not seem to continue the vertical penetration of the substratum, being restricted to the uppermost 1-2 cm. In rubble, papillated *C. aprica* grow tissue extensions that bind fragments across several centimeters, conforming an agglutinating γ stage. The greatest lateral advance for *C. langae* was 10.2 cm in 6 months, and for the β stage *C. aprica* 2.2 cm in 6 months. Vertical rates of advance were within the limits of measurement precision (max. 1-2 mm in 6 months). The sponge coral boundaries which had minimal or null lateral advance were those in which the angle of encounter was lower than 180 degrees. In these cases the corals had been able to grow above the advance plane of the sponge, avoiding erosion of the supporting skeleton directly below the polyps. In the cases in which the angle was appropriate for lateral advance, sponges grew less against certain species of crustose algae, corals and hydrocorals, some of which are known to be good space competitors. Some encrusting gorgonians, zoanthids and sponges were able to overgrow the sponge tissues. There was also little or no lateral advance of the sponges in steeply inclined, lower or shaded surfaces. In conclusion, the proximate factors responsible for the current rates of lateral excavation and advance of the studied sponges are the angle of confrontation, the amount of incident light, and the competitive ability of the neighbour being excavated.

COMPARISON OF RNA EXTRACTION METHODS FROM SPONGES

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Molecular biology techniques are increasingly being used in sponge systematics and other areas of sponge research. Sponges are known to produce a variety of chemical compounds that are known to inhibit downstream applications of both DNA and RNA, such as PCR and RT-PCR reactions. Therefore, robust methods that allow for downstream application of both DNA and RNA need to be developed. RNA by nature is unstable and easily degraded by environmental RNAses. Previously RNA was extracted using guanidine thiocyanate, but more recently this has been tended to be replaced by less hazardous chemicals.

Three extraction methods were compared including TRIzol® reagent (Invitrogen), TRIzol® reagent plus the extraction columns of the FastDNA® Spin kit for soil (Q-BIOgene) and the FastRNA®Green kit (Q-BIOgene). We investigated these extraction methods in terms of yield of RNA recovered and quality of RNA as measured spectrophotometrically and by gel electrophoresis. Presence of inhibitory compounds was ascertained by the ability to produce double stranded cDNA using SuperScript II reverse transcriptase (Invitrogen).

Results indicate that all methods were able to extract stable RNA that could be converted to cDNA. However, the size of RNA, amount and ratios varied between the methods. The FastRNA®Green kit extracted 700 µg per gram sponge (wet weight) compared to 181 and 560 µg per gram sponge (wet weight) for TRIzol® with spin column and TRIzol® only respectively. The size of RNA as analysed by gel electrophoresis was similar for all three extraction methods. The SuperScript™ choice system for cDNA synthesis (Invitrogen) was used to convert 4 µg of each of the total RNA to cDNA using Oligo(dT). Yields of cDNA varied from 900 ng to 500 ng of cDNA with the FastRNA®Green kit again showing the best performance.

In conclusion the FastRNA®Green kit produced not only largest amount of RNA per gram of sponge sample but also the highest quality based on conversion to cDNA.

GEMMULAR MORPHOLOGY, PERSISTENCE AND DISPERSAL STRATEGIES IN A *CORVOSPONGILLA* SPECIES FROM THE WESTERN PALAEARCTIC

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In the framework of a biodiversity evaluation in the river Tigris catchment basin (Mesopotamia, N-E Iraq) freshwater sponges were recorded from eutrophic lentic waters. Specimens were ascribed, according to skeletal and gemmular diagnostic traits, to the genus *Corvospongilla* Annandale, 1911 and belong to a species probably new for science. This first finding in the Palaearctic region reduces the disjunction in the geographic range of this widespread genus known till now from the Afrotropical, Oriental, Neotropical and Nearctic regions. The analysis by Scanning Electron Microscopy (SEM) highlighted the existence of two gemmular morphs diverging for distribution within the sponge body, architecture, and functional role. Sessile sub-oval gemmules, with lateral foramen and gemmular theca of compact spongin, are grouped at the sponge basis and some of them strictly adhere to the basal spongin plate. Free sub-spherical gemmules, with apical foramen and pneumatic layer in the gemmular theca, are usually scattered in the choanosomal skeletal net. These two gemmular morphs appear to drive cryptobiosis towards different survival strategies, improving, respectively, the regeneration of the mother-sponge *in situ* by sessile gemmules and the species passive dispersal. The trait "gemmular dimorphism" displayed by some species of the genus *Corvospongilla* diverges from the gemmular monomorphic condition typical of spongillids, metanids and potamolepids in which the single gemmular morph perform both roles of propagule and resistant body. These results stress the problem of the systematic status and phylogenetic relationships of the genus sharing the traits "free gemmule with pneumatic layer" and "sessile gemmule with a compact spongin theca" with, respectively, Spongillidae-Metanidae and Potamolepidae.

FRESHWATER SPONGES ASSOCIATED TO SUB-LACUSTRINE HYDROTHERMAL VENTS (NEW ZEALAND)

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An investigation by the submersible *Jago* of a lacustrine hydrothermal system in Lake Taupo, New Zealand, resulted in the discovery of two hydrothermal vent fields (Te Hoata and Te Pupu). Elevated concentrations of silica, salt and hydrocarbons together with high temperatures (44°C) characterised waters at a depth range of 126-173 m. In these environmental conditions, benthic communities were dominated by a high density of sponges with extensive cover area and biomass. In some areas sponges displayed a notable size (15 x 25 cm), showing a maximum density of 25 specimens/m², and covering 60-70% of the available substrata represented by inactive chimneys and rocky crops. The walls of the chimneys recovered from the sites are rich in Mn, Hg, As, S, Sb, Tl, Ba, and Zn and are dominated by amorphous silica, silicified diatoms, and strands of filamentous bacteria. *In situ* observations show that the sponges range in body shape from encrusting to massive. Their colour was white with pinkish and bluish patches suggesting a symbiosis with bacteria. Dermal membrane within living specimens appeared notably detached from the underlying tissues suggesting an active water pumping. The limited sampled specimens were frozen and some fragments used for LM and SEM morphological analysis of skeletal architecture, spicules, and when present, larvae and gemmules. Skeletons and spicules are characterised by comparable traits in all samples. Two gemmules found in one sample enabled us to ascribe these sponges to the poorly known genus *Heterorotula* Penney & Racek, 1968 endemic of the Australian-New Zealand Regions. Variations in body shape could be interpreted as different growth phases in the life cycle, or different species. The few sponge samples and the absence of gemmules did not allow a definitive identification at a species level. Sponges appear to perform a key structuring role of the benthic community in these extreme habitats, according to the presence of a rich associated fauna of protozoans, annelids, ostracods, copepods, amphipods, and other taxa not identified at present. This record suggests that Spongillidae are able to successfully colonize sub-lacustrine hydrothermal vents as also reported for Lubomirskiidae in the depths of the Lake Baikal. Additional dives are needed to evaluate sponge biodiversity at the species level and to better understand the life style strategies selected by these sponges to survive, and thrive, in such extreme environments.

PHYLOGENY AND EVOLUTION OF CALCAREOUS SPONGES: MONOPHYLY OF CALCINEA AND CALCARONEA, MULTIPLE MORPHOLOGICAL CONVERGENCES, AND THE PRIMITIVE NATURE OF AXIAL SYMMETRY

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Because calcareous sponges are triggering renewed interest with respect to basal metazoan evolution, a phylogenetic framework of their internal relationships is needed to clarify the evolutionary history of key morphological characters. For the first time, we have attempted to code morphological variation at the suprageneric level within Calcispongia. We show that very few phylogenetic information can be retrieved from the cladistic analysis of morphological characters. Noteworthy, morphology cannot help to resolve the long-standing disagreement about the main subdivision of Calcispongia, i.e. between a classification based upon the aquiferous system (Homocoela/Heterocoela) and an alternative classification based upon cytological and embryological characters (Calcinea/Calcaronea). We then analyze 18S and 28S rRNA data, alone and in combination with morphological characters. The monophyly of Calcispongia is highly supported. The monophyly of both Calcinea and Calcaronea is retrieved, while the data strongly reject the competing Homocoela/Heterocoela hypothesis. The topology obtained within Calcaronea suggests that major rearrangements of the current classification scheme will be needed in the future. In particular, the well-supported polyphyly of the genus *Sycon* will have nomenclatural consequences. The phylogeny is used to assess preliminary hypotheses about the evolution of a few important morphological characters. Characters of the skeleton architecture are highly homoplasic, together with characters of the aquiferous system. On the contrary, axial symmetry seems to be primitive to all Calcispongia. The latter conclusion potentially has deep implications on considerations of early body plan evolution in Metazoa.

RESULT COMPARISON OF TOXICITY BIOASSAY AND COMPOUND QUANTIFICATION IN *DYSIDEA AVARA* AND *IRCINIA VARIABILIS*

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We assayed two methods, the Microtox® assay and chemical quantification, to assess the natural toxicity of two sponges whose secondary chemistry is known: *Dysidea avara* and *Ircinia variabilis*. We used Microtox® to compare the toxicity of the acetone and methanol extracts of *D. avara* versus that of the major metabolite of the sponge (avarol). The methanol extract was more toxic than that of acetone and was as toxic as pure avarol. We conclude that the toxicity of *D. avara* is mainly due to avarol and that methanol rather than acetone extracts are more suitable for the detection of species toxicity by Microtox®. We also quantified palinurin, the major metabolite of *I. variabilis*, in specimens from several habitats. The concentrations of this metabolite ranged from 0.75 % to 1.75 % of sponge dry weight. With the same methanol extracts used for palinurin quantification, we ran the Microtox® assay and found a positive significant regression between toxicity (negative with respect to the EC50) and concentration of this metabolite. Pure palinurin was tested at the same concentration present in the pure extract assayed and the toxicity recorded was higher than that of the methanol extract. Palinurin is the main secondary metabolite that confers toxicity to *I. variabilis*, as avarol conferred it to *D. avara*. Our results confirm that the standardised Microtox® assay is an accurate and repeatable tool for assessing the toxicity of crude extracts and pure metabolites of marine species. This method is faster and easier to perform than chemical quantification even when the sponge chemistry is known, and is appropriate for studies on variation in natural toxicity in a range of environmental conditions. Although in the species studied here the bioactive responses of the main metabolites were similar to those of the crude extracts, analyses of the latter are preferable in preliminary screenings to avoid that toxicity caused by minor metabolites be disregarded.

THE ROLE OF SPONGE NATURAL TOXICITY IN COMMUNITY STRUCTURE AND DYNAMICS

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Both antifouling and space competition functions of secondary metabolites may make their production relevant for community structure and dynamics. We investigated whether sponge toxicals (i.e. natural toxicity) play some role in structuring animal-dominated communities. We related mean toxicity of benthic communities from Mediterranean caves with several abiotic parameters such as irradiance, water movement and organic matter in the water. The study was carried out in June and November for assessing seasonal variation. We also correlated community toxicity to structural parameters of the community such as α diversity, mean patch size and number of significant positive contacts between species (i.e. contacts which were more frequent than it would be expected according to species abundance). The relationships of community toxicity with abiotic and biotic parameters greatly varied in different caves. In Cabrera cave (located in an oligotrophic environment) the only parameter that presented a significant correlation with toxicity was the percentage of positive associations, and this correlation was negative. In contrast, in Medes cave (located in an eutrophic environment) mean toxicity of communities correlated positively with irradiance, alfa diversity and mean patch size. The significant relationship detected between toxicity and the percentage of significant positive associations is an indication of the importance of neighbours to partially explain the toxicity variation recorded. We also related the species natural toxicity to the non-random positive associations featured by each species at every community of each cave separately. As we found at the community level, only sponges from Cabrera cave displayed a significant negative correlation with the number of significantly positive contacts. In contrast, sponge species from Medes cave did not have this relationship. The significant negative correlation between mean toxicity of sponge-dominated communities from Cabrera cave was stronger than the same correlation at species level, indicating a species-specific variability in the response and the existence of other factors that may influence the associations. The less toxic sponge species tend to be in contact with more species than those with a higher toxicity. It is known that inter-species interactions are not simple hierarchical but non hierarchical competitive networks. Thus, a toxic species may interact with many other species and its toxicity does not necessarily represent a generalist mechanism of competition. Taken together our results show a complex pattern in which the relative importance of toxicity varies with species, community, season and cave. However, they also indicate that natural toxicity may play a decisive role in structuring benthic communities, despite it has rarely been considered in previous community studies.

FREQUENCY OF SPONGE AND CORAL COLONIES ON THREE REEF HABITATS OF THE RAJA AMPAT ISLANDS, INDONESIA

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The Raja Ampat Islands in Papua Province, Indonesia, have some of the most biologically diverse coral reefs in the world with very low anthropogenic impact. This provided a unique opportunity to examine frequency of sponge and coral colonies across three habitats: fringing, platform and sheltered reefs. I sampled the macrobenthic community at 1m intervals on four 25 m transects placed at two depths (4-6 m and 18-20 m) on each reef site. Variation in frequency of both coral and sponge colonies was high. As expected, frequency of coral was higher than sponges across habitats. Sponges were more common at the deep than shallow depth on the fringing and platform reefs. The sponges, *Phyllospongia lamellosa* and species of *Dysidea*, were the most frequently observed during the survey. Boring sponges, *Cliona* spp., were found to be locally common at one fringing reef site in the North Fam Island region.

REVISION OF *PLEUROCHORIUM ANNANDALEI* (PORIFERA, HEXACTINELLIDA)

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Pleurochorium annandalei, known previously from the N Indian Ocean, has now been found to be widely distributed in the W Indian Ocean as well. Two new subspecies of *P. annandalei* are described. Discovery of a more complete, branching specimen and examination of its spicule content show that *Pleurochorium* has a much more complex body form than has previously been accepted. The type of tubular branching observed in this genus cannot be considered dichotomous or any of its variations, but should be interpreted as a regular emission of tubular branches from the side of the wall. Hence the genus should be retained in its present position within Chonelasmatinae. The new data allow refinement of the diagnosis of this genus and clarification of differences between the subfamilies of Euretidae.

SPONGE DISTRIBUTION IN CAGARRAS ARCHIPELAGO, RIO DE JANEIRO, BRAZIL

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In this study we describe the structure and distribution of the sponge community in Cagarras Archipelago, Rio de Janeiro, Brazil. The archipelago has three major islands (Cagarra, Palmas and Comprida) and four islets. Qualitative samples were taken through SCUBA diving from January 2001 to February 2002 in the three islands and two islets. Quantitative samples were taken in 51 quadrates of 0.25 m^2 from December 2001 to February 2002 in Palmas Island. Cluster analysis using Jaccard's coefficient on qualitative (presence/absence) data grouped the two islets (Lage da Cagarra and Filhote da Cagarra), which are more exposed to wave action, and the three islands which form a group of sheltered sites. Cluster analysis using Bray-Curtis coefficient on quantitative data in Palmas Island allowed distinction of three groups of samples: overhangs, shallow horizontal surfaces (6 m depth), and vertical walls plus deeper horizontal surfaces (17 m depth). Sponge abundance was greater in overhangs (15.3 ind x m^{-2}), which were dominated by Protosuberites sp. (33 % of the sponge individuals in this habitat) and *Clathrina* sp. (23 %). Abundance was reduced in vertical walls (8.0 ind x m^{-2}) and deep horizontal surfaces (4.2 ind x m^{-2}). *Pachychalina* sp. dominated the deep horizontal surfaces (43 %), whereas *Clathrina* sp. dominated in the overhangs (19 %). Shannon's diversity in overhangs (H'=1.5 nits x ind^{-1}), vertical walls (H'=1.5 nits x ind^{-1}) and deep horizontal surfaces (H'=1.2 nits x ind^{-1}) were not significantly different ($P < 0.05$), whereas in shallow horizontal surfaces the sponges were significantly less diverse (H'=0.25) and less abundant (2.1 ind x m^{-2}). Shallow horizontal surfaces were dominated by *Hymeniacidon heliophila* (70 %). The results indicate that 1) wave action affects the composition of sponge species; 2) depth is a more important "factor" structuring sponge communities in horizontal than in vertical surfaces and overhangs; and 3) substrate inclination has a stronger influence on the species composition and abundance than on the diversity.

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PORIFERA FROM THREE OCEANIC ISLANDS OFF NORTH EASTERN BRAZIL

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Recent studies on the taxonomy of sponges from oceanic islands off Brazil have raised significantly the number of species previously known in the area, including several new species. The islands studied here, Atol das Rocas (AR), Fernando de Noronha (FN) and São Pedro e São Paulo Archipelago (SPSP), have great environmental, scientific, economic and strategic value, being classified respectively as Biological Reserve, National Park and Environmental Protected Area. They have distinct geomorphological features and range from 260 to 1010 km from mainland. These islands were visited by the authors in 1996, 1998, 1999, 2000 and 2001, and were sampled from 0 to 50 m depth by SCUBA and free diving. A total of 376 specimens were collected and are deposited in the Porifera Collection of Museu Nacional-Universidade Federal do Rio de Janeiro; 154 of them from AR, 121 from FN and 101 from SPSP. Underwater photographs were taken with a Nikonos V camera, 35 mm and close up lenses, 1:1 extension tube and SB-105 strobe, using Provia 100 film. Taxonomic identification was made by comparison with the literature and museum specimens. The 92 species recorded here represent two classes (Demospongiae and Calcarea), 13 orders, 40 families and 60 genera. At least six species are new to science, including *Clathrina* sp.1, *Clathrina* sp.2, *Plakortis* sp.1, *Plakortis* sp.2, *Plakortis* sp.3, and *Aplysina* sp. *Clathrina* sp.1, *Plakortis* sp.1 and *Aplysina* sp. have only been registered to Atol das Rocas, and *Clathrina* sp.2 is endemic of São Pedro e São Paulo so far. Fernando de Noronha showed the highest sponge diversity (53 spp), followed by Atol das Rocas (44 spp.), and São Pedro e São Paulo Archipelago had the lowest (21 spp.). The majority of species identified in this study from AR (40) and SPSP (15) are new records to the areas. The sponge species from these islands are probably more than those here presented, therefore further collections and a more detailed taxonomic study may increase their total number.

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A NEW LOOK ON THE BIOGEOGRAPHY OF BRAZILIAN MARINE SPONGES (PORIFERA, DEMOSPONGIAE)

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The biogeography of Brazilian marine demosponges has been studied in the 1970's and early 90's. The recent growth of the knowledge on sponge diversity in Brazil may allow the detection of possible new biogeographic areas in the Brazilian coast. This study is discussed here based on an analytical perspective. The units of analysis were 15 coastal states, spreading from Amapá in the north, to Rio Grande do Sul in the south (Sergipe and Paraná states have no published records), plus the oceanic archipelagos of Fernando de Noronha, and St. Peter's & St. Paul's Rocks (on the Mid-Atlantic Ridge). The Caribbean and the Patagonian region were used as outgroups. Political states were chosen as area units, as collecting data is often restricted to these (richest Challenger Brazilian station reads only "off Bahia"). Over 300 species records had their occurrences tabulated, 111 of which were found to occur in more than one unit area, and were thus used as characters for a Parsimony Analysis of Endemicity (PAE), run in the computer program Hennig 86. Two most parsimonious trees were obtained (246 steps, CI-45, RI-48), which makes clear the large amount of homoplasy present in the data. A successive weighting scheme (SWS) was adopted generating a single tree (CI-67, RI-80). States on the north of the country (ca. 2°S-5°N), viz. Amapá, Pará and Maranhão, formed a monophyletic triplet, already apparent prior to the SWS. These are all under the influence of the warm North Equatorial current. Interestingly, eastern Brazil and Fernando de Noronha (ca. 2-9°S) clustered with the Caribbean fauna (also prior to the SWS), thus suggesting a discontinuous distribution of this biota. The possibility cannot be discarded though that this might have happened through some phenomenon analogous to "long-branch attraction". There are another two major clusters in the outcome: central and southeastern Brazil (ca. 12-29°S), a sister clade to the previous one mentioned, and the extreme south (under 29°S). The former comprises a known transitional biogeographic area, the northern limit of which found here is surprising, as it includes the State of Bahia clustered with the States of Rio de Janeiro and of São Paulo. Again, "long-branch attraction" could be an explanation. At the base of this clade are the States of Espírito Santo (ca. 18-22°S) and of Santa Catarina (ca. 26-29°S), from which smaller species lists are available this far. Lists of molluscs and of reef fishes point to the former as one of the richest areas in Brazil. Finally, the clustering of the State of Rio Grande do Sul (ca. 29-33° S) with the Patagonian region is not unexpected. Brazil's extreme south is mostly devoid of shallow-water hard substrates, thus making the influence of colder waters even more notorious. St. Peter's & St. Paul's Rocks and the State of Paraíba form a basal polytomy possibly due to their small lists of species.

SPONGES OF THE N-NE BRAZILIAN CONTINENTAL SHELF (PORIFERA,DEMOSPONGIAE, POECILOSCLERIDA)

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Knowledge of the Brazilian shallow-water poecilosclerid fauna ranging from the state of Amapá in the far north, to the state of Maranhão is limited to a few records made by Collette & Rützler (1977), Hajdu & Desqueyroux- Faundez (1994) and Mothes, Hajdu & Van Soest (2000). Tendal (1973) working on material gathered by the Albatross Swedish Expedition described *Chondrocladia albatrossi* from deep-water (4474-4430 m) off the coast of the state of Ceará (02°26' N / 39°26' W to 02°24' N / 39°12' W). In the present study, eight species are described, four of which are new species, and two are new records for the area. The studied specimens were collected between 23 and 103 m depth between the latitudes 04°13'N and 02°17'S and the longitudes of 50°31' W - 41°37' W, off the coasts of the states of Amapá, Pará and Maranhão, during the Federal Governament Oceanographic Cruises: Comissão Pesca Norte I (DHNM, R/V "Almirante Saldanha") and Comissão Maranhão (SUDENE, fishing trawler "Barco Pesqueiro IV"), in 1968 and 1973, respectively (Kempf, 1972). The studied material has been deposited in the Porifera collections of the Museu de Ciências Naturais (Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil) and the Zoological Museum of Amsterdam (University of Amsterdam, Amsterdam, Netherlands). Skeletal slides and dissociated spicule mounts were made following Mothes-De-Moraes (1978) and Mothes (1996). The SEM study was made according to Mothes & Silva (2002). An annotated comprehensive list is provided of poecilosclerids sponges collected and previously recorded from the area studied. A key for the identification of the brazilian poecilosclerid species is included.

MOLECULAR MARINE BIOTECHNOLOGY WITH SPONGES

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Members of the phylum Porifera contain the largest number of bioactive compounds among all Metazoa. However, despite the large number and the high variety of structurally different natural products only very few of these marine secondary metabolites have been tested in clinical trials. So far ara-A a derivative of ara-U, isolated from the sponge *Tectitethya crypta* is the only secondary metabolite which has been approved for human application in clinics. Some other sponge metabolites, including avarol have reached clinical evaluation.

It became clear that the limited availability of larger quantities of refined starting material from a certain sponge species for extraction of bioactive compounds is the major cause for the low attractiveness of such secondary metabolites for commercial exploitation. Four routes can be followed to obtain greater amounts of sponge secondary metabolites. First, chemical synthesis; this approach has successfully been undertaken but, in many cases, requires many steps with only low yields. Second, cultivation of sponges in the sea, in mariculture. In general, the mariculture of sponges for commercial use has a long tradition. However, only the cultivation of bath sponges was successful and realized profits. Only very recently farming of sponges in a sustainable manner, following the rules of the Agenda 21 (United Nations - Sustainable Development; www.un.org/esa/sustdev) for the production of bioactive compounds has been started. Third, the cultivation of sponge specimens in a bioreactor has been investigated during the last years. Fourth, the production of secondary metabolites in bioreactors using sponge cells in culture (in analogy to the production of bioactive compounds from bacteria and fungi) is another route that indicates progress. The first successful approach to show that sponge cells can proliferate and grow *in vitro* was recently begun with the demosponge *Suberites domuncula*. One crucial step towards a solution of this problem was the finding that single sponge cells, obtained by dissociation of sponge tissue, have lost their telomerase activity and hence their potency for an (unlimited) cell division. After formation of aggregates the cells regain telomerase activity and with this their growth potential; these cells also are able to differentiate in the aggregates. Such assemblies were termed primmorphs. In addition, and finally, cloning of the functional genome of the sponge *Suberites domuncula* will be performed to approach the production of recombinant, low-molecular weight bioactive compounds sponges.

This work has been performed with the contribution of the "Consortium -German Center of Excellence (Biotecmarin)".

NEW PERSPECTIVES ON FARMING OF PHARMACEUTICALLY RELEVANT MEDITERRANEAN SPONGES. PRELIMINARY RESULTS OF AQUACULTURE IN THE BAY OF CALVI (CORSICA)

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Marine sponges have been recognized as an attractive source of numerous novel compounds exhibiting bioactivity.

Main difficulties in processing these metabolites for pharmaceutical purposes, however, arise from the high amount of raw sponge material needed for extraction and isolation of the relevant compounds. To protect natural sponge populations from harvesting and over-exploitation, *in situ* sponge aquaculture is still the most practicable and promising method to produce sufficient sponge biomass.

Within the EC-funded NOMATEC project a new farming method is developed. Some common Mediterranean sponge species with potential pharmaceutical relevance have been taken in culture at depth between 12 and 20 m. Cuttings are farmed in mesh. Survival and growth rates are investigated applying two different observation methods (drip wet weight and projected body area).

The here presented preliminary results of the first project year show that our mari-culture units are a promising tool for sustainable farming of sponges for biomedical purposes.

THE UTILITY OF NUCLEAR MARKERS FOR PHYLOGEOGRAPHIC ANALYSES OF *PLACOSPONGIA*

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Marine sponges often have broad distributions despite the fact that their larvae do not persist in the plankton for more than a few days. Sponges also asexually reproduce via budding and fragmentation and little is known about the passive dispersal of adult fragments. We can make few predictions about sponge/area relationships because of our incomplete knowledge of sponge dispersal potential. Traditional morphological characters are not variable enough to resolve the fine scale phylogenetic relationships that are necessary to address dispersal potential within clades. Similarly, mitochondrial genes that are informative for phylogeographic analyses of many metazoan lineages are nearly invariable between closely related sponge lineages. Recently, the internal transcribed spacer (ITS) region of the nuclear genome has proven informative for phylogeny reconstruction at some levels in marine sponges. Sequence data from the globally distributed clade of *Placospongia* similarly indicates that ITS is informative at some levels of sponge phylogeny, but intra-individual divergence combined with incomplete sorting may limit utility in intraspecific phylogeographic analyses. Here, I compare topologies resulting from maximum parsimony analyses of partial 28s rDNA and ITS sequence data. Phylogeographic analyses using molecular sequence data may finally shed light on how far sponges actually disperse, and what types of barriers prevent them from dispersing farther.

CELLULAR ORGANISATION AND DYNAMICS OF BODY EXTENSIONS AND THEIR PUTATIVE ROLE IN MOVEMENT OF *TETHYA WILHELMA*

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Though several works on the active movement of sponges have been carried out during the last century, locomotion of sponges has not drawn much attention. In fact, most authors of zoology textbooks ignored this phenomenon an called sponges "motionless" animals. Nevertheless, from some works it can be estimated that all sponges locomote, even if the speed of motion is so low that it is extremely difficult to detect. Among the faster locomoting sponges, specimens of the genus *Tethya* display the highest movement activity. For our investigations we used the recently described species *Tethya wilhelma* Sarà, Sarà, Nickel & Brümmer 2001, which inhabits several aquaria of the zoological garden "Wilhelma" of Stuttgart at high population density. Like other species of the genus, *T. wilhelma* features the production of long thin body extensions. Since these extensions are able to attach to surrounding substrate, they have been discussed to be the origin of the movement. Our work focused on the cellular organisation of the body extensions. By immunofluorescent labelling and confocal laser scanning microscopy (cLSM) we characterised the morphological structure of the extensions. In addition we retrieved data on the cellular dynamics *in vivo* by digital or analog-to-digital time-lapse microscopy recording. Time-lapse observation on a macroscopic level on whole animals in the aquarium habitat and on attachment points of the extensions supplemented the microscopical data.

Beside the bundles of strongyloxeas and anisostrongyles, the body extensions of *T. wilhelma* are structurally built by a limited number of cell types, which are addressed as pinacocytes, granulose amoebocytes, multipolar fusiform cells and extremely long (up to 200 µm and more) slender, fusiform actinocytes (myocytes). The latter type cores the body extensions at very high numbers together with the spicules, both arranged more or less in parallel. *In vivo* time-lapse microscopy verified the sliding telescope mechanism for the elongation of the body extensions. At high magnification the dynamics of the actinocytes was recorded. Contraction and active movement of the actinocytes was demonstrated. From our observations we postulate that the sliding movement of the strongyles originates from a contraction and movement cycle of a set of actinocytes. How this is co-ordinated needs further research.

Though many extensions may originate to any direction, the specimens move only to one direction when locomotion is initiated. Neither is the direction of movement linked to a higher number of body extensions, nor are the extensions able to move something towards the sponge. Both indicate that locomotion does not originate from contraction of the body extensions. Nevertheless, they seem to play a role for the movement. Macroscopic observations revealed three types or stages of the body extensions. Beside the well known extensions producing buds (type I) we found thin extensions which can extend extremely long and are able to attach to substrate. We called them "scout extensions" (type II). This type can be transformed to a type we called "guide extensions" (type III), characterised by thickening and a high cell density as well as an expanded basis at the junction to the sponge body. Combining these observations with additional ones on the very high degree of morphological reartangement activities in *T. wilhelma*, we postulate that the body is "gliding" or "flowing" on this extension type III by extensive local cellular reorganisation. A schematic hypothetical movement model is presented.

3-DIMENSIONAL SPONGE *IN VITRO* CULTURES: CURRENT APPROACHES AND PERSPECTIVES

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In vitro sponge cultivation is one possibility to increase access to bioactive compounds produced by sponges or their associated microorganisms. Beside this *in vitro* cultures can be used for experiments on physiology, morphogenesis, spiculogenesis and various other fields of basic sponge research. Anyway the biotechnological background is the main driving force of recent research on *in vitro* cultivation. In our recent research we have laid emphasis on the development and advancement of 3D sponge *in vitro* culture (3D-SiviC) systems, using mainly Mediterranean sponges.

Our group has been involved in the development of the Primmorph® culture system in which dissociated sponge cells are reaggregated to produce small multicellular aggregates which can be maintained as floating elements or attached to culture dishes or special designed culturing substrates. In this way the aggregates can be maintained for several months and used for experimental procedures to determine optimal culture condition and medium composition. The Primmorph® method has been applied so far on many sponge species by us and other groups. Recently we adapted the method of sponge fragmentation to the conditions of *in vitro* cultivation and established a method to regenerate the fragments in closed systems under controlled conditions. We have recently shown that cultivated fragments ("Fragmorphs") of *Chondrosia reniformis* can be maintained for more than one year. During this period significant changes in morphology and histology take place. Though cellular density decreases, most probably due to insufficient nutritional supply in the preliminary culture medium, remaining cells are viable. Requirements and methods for cell viability checks as well as examples of available molecular and metabolic markers will be presented and discussed. The major aim for our ongoing research on 3D-SiviC systems is to develop suitable culture media and nutritional strategies as well as to test growth promoting substances and culturing substrates. Basic physiological research on sponges will be indispensable to understand the needs of sponge cell communities in artificial environments and thus influence the progress of sponge biotechnology significantly.

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MULTIDETECTOR X-RAY COMPUTED TOMOGRAPHY AS A NON-INVASIVE TOOL FOR BIOMETRIC STUDIES OF SPONGES: EXAMPLE *SUBERITES DOMUNCULA*

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Though X-ray computed tomography (CT) methods have been mainly developed for medical imaging and material science they have also been used in marine ecology to study environmental pollution problems and in marine biology to determine the structure and age of corals as well as the porous structure of a Mediterranean pre-coralligenous structure. Except some trials to determine excavating sponge body volumes the method has never been used for sponges. For our recent work we used multidetector computed tomography (MDCT) to image living specimens of *Suberites domuncula* associated with hermit crabs. The individuals have been collected with permission in Rovinj (Croatia) and kept in seawater aquariums in Stuttgart. For our investigations living sponges were transported to the University of Tübingen in batches of 5-7 animals in approximately 50 l of seawater. For imaging in MDCT-prototypes (Siemens Somatom Series: VolumeZoom® and Sensation 16®, Siemens AG, Forchheim) the specimens were transferred to 1 litre plastic containers which were mounted to the head holder of the machines. Spiral x-ray scanning was performed using an "inner ear mode" at various intensity settings and slice thickness between 0.6 and 1.0 cm. Serial sections have been reconstructed and stored in the DICOM 3 file format. Image processing and rendering of 3D volume- and surface-models were performed on a Silicon Graphics Workstation using 3D-Virtuoso® - Software (Siemens AG, Forchheim) or on a PC using "3D-Doctor" (Able Software, Trial Version). Pseudo colour representation of the serial sections as well as the 3D reconstruction allows immediate recognition of differences in density, e.g. by canal structures or incorporated foreign material. Real-time 3D-handling at the Workstation using computer controlled shutter glasses enables to view the model of the individuals by any desired angle in the volume rendering mode (VR). Virtual sections in any direction, length measurements and volume calculations are possible.

MDCT allowed us to determine *in vivo* the position, size, condition of the snail shell initially used by the hermit crab. In most cases, it is possible to determine at least the taxonomic group if not the genus of the snail shell. The helical living tube of the hermit crab built by *S. domuncula* can be visualised as well as the detailed body structures of the crab itself. In the case of large crab-sponge association the crab was no longer inhabiting any part of the shell, but only the helical tube, since the opening of the shell was too small. In case of younger crabs the helical tube was not that extended and the cavity of the shell was still used by the crab.

The surface structure of *S. domuncula* with its oscula as well as its internal morphology of the aquiferous system can also be visualised by pseudo colour.

However, the method is limited to the larger canals and cannot visualise single ostia nor canals of smallest diameter. None of the specimens of *S. domuncula* was damaged by the procedure and all of them were brought back to their aquarium habitat.

MDCT is an excellent method to perform biometric and structural examination of *S. domuncula* and other sponges.

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THE INFLUENCE OF THREE SPONGE SPECIES ON THE SETTLEMENT AND GROWTH OF BRYOZOANS

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The influence of three sponge species (*Acarnochalina hooperi*, *Plakortis lita*, and *Xestospongia vansoesti*) on the settlement of two species of bryozoans was investigated during the summers of 1996 and 1998 in a coral reef off Mactan Island, Philippines. In an earlier study these three sponge species were demonstrated to release allelochemicals that were toxic to specific hard coral species. Settlement plates made from Mactan Stone (a local substratum) were set out at the study site in June 1996 for a one-month period. Plates were hung from a rack and for each plate either one of the sponge species or a control (a synthetic sponge or an acrylic stick) was placed near each plate's surface. The sponge treatments had no significant effect on the bryozoan densities on the plates. One bryozoan species was, however, significantly smaller in the *A. hooperi* treatment as compared to the control plates. The other bryozoan species was also significantly smaller than in control treatments when exposed to *X. vansoesti*. To determine if sponge allelochemicals were responsible for the settlement patterns observed, another settlement study was conducted beginning in June 1998 where settlement plates were constructed from acrylic. Instead of whole organisms, chemical extracts from each sponge species were incorporated into an agar gel, which was placed in plastic holders and separately affixed to each plate. The extracts slowly leached from the gels exposing the plates to sponge allelochemicals for a ten week period. This release was not quantified. Controls were comprised of plates with gels lacking extracts. As with the Mactan Stone settlement plates, the densities of both species of bryozoans were not affected by the sponges. However, one species of bryozoan experienced a reduction in size when exposed to extracts from *X. vansoesti* as compared to bryozoans on the control plates. The sponges *A. hooperi* and *P. lita* had no influence on either density or size of the two bryozoan species. This study demonstrated how sponge allelochemicals could influence the growth of neighbouring organisms.

NEW RECORDS OF MARINE SPONGES (PORIFERA, DEMOSPONGIAE) IN THREE ISLANDS OF THE SOUTH WESTERN ATLANTIC

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In Santa Catarina's coast (southern of Brazil) sponge fauna studies are scarce and limited to tidal zones and isolated areas. Knowledge is restricted to a few records made by Volkmer-Ribeiro & Mothes-de-Moraes (1975); Mothes-de-Moraes (1987); Mothes & Lerner (1994); Lerner (1996, 2001); Lerner & Hajdu (2002). With the aim of contributing to the understanding of shallow-water sponges in the south-western Adantic, we surveyed several localities where Porifera have never been studied before. The samples were collected by scuba diving in Coral Island, Moleques do Sul Island and Cachoeira do Bom Jesus beach, all situated close to Florianópolis (27°50' S/48°30' W). The water temperature was between 16° to 25° C and the depths between 2.5 to 16 m. The taxonomic study was made based on skeletal slides, dissociated spicules mounts and SEM study. The samples are deposited in the Porifera Collection of the Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil. We could examine 28 specimens that are distributed in seven families: Clionidae Gray, 1867; Suberitidae Schmidt, 1870; Trachycladidae Hallmann, 1917; Raspailiidae Hentschel, 1923; Dictyonellidae van Soest, Diaz & Pomponi, 1990; Callyspongiidae De Laubenfels, 1936; Dysideidae Gray, 1867. Five species are new to the science.

FEEDING SPONGES IN BIOREACTORS: WHAT DO THEY EAT?

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Information on the feeding biology of sponges is important for the development of controlled sponge cultures in closed bioreactor systems. Qualitative aspects of feeding, i.e. the appropriate mimicking of the natural food composition, and quantitative aspects of feeding (finding the optimal amount of food to obtain maximal growth) are considered as key factors for successful culture. Our work during the last few years has focused primarily on quantitative aspects, using the tropical Demosponge *Pseudosuberites andrewsi* as a model organism and several microalgae species as food. Promising results were obtained when explants of *P. andrewsi* were fed with the marine diatom *Phaeodactylum tricornotum*. The food was supplied either as intact algae or as a filtered crude extract. Growth (measured as increase in underwater weight) was found in both experiments. The explants fed with intact algae increased up to 255 % (average underwater weight) of the initial weight in 45 to 60 days. The explants fed with crude extract increased to an average of 200 % of the initial weight in 30 days. These results show that it is possible to grow a sponge using a single microorganism species as a food source. In addition, it was demonstrated that sponges are also capable of growing on non particulate food.

The relevance of non particulate food for sponges is further discussed. Calculations based on literature data and measurements of food availability in natural systems and public aquaria indicate that many sponges would suffer from starvation if they would depend solely on the uptake of particles such as bacteria and algae. Elucidation of the nature of the pool of non particulate organic material in natural systems and its role in the feeding biology of sponges is an interesting area for further studies related to the design of controlled sponge cultures in bioreactors.

ASSOCIATED MICROFLORA AND ANTIMICROBIAL ACTIVITY OF THREE SPECIES OF MARINE SPONGES

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The heterotrophic bacterial population associated with 3 species of marine sponges, *Axinella tenuidigitata, Dysidea herbacea* and *Sigmadocia fibulata* inhabiting a coral reef of the south-east coast of India was enumerated. The total heterotrophs associated with *A. tenuidigitata* recorded a maximum of 4.81 x 10^4 CFU gm^{-1} and for *D. herbacea* it was 6.44 x 10^5 CFU g^{-1}, but for *S. fibulata* the population was 8.11 x 10^6 CFU g^{-1}. The bacterial population of ambient water ranged between 3.82 x 10^3 and 1.43 x 10^4 CFU ml^{-1}. The composition of bacterial genera was dominated by *Vibrio* (31.45 %), followed by *Pseudomonas* (18.5 %), *Flavobacterium* (13.31 %), *Aeromonas* (11.69 %), *Corynebacterium* (9.68 %), *Micrococcus* (9.27 %) and *Bacillus* (6.45 %). The vibrios were composed of *Vibrio parahaemolyticus* (55.13 %) and *V. alginolyticus* (20.51 %) as dominant species, in addition to *V. cholerae* (5.13 %), and *V. fischeri* (2.56 %). The evaluation of antibacterial activity of sponges revealed that *A. tenuidigitata* as the most active species inhibiting 46.75 % of the total isolates. On the other hand, *D. herbacea* inhibited 24.19 % and *S. fibulata* was active over 8.87 % of the bacterial isolates. Interestingly all the *Bacillus* strains were sensitive to the sponge metabolites, but *Vibrio* showed highest susceptibility (80.77 %) and *Pseudomonas* was the most resistant species. The range of susceptibility varied with sponge species. Though the sponges are chemically defended against bacteria, there exists effective association of both resistant and sensitive strains, especially vibrios that are the most dominant and most sensitive associated bacteria.

GLYCEROL ETHERS IN MARINE SPONGES - BIOMARKERS OF ARCHAEA AND SULFATE-REDUCING BACTERIA

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Sponges are considered to be the most ancient metazoans and are known to host diverse microbial communities. Numerous studies were performed to characterize the phylogenetic distributions of sponge associated microorganisms (SAM). In particular for the Demospongiae it was shown, that most of the SAM belong to the domain Bacteria comprise a variety of phylogenetic groups. Recently, symbiotic psychrophilic Crenarchaea were also detected in Demospongiae (Preston *et al.*, 1996).

In a recent comprehensive study based on lipid biomarker data we suggested a close affinity of Hexactinellida to Archaea, whereas bacterial communities were dominant among Demospongiae (Thiel *et al.*, 2002). In order to get further information on the phylogenetic affiliations of SAM we continued our investigations of microbial biomarkers. Here we present the occurrence of mono alkyl ether lipids and dialkyl diglycerol tetraether lipids in sponges of the classes Hexactinellida and Demospongiae.

While isoprenoid diglycerol tetraethers are diagnostic for members of the Archaea, prokaryotic as well as eukaryotic sources are discussed for glycerol monoethers and their natural derivatives in sponges.

(I) Substantial amounts of acyclic and cyclic biphytanes (C40 isoprenoids), which are indicative of representatives of marine non-thermophilic crenarchaeotes, were observed after cleavage of the tetraether lipids in the axinellid and hexactinellid sponges (e.g. *Phakellia ventilabrum*; *Aulosaccus* cf. *mitsuk.*, subclass Hexasterophora). These results substantiate the presence of associated Archaea in Hexactinellida. To our knowledge, this is the first chemical report on the presence of sponge associated Archaea in general.

(II) Mono alkyl glycerol ethers were found in the Demospongiae. Previous studies reported the microbial synthesis of non-isoprenoidal mono alkyl glycerol ethers in Bacteria affiliated with the deeply diverging orders Aquificales and Thermotogales and in the sulfate-reducing Bacteria *Desulfosarcina variabilis* and *Desulforhabdus amnigenus*. In our samples these ether lipids are present in highest amounts in morphological "compact" Demospongiae like members of the family Geodiidae, which are considered to contain dense bacterial populations within their oxygen-depleted tissue. Sulfate Reducing Bacteria (SRB) are an integral part of their symbiotic communities, and in addition, enrichments of mono alkyl glycerol ethers were observed for sponges comprising relatively high amounts of biomarkers for SRB. In contrast to previous studies we suggest, that these unusual ether lipids are not solely produced by the host organisms and that esssential portions are derived from sponge associated SRB.

TAXONOMY AND PHYLOGENY OF THE GENUS *AGELAS* (PORIFERA, DEMOSPONGIAE, AGELASIDAE) IN THE SOUTHERN CARIBBEAN SEA

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The genus *Agelas* (Porifera, Demospongiae, Agelasidae) is an interesting group of closely related tropical sponges, whose extraordinary morphological similarity contrasts with a yet uncertain phylogenetic relationship with other groups of demosponges. Its great diversity in the Caribbean Sea (14 species, vs. 8 species in the Indo-Pacific) and the existence of geographically distinct morphotypes in several species, has led us to hypothesize the occurrence of a Caribbean radiation of the genus during the Neogene, possibly related to the closing of the isthmus of Panama and the subsequent changes in sea-level during the glaciations of the northern hemisphere. To test these hypotheses, we undertook an extensive sampling in several localities throughout the Caribbean, to obtain morphological, biochemical and molecular data to reconstruct the phylogeny of the genus and associate the branching events with Neogene history. In this paper, we present the taxonomic description of the species existing in the Southern Caribbean and a preliminary cladogram based on morphological characters. Samples were obtained from several localities in Venezuela, the continental coast of Colombia, the San Andrés and Old Providence Archipelago (Colombia) in the SW Caribbean, and from Belize. Specimens were observed and photographed *in situ* and fragments of them collected. From the fraction fixed in 96 % ethanol, to be used in the morphological analysis, clean spicule mounts were made, as well as dehydrated and stained thick sections mounts for microscopical examination of the skeleton. Seven species were collected and described: *Agelas clathrodes*, *A. citrina*, *A. conifera*, *A. dispar* (brown morphotype, orange morphotype), *A. sceptrum*, *A. sventres* and *A. wiedenmayeri*. While most species show a rather constant morphology throughout the sampled localities, there is a strong geographical as well as local variation in *A. conifera*. In this species in some areas there are locally unique morphotypes, but in others there are two or three morphotypes coexisting sympatrically. The latter is indicative of the existence of more than one species or of a complex of species. The morphotypes of *A. dispar* are morphologically quite distinct and do not coexist within the same geographical area in the Southern Caribbean. However, their sympatric occurrence in The Bahamas indicates that they are different species. Morphological characters are now being analyzed and polarized to reconstruct the internal phylogeny of the group.

WHAT CAN SPONGE CHEMISTRY TELL US ABOUT SPONGE ECOLOGY?

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Sponges are important components of benthic marine ecosystems and are particularly abundant on Caribbean coral reefs. Over the past decade, my research group has studied the effects of sponge secondary metabolites in determining the distribution of sponge species through their functions as chemical defences against potential predators, competitors and pathogens. Our results diverged from traditional views that the impact of fish predation on Caribbean reef sponge populations was minimal, demonstrating that spongivorous fishes, including parrot fishes, feed preferentially on chemically undefended species, limiting them to cryptic habitats and refugia. Generalist predatory fishes were deterred by sponge chemistry, but not by structural elements, toughness, or the nutritional quality of sponge tissue. In general, potential invertebrate predators (crabs, sea stars) were deterred by the chemistry of the same sponge species that deterred fish predators. Well known phenomena from studies of the chemical ecology of terrestrial plants and insects were not apparent for sponge species we examined: we found no evidence of aposematic (warning) coloration, of differential elaboration or induction of metabolites, or of biotransformation of precursors into deterrent compounds. Most recently, we have used a novel technique to test the ability of sponge metabolites to inhibit the overgrowth of adjacent species: extracts of 6 of 20 species consistently inhibited overgrowth by sponge competitors, while extracts of 3 species consistently promoted overgrowth. Field surveys corroborated these assay results, demonstrating that the former 6 species were infrequently involved in contact interactions, while the latter 3 species were frequently overgrown by other sponges, suggesting an associational defence.

Our results may be important for understanding recent changes in Caribbean reef ecosystems. General overfishing (trapping, netting) on some reefs may eliminate spongivorous fishes (particularly angelfishes and parrot fishes), allowing some chemically undefended sponge species to grow unchecked. If some of these species are competitively dominant (through fast growth, or by virtue of chemical defences), they may out-compete adjacent sponges and corals for space. Alternatively, selective "sport" fishing for piscivorous fishes (barracuda, grouper, snapper, grunts) may eliminate predators of spongivorous fishes, resulting in their increased populations, and more intense predation on sponges. Therefore, fishing activities may alter sponge and coral community structure through direct and indirect means.

AN UNKNOWN POESCILOSCLERID SPONGE IS INVADING THE GULF OF MORBIHAN (SOUTH FRENCH BRITTANY)

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An unknown Poecilosclerid sponge has been observed for the first time in the Gulf of Morbihan in 1999. Since then, this sponge, which seems to have a fast dynamic, becomes very abundant with tens of large specimens. Considering several criteria, this sponge is thought to be an introduced species: (1) it is new to the area, (2) its distribution is rather localized; (3) from the first localized station, its spread follows a logical pattern; (4) it has a strong tendency to proliferate; (5) there is a potential introduction source (aquaculture farms) close at hand. An ecological survey is running.

For the moment, the taxonomic status of this sponge is quite obscure. It is yellowish, encrusting to massive and its thickness may reach 50 cm. The surface is rather smooth, often colonized by epibionts and exhibits an excessive mucus production. Oscula are not detectable. The skeleton is plumo-reticulate. Megascleres are mainly represented by acanthostrongyles of two different sizes, 180-240 µm and 260-330 µm, and few acanthostyles, 260-300 µm. Microscleres are represented by two "antagonistic" types : palmate chela, 25-60 µm, and onychaetes, 55-90 µm. The last character is only known from the family Tedaniidae. Actually, we are thinking to at least a new genus. The 28S rRNA sequences will help us to demonstrate the affinity of this sponge with the Tedaniidae or an other family of the Poecilosclerida.

IN SITU COMPARATIVE STUDY OF SEVERAL MEDITERRANEAN SPONGES AS POTENTIAL BIOMONITORS OF HEAVY METALS

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The heavy metals content of sponges is investigated in order to assess their suitability as biomonitors with regard to their concentration ability, inter-individual variability and indication of pollutant bioavailability. The concentration of 10 elements is determined in six species of massive Demosponges well represented in a polluted and a clean site: *Cliona viridis*, *Caminospongia scalaris*, *Chondrosia reniformis*, *Spongia officinalis*, *Spongia agaricina* and *Aaptos aaptos*. Wide interspecific variations are observed between them, no doubt as a result of their morphological and physiological differences. *Cliona viridis* appears to be unique with regard to the higher concentrations found for several elements, especially Cd which is from 42 to 375 times more concentrated than in the other species. However, this species displays highly variable results and gives a rather poor image of the level of contamination of the sites, as only three trace elements out of ten have a significantly higher concentration in the polluted site. Three species, *S. officinalis*, *S. agaricina* and *A. aaptos*, which give a consensual indication of the metals bioavailability, with sufficiently high and homogenous concentrations, appear to be well suited for consideration in the overall assessment of the health of assemblages from Mediterranean rocky habitats.

CELL TYPES AS TAXONOMIC CHARACTERS IN *APLYSINA* (APLYSINIDAE, VERONGIDA)

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Some sponge groups are known to present a complex taxonomy, commonly associated with few morphological taxonomic characters, viz. low variety of spicule types (Haplosclerida), skeletons composed by spongin fibers only ("keratosa" sponges) or even lack of a skeleton altogether (*Oscarella*). Consequently, techniques other than traditional morphology have been sought to enhance taxonomic diagnoses of these groups. Techniques used successfully were isozymes, DNA fingerprinting and cytological characterizations aided by transmission electron microscopy. Nevertheless, most require expensive equipment or are time consuming procedures. In this work we used a simple technique, based on the characterization of definite cell types obtained by tissue dissociation and cytospins. Two species were used: *Aplysina caissara* Pinheiro and Hajdu, 2001 and *Aplysina fulva* (Pallas, 1766). Both species show similar external and internal morphology, principally on smaller individuals, leading to erroneous identifications. Three cell types shared by both species were used, namely spherulous cell I, spherulous cell II, and microgranular cell. Both species can be clearly distinguished based on the observed characteristics of these cell types (general size and variety of inclusions). The results show that the morphology of definite cell types, as observed on simple cytospins, can be an additional taxonomic character to the differentiation of cryptic species.

WHAT CAN WE LEARN ABOUT SPONGES FROM PALAEONTOLOGY

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In the age of molecular systematic and phylogeny, palaeontological data are sometimes considered as less important. Palaeontology has, however, one important advantage over them, it gives us time dimension. This can tell us directly about the antiquity of particular sponge groups or lineages, and now we know that two large sponge groups, i.e. Hexactinellida and Demospongiae existed already in the Precambrain, while Calcarea are probably slightly younger. It allows us, however, to understand also other aspects of sponge evolution and ecology. Past occurrences of large sponge faunas show that general pattern of their depth distribution, i.e. demosponges dominating in more shallow settings and hexactinellids in deeper environments, is today the same as in the past. It seems however, that some groups of sponges with solid silica skeleton, such as lithistids and hexactinellids with fused skeleton, inhabited in the geological past more shallow environments than today, what can be associated with higher silica contents in the Paleozoic and some of Mesozoic seas. An example of non-actualistic ecological occurrence of siliceous sponges is the Eocene lithistid fauna of SW Australia. It is a very rich and diversified lithistid sponge assemblage which clearly inhabited extremely shallow water, in a near-shore environment, while today's lithistids occupy, with some exceptions only, deep-water habitats. Some Upper Cretaceous lithistid faunas known from the chalk clearly inhabited muddy soft bottom, rather than hard rocky bottom as it is the case with most Recent lithistids. The fossil record of sponges is very broken and of discrete character but it concerns mostly bodily preserved sponges. Very important information can be gathered from studies of loose, disassociated spicules, which are much more common. The Cambrian bodily preserved sponges, for example, display very simple spiculation, while some assemblages of disassociated spicules of the same age display much more advanced spicule types and higher spicule diversity, with some strange morphologies unknown in fossil bodily preserved sponges. Thus we must be cautious in our perception of very simple sponges in the Cambrian. Palaeontological data give us also insight into morphological potential of particular sponge groups, as revealed by a wide variety of fossil sponge morphologies. Lack of data about sponge evolution during some intervals of geological time follows perhaps not so much from the poor fossil record, or poor quality of the palaeontological material as sometimes believed, but rather from the fact that fossil sponges have not been the subject of numerous studies, and that there were and are very few, too few, palaeospongologists studying them.

BIODIVERSITY AND DISTRIBUTION OF POLYMASTIIDAE (DEMOSPONGIAE, HADROMERIDA) IN THE ARCTIC AREA

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Biodiversity of Arctic sponges has been subjected to very few studies (Koltun, 1959, 1966, Ereskovsky, 1995) and evidently needs revision. The present work is focused on the family Polymastiidae Gray, 1867 whose fauna was recently revised in NE Atlantic (Boury-Esnault, 1987; Boury-Esnault *et al.*, 1994) and SW Pacific (Kelly-Borges & Bergquist, 1997), but is poorly known in other areas. 17 nominal species and subspecies from 10 nominal genera have been included in the family checklist for the Arctic area at one time or another. Now only 12 species from 6 genera of the list are considered to be valid. The genus *Vosmaeria* Fristedt, 1885 has been excluded from the family due to the absence of typical polymastiid cortex. Two species are included in the genus *Sphaerotylus* Topsent, 1898: *S. capitatus* (Vosmaer, 1885) and *S. borealis* (Swarczewsky, 1906). The latter possesses grapnel-like exotyles together with typical sphaerotylostyles in the cortex and therefore few authors (Swarczewsky, 1906; Boury-Esnault, 2002) include it in *Proteleia* Ridley & Dendy, 1886. Arctic sponges of the genus *Radiella* Schmidt, 1870 belong to two species, *R. hemisphaerica* (Sars, 1872) and *R. sarsi* (Ridley & Dendy, 1886). The latter was erroneously confused with *R. sol* Schmidt, 1870 by Koltun (1966). No changes concern the genus *Tentorium* Vosmaer, 1887 with a single species in the northern hemisphere: *T. semisuberites* (Schmidt, 1870). *Weberella* Vosmaer, 1885 previously synonymised by Koltun (1966) and Ereskovksy (1995) with *Polymastia* Bowerbank, 1864 is considered to be a valid genus with the only Arctic species *W. bursa* (Mueller, 1806). As to the genus *Quasillina* Norman, 1869 the Arctic species described as *Q. richardi* Topsent, 1913 is put here in synonymy with *Q. brevis* (Bowerbank, 1862). Five species inhabiting the Arctic area belong to the genus *Polymastia* Bowerbank, 1864. *P. arctica* (Mereikowsky, 1878) previously confused with *P. mamillaris* (Mueller, 1806) and *P. penicillus* (Montagu, 1818) (Vosmaer, 1887; Swarczewsky, 1906; Koltun, 1966; Ereskovsky, 1995) are considered to be a valid species. These sponges produce buds which are unknown in other northern *Polymastia*. *P. euplectella* Rezvoj, 1927 previously confused with *P. robusta* (Bowerbank, 1861) by Koltun (1966) quite differs from the latter by the size and structure of the papillae as well as by spicule composition. The validity of *P. thielei* Koltun, 1964 is also re-established. These sponges are characterised by the presence of an aquiferous cavity layer in the cortex in contrast to *P. uberrima* (Schmidt, 1870), which is also found in Arctic. The status of *P. grimaldi* (Topsent, 1913) needs further examination as this species is very close to *Radiella* by growth form, structure of basal cortex and presence of fringe. All of the revised Polymastiidae species inhabit both the Arctic and the NE Atlantic with the exception of *P. arctica* and *P. euplectella* which ere known only from the Barents and White Seas.

NEW SESQUITERPENES FROM THE MARINE SPONGE *PHAKELLIA VENTILABRUM*

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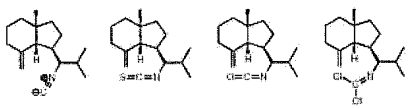
Sponges of the orders Axinellida and Halichondrida are known to produce a variety of sesquiterpene isonitriles. These are usually accompanied by isothiocyanates and formamides showing the same carbon skeletons . In one case a sesquiterpene isocyanate has been reported.

In *Phakellia ventilabrum* we now identified groups of sesquiterpene isonitriles and isothiocyanates as well as isocyanates.

In addition, we found dichloroimines which, too, seem to be biosynthetically related to the isonitriles. Dichloroimines proved to be very rare among marine natural products .

In *P. ventilabrum*, sesquiterpenoid dichloroimines were detected as trace compounds, which strongly suggests the presence of "Isonitrile-Isothiocyanate-Isocyanate-Dichloroimine-Quartetts", rather than the frequently found "Isonitrile-Isothiocyanate-Formamide-Triplets".

The isolation of these compounds was carried out by the combination of flash chromatography, HPLC and preparative GC. Structure elucidation was based on GC-MS and GC-FTIR investigations, as well as on NMR data. The development of micro-reactions for the interconversion of isonitrile-derivatives facilitated the identification of minor and trace compounds. As an example the structures of typical derivatives of the known axisonitrile-1 are shown below.



DEMOSPONGES ASSOCIATED TO THE MACROALGAE *LAMINARIA OCHROLEUCA*: DISTRIBUTION PATTERNS IN TIME AND SPACE

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We studied the demosponge fauna associated with a small North-Atlantic population of the macroalgae *Laminaria ochroleuca* from 1996 to 1999. Both sponges and algae, the rhizomes of which serve as a substratum for the sponges, were sampled by SCUBA diving using 2500 cm^2 quadrates. We examined differences in sponge abundance (biomass in gm^{-2}), species richness, and species diversity as a function of time (year 1 to year 4), depth, exposure to waves (exposed *versus* protected sites within the algal bed), and silt (weight of sediment on the bottom of the sampling sites). Because an unidentified mortality agent caused a dramatic decrease in both number and biomass of algae during 1998 and 1999, we took this opportunity to investigate potential qualitative and quantitative changes in the sponge population linked to such a *Laminaria* decay.

We identified a total of 47 demosponges from the *Laminaria* bed. Depth was the only environmental variable having a significant effect on sponge distribution, with detectable differences in species diversity between depth ranges. We also found differences in sponge biomass and species richness as a function of time, In both cases, values peaked in 1998, showing temporal patterns apparently uncorrelated with those of the *Laminaria* biomass. Classification and ordination analyses detected differences in the taxonomic distribution of the sponge fauna between both years and the various microhabitats defined by the small set of environmental variables considered in the study. In the classification analysis, the sponge fauna of years 1997 and 1998 showed the greatest similarity, again following a pattern apparently unrelated to the *Laminaria* mortality. In the ordination analyses, depth and algae biomass explained better than other environmental variables the pattern of sponge distribution. The canonical ordination analysis also revealed that the abundances of a small group of sponge species increased coincidentally with the *Laminaria* decay. The implications of the various distribution patterns detected in the study are discussed in a broader ecological context.

A CLIMBER SPONGE

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Chondrilla nucula Schmidt, 1862 is a photophylous sponge species living in Mediterranean shallow waters; its growth system is modular and shows extreme body remodelling, particularly evidenced during the production of new clonal individuals. In natural environments long stretched filaments allow large buds to slide downward, settling on a new substrate.

In the sponge farming (USAMA ®) of Portofino were settled 20 plastic tubes (10 x 5 cm) each one containing 2-4 fragments (modules) of *Chondrilla nucula*. After one year of permanence in tubes the modules fused each others and their number doubled; the bottom of each tube was completely covered by sponge biomass showing a clear tendency to colonise also vertical walls. Sponge mortality was lower than 10%. In a successive period of 4-6 months all sponges climbed on wall tubes reaching the superior free rim that was completely covered by 10-12 modules in all tubes. Three years after the experiment starting (June, 27, 2002) sponges were settled on all available surfaces (also external) of tubes, showing an inversion of geotropism (firstly negative, finally positive).

Some results are evident: 1) the movement is active, being against the gravity in a first phase; 2) the direction (upwards) is the same for all specimens, as consequence is not casual; 3) the geotropism inversion is the consequence of different growing strategies exploiting opposite opportunities of substratum colonization proposed by inner and outer vertical walls of tubes.

In conclusion, this is the firsts evidence of sponge "voluntary" movement.

THE PROBLEM OF CONSPECIFICITY AND THE TAXONOMIC STATUS OF *IRCINIA FELIX*, *IRCINIA FASCICULATA* AND *IRCINIA VARIABILIS*

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De Laubenfels (1948), in his synopsis on horny sponges, proposed the conspecificity of *Ircinia variabilis* (Schmidt, 1862) and *I. fasciculata* (Schmidt, 1862) with *I. fasciculata* (Pallas, 1766) and *I. felix* (Duchassaing & Michelotti, 1864). The two Mediterranean Schmidt's species and the Caribbean one of Duchassaing & Michelotti were considered junior synonyms of Pallas' *fasciculata*; for this last, according to the lack of the holotype, was designed a Caribbean neotype (De Laubenfels, 1948).

Successively Wiedenmayer (1977) and Van Soest (1978) considered the conspecificity of the previously cited Mediterranean and Caribbean *Ircinia* species with caution.

To characterise the traits of *I. felix* a morphological analysis was performed on collections from Florida and Belize. These data were compared with a Mediterranean collection of specimens ascribed to *I. variabilis*. Further observations were carried out on materials belonging to historical collections, including Schmidt's, Duchassaing & Michelotti's and de Laubenfels' type materials.

In our study, the neotype of *I. fasciculata* (Caribbean Sea) resulted clearly to be an *I. felix* specimen, according with the skeleton morphology of Duchassaing & Michelotti type specimens and the Florida collection.

The lectotype of *I. variabilis*, together with other three Schmidt's specimens from the Adriatic Sea, share the diagnostic traits with the specimens of *I. variabilis* collected in different sites of the Mediterranean Sea.

The single specimen of *I. fasciculata* belonging to Schmidt's collection is here ascribed to the genus *Sarcotragus* (*sensu* Ruetzler, 1965; Boury Esnault, 1971; Pulitzer-Finali & Pronzato, 1980), according to the presence of very thin filaments and the exclusive presence of spicules inside primary fibres. Conformably *I. fasciculata* (Esper, 1794) was ascribed to the genus *Sarcotragus* by Topsent (1920, 1930).

The type material of *I. fasciculata* (Pallas) is missing, the original description is too short and generic and the type locality is unknown.

Following these evidences the systematic position of *I. fasciculata* (*sensu* Pallas, Schmidt, Esper), *I. felix* (Duchassaing & Michelotti) and *I. variabilis* (Schmidt) is discussed and emended.

MORPHOLOGY AND TAXONOMY OF CARIBBEAN AND MEDITERRANEAN COMMERCIAL SPONGES

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According to the recent literature on Dictyoceratida, commercial sponge species belong to the genera *Spongia* and *Hippospongia* (de Laubenfels & Storr, 1958; Vacelet, 1987; Pronzato et al., 2000). Their distribution is almost exclusive of Mediterranean and Caribbean Seas.

The Mediterranean area hosts seven species, five of which of commercial value: *Spongia officinalis* Schmidt, 1862; *S. mollissima* Schmidt, 1862; *S. agaricina* Pallas, 1766; *S. zimocca* Schmidt, 1862; *Hippospongia communis* Lamarck, 1813.

The Caribbean area hosts twelve species, nine of which regularly traded: *Spongia barbara* D. & M., 1864; *S. graminea* Hyatt, 1877; *S. tampa* de Laubenfels & Storr, 1958; *S. cheiris* de Laubenfels & Storr, 1958; *Hippospongia lachne* de Laubenfels, 1936; *H. gossypina* (D. & M., 1864).

In spite of these common species has been the object of several studies (Schmidt, 1862, 1864, 1866, 1868; Duchassaing & Michelotti, 1864; Hyatt, 1865; Lendelfeld, 1889; Topsent, 1930, 1932, 1933; de Laubenfels, 1948, 1950; Wiedenmayer, 1977; Van Soest, 1978) an exhaustive revision of their taxonomic status had not been performed.

The morphological analysis of macro- and micro-characters was carried out on type materials and recent collections from both geographic areas. Studied specimens are mostly in dry status or exclusively represented by cleaned skeletons.

Our overview evidences, once again, that gross external morphology is one of the major diagnostic traits for bath sponges. On the contrary the variability of micro-morphological traits, such as the core of primary fibres and the secondary network organization, make difficult a precise diagnosis.

SPICULAR CHARACTERS IN FOSSIL AND LIVING SPECIES OF MEDITERRANEAN *GEODIA*

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The genus *Geodia* Lamarck, 1815 encompasses about one hundred described species with a very wide bathymetric and geographic distribution.

Four species live in the Mediterranean Sea: *Geodia cydonium* (Jameson, 1811), *Geodia conchilega* Schmidt, 1862, *Geodia tuber* Lendenfeld, 1894 and *Geodia micropunctata* Row, 1911.

Species belonging to this genus share a complex body architecture where the ectosome is differentiated into a thick cortex reinforced by collagenous fibres and microscleres; the choanosome is sustained by megascleres, radially arranged, with different kinds of microscleres. Our SEM study of living Mediterranean species evidenced that they differ in shape, skeleton architecture, spiculation and ecological distribution, but they don't show marked differences as far as sterrasters are concerned.

Further observations, focused on sterrasters, allowed to understand their building system involving a complex process of silica deposition on protein cores radially organized; the last phase is the formation of apical thorns. The complexity and diversity of these ornamentations did not define different kinds of spicules, but were considered different phases of their growth.

SEM comparison between sterrasters of Mediterranean living species and Miocenic ones (Serravalle Scrivia area, Northern Italy) show that fossil sterrasters are much bigger than present ones. A progressive spicular reduction during the evolution of these Mediterranean species is here suggested. The fossil sponge spicules at Serravalle Scrivia are so abundant that it is possible to extend the distribution area of the fossil species belonging to the genus Geodia to the north-western Mediterranean.

HEXACTINELLIDA AFTER 132 YEARS OF STUDY: WHAT ' S NEW?

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Hexactinellid sponges were first recognized to constitute a natural group by Thomson in 1868 as his Vitrea, but authority for the more accepted name, Hexactinellida, is accorded to Schmidt, 1870. Zittel's (1877) primary division of the group into forms with fused rigid skeletons, Dictyonina, and those with only separate spicules, Lyssakina, was used by Schulze (1886) as the basis of his first modern taxonomic scheme for the group. Subsequent important modifications were 1) Schulze's (1899) recognition of primary phyletic division of the group based upon microscleres, Amphidiscophora and Hexasterophora, instead of fusion/non-fusion of main spicules, 2) Schrammen's (1902) recognition of the lychnisc-based group as a distinct high-level taxon (now order), 3) Ijima's (1927) reorganization of above-family taxa to the presently used arrangement; and 4) Tabachnick & Reiswig's (2000) recognition of a distinct pattern of spicule fusion in formation of the order Aulocalycoida. Continuing changes in higher level arrangements are expected in view of the many remaining taxa to be discovered (estimate only 50 % of species described) and the low level of detailed character analysis of the "known" species. Previously unexpected variation in patterns of spicule fusion in dictyonine sponges should provide a better basis for recognition of phyletic groups. The place of Hexactinellida within the Porifera remains controversial in spite of advances in knowledge of soft-tissue structure and several molecular sequence studies. The syncytial nature of all well-fixed hexactinellids was strongly suspected by both Schulze and Ijima from their light microscope studies in the late 1800's and early 1900's. Convincing proof of syncytiality was not available until the electron microscope study of Mackie & Singla (1983) which formed the basis of the distinction of Hexactinellida from other cellular sponges. Arguments for the sister-group Demospongiae - Calcarea based upon their cellularity (Cellularia) syncytial organization of Hexactinellida, and sister-group Demospongiae - Hexactinellida based in part upon siliceous skeleton (Silicea) versus calcareous skeleton in Calcarea remain competitive. Although recent molecular sequence studies have generally favored the second of these hypotheses, a pattern of consistent high-level support for one, and consistent rejection of the other has not yet let to consensus. Hexactinellid species populations are generally thought to be sparsely distributed over fairly large geographic ranges. How such dilute populations maintain functional reproductive contact remains a mystery for hexactinellids as well as other sparsely distributed megafauna. In contrast, historic early dredging and recent photographic sled and submersible surveys indicate significant occurrence in many regions of dense hexactinellid populations of single species and multi species communities in relatively large patches often with dimensions of kilometers. Factors conducive to large patch development and maintenance are not intuitive but are under investigation. The obviously important components of larval behavior (dispersion, settlement, attachment site selection) and post-larval development remain areas of basic hexactinellid biology containing little factual information. The *Oopsacas* populations of the cave near Marseille, from which Boury-Esnault and Vacelet (1994, 1999) have provided the detailed description of the distinctive trichimella larva, offer the best hope for filling these voids.

MICROBIAL DIVERSITY IN *PETROSIA FICIFORMIS* INVESTIGATED BY DIFFERENT TECHNICAL APPROACHES

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To define the microbial community associated to photophylous populations of the common Mediterranean sponge *Petrosia ficiformis* (Poiret, 1789), we choosed a different approaches using both classical and molecular techniques.

A total amount of about 70 bacterial strains were isolated both from the sponge surface and mesohyle and cultured on Marine Agar. Bacteria were identified according to biochemical and morphological screenings. Morphological observations by Light Microscopy (LM) and Scanning Electron Microscopy (SEM) showed a strong dominance by cocci and rods strains, whose localization and relationship with the host sponge was investigated by Transmission Electron Microscopy (TEM).

Molecular analysis (RAPD) allowed similarity clustering within the culturable microbial community.

It was highlighted that P. ficiformis hosts large and complex communities of bacteria belonging to the genera Vibrio, Pseudoalteromonas, Corynebacteria, Bacillus, Micrococcus, Actinomyces, and Flavobacterium. Our results confirm that classical microbiological approaches are needed to identify bacteria, though these techniques can only lead to the isolation and cultivation in vitro of small percentages of the bacterial community associated to the sponge. Molecular studies, on the other hand, can perform a key role in the identification of even hardly culturable and unculturable species improving also our knowledge on bacterial taxonomy. From an applied point of view the isolation, cultivation and characterization of bacteria symbiotic with sponges can be of major importance in discriminating new sources of novel bioactive compounds.

DISTRIBUTION OF ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES IN MARINE SPONGES

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Among marine invertebrates, sponges are considered to be the most productive source of bioactive compounds for potential industrial and pharmacological applications. In this view, sponges from different geographical locations were screened for the presence of such compounds. The correlation between bioactive properties and the geographical origin and systematic position of the sponges was also investigated.

A total of 216 samples of sponges corresponding to 202 species distributed throughout 13 orders were collected from 5 geographical locations. They were extracted and submitted to bioassays against a panel of 14 organisms. General cytotoxicity was assessed by means of the brine shrimp *Artemia salina* lethality assay. Antimicrobial assays were carried out by the disc-diffusion method against Gram-positive, Gram-negative bacteria, yeast and 10 filamentous fungi. About three-quarters of the screened sponges inhibited at least one of the tested organisms while about a third exhibited a specific toxicity. The Caribbean sponge *Haliclona vansoesti* distinguished itself from all other species by its broad-spectrum of activity. The general pattern of sensitivity towards sponge methanolic extracts revealed that *Artemia* larvae were the most sensitive organisms followed by filamentous fungi, yeast and bacteria. About a third of the active extracts were toxic only against *Artemia* while only a few sponges displayed specific activity against bacteria and yeast but not against filamentous fungi. Thus *Artemia* lethality assays proved to be effective in detecting bioactive compounds from sponges, but these tests were not necessarily correlated with antimicrobial activities. No relation was found between antibacterial, antiyeast or antifungal activities. No major differences in the number of toxic species were observed according to sponge collection origin, but the patterns of activities and specificities were different. Bioactive species were found in all orders of sponges. Hadromerida included the greatest percentage of bioactive species against one or more assay organisms, followed by Halichondrida, Verongida, Haplosclerida, Dendro/Dictyoceratida and Poecilosclerida. Hadromerida and Dendro/Dictyoceratida species displayed high specific toxicity contrary to

Halichondrida, which exhibited a broad range of toxicity.

Bioactive compounds from 76 sponges belonging to 63 species were isolated and their structures elucidated using a combination of spectral methods. Novel sponge compounds were detected for *Axinyssa* spp., *Callyspongia pseudoreticulata*, *Esconemna* spp., *Haliclona toxius*, *Haliclona wansoesti*, *Hyrtios* spp., *Hyrtios tubulatus*, *Hyrtios erectus*, *Hyrtios reticulatus*, *Niphates recondita*, and *Suberea* aff. *praetensa*. Three types of chemosystematic patterns were recognized: 1) all taxa of a group possessed the same family of compounds, 2) the compound was found exclusively or dominantly in a limited group of related sponges, but not all representatives of the group possessed the compounds, 3) the compound was unique to the species and no related compounds occurred in other sponges.

IDENTIFICATION OF HOMOLOGUES OF THE EMH-3 HOMEOBOX-CONTAINING GENE IN DEMOSPONGES

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In recent years, the study of the structure and function of homeobox-containing genes in basal metazoans has appeared to be fundamental to understand the evolution of these genes throughout the animal kingdom. In this context, we have continued the study of the EmH-3 homeobox-containing gene isolated from the freshwater sponge *Ephydatia muelleri* and determined the role this gene might play in sponge morphogenesis. As previously reported, we have established that the EmH-3 gene is differentially expressed during sponge development, from almost undetectable levels in gemmules to high levels at the moment of hatching and throughout the sponge's life. EmH-3 expression is cell-specific, only being strongly expressed in the undifferentiated and pluripotent archaeocytes. It has been found to be necessary for the differentiation of archaeocytes into choanocytes and hence for the completion of a functional sponge. EmH-3 presents a high homology with EfH-1/prox 2 and Spox TA1 isolated from the freshwater sponge *Ephydatia fluviatilis* and the marine sponge *Tethya aurantium* respectively.

In the present work homologues of the EmH-3 gene have been identified and characterized in three other freshwater sponge species, *Spongilla lacustris*, *Eunapius fragilis*, and *Trochospongilla horrida*, by PCR and RT-PCR with specific primers and sequencing. They were designated EmH-3Sl, EmH-3Ef and EmH-3Th respectively. The lengths of the amplified PCR products varied from 740 bp to 900 bp according to the species but all the transcripts had about the same length i.e. 440 bp as the EmH-3 transcript. The genomic DNA sequences comprised three exons separated by two introns of different lengths, the homeobox lying in the third exon. Splicing sites were identical to those found in the EmH-3 gene but differed from those of prox 2 in *E. fluviatilis*, the first exon being 50 bp shorter in the latter species. Comparative analysis of the nt and the aa sequences obtained from *S. lacustris*, *E. fragilis*, and *T. horrida* revealed a high degree of identity with the EmH-3 gene from *E. muelleri* and with each other, especially in the homeobox region. As to their expression, all the identified EmH-3 homologues were expressed differentially during gemmule germination and hatching as observed for EmH-3 in *E. muelleri*. High conservation of gene structure, sequence and expression patterns suggested that the EmH-3 homologues might have the same function as the EmH-3 gene in cell differentiation and sponge development. Parsimony and Neighbour-joining analyses based on nt sequences revealed a grouping of *E. muelleri* and *E. fluviatilis* on one hand, of *S. lacustris* and *E. fragilis* on the other hand, *T. horrida* being outside both groups. Such grouping was supported by high bootstrap values. EmH-3, its homologues and EfH-1/prox 2 homeodomains appeared to be most closely related to the Hox 11/Tlx class of higher Metazoa. The search for EmH-3 homologues in different orders of marine sponges is in progress.

BIOLOGY OF SPONGE NATURAL PRODUCTS

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Many sponge secondary metabolites display interesting bioactivity, which gives them potential applications as new drugs, anti-fouling substances, or tools for chemical engineering for a variety of human uses. A few synthetic analogues of such sponge compounds have already found their way into industry, but synthesis of many others appears to be cost prohibitive or even impossible. Supply of such compounds has to come from the source organism or its cells, but incomplete understanding of the biological, ecological and physiological features of sponge secondary metabolites has resulted in failure to obtain a steady supply. In the "Symbiosponge" project (EC-MAS3-CT97-0144) the biological and chemical aspects of sponge natural products of interest for human use have been studied in order to obtain understanding of the cellular origin, the possible microsymbiont involvement and the ecological significance of sponge secondary metabolites, and their distribution patterns among other sponges.

We have been able to demonstrate involvement in secondary metabolite production of sponge cells (archaeocytes and spherulous cells) in two of the sponges studied (*Haliclona vansoesti* and *Agelas conifera*). Involvement of heterotrophic bacteria and possibly cyanobacteria has been shown to be likely, but not been proven beyond doubt in two other sponges (*Xestospongia muta* and *Ircinia felix*).

In cases where the sponge cells themselves are involved, the ecological purpose of the compounds has been clarified. Sceptrin and oroidin (*A. conifera*) are predator deterrent compounds and may also have a function in space competition. Similar compounds are found in all closely related sponge taxa. (2R, 3R, 7Z)-2-aminotetradec-7-ene-1, 3-diol (*H. vansoesti*) is a competitive defensive compound and is unique for this particular sponge species. The polyacetylenic (*X. muta*) and variabilin-type (*I. felix*) compounds of suspected bacterial origin appear more difficult to classify in an ecological sense. Their pattern of distribution is less clear as these compounds appear to be taxon-linked, but not all closely related taxa possess them.

PORIFERA (POECILOSCLERIDA) OF SPANISH ANTARCTIC EXPEDITION BENTART-94

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Porifera represent one of the most important elements in the Antarctic biota due to their diversity and to their dominance in diverse areas (Sarà *et al.*, 1992). In the Antarctic benthos, at depths of 100 m, sponges can attain values of biomass comparable to the highest values recorded from tropical areas (Beliaev & Ushakov, 1957). Demospongiae and Hexactinellida are evenly distributed and more abundant than calcareous sponges in Antarctica, where the silica rich waters facilitate the increase of spicule size.

In this communication the results of the investigations on the material collected by the Spanish research vessel "Hespérides" during the expedition BENTART-94, in the South Platform of the Livingston and Deception Islands are presented.

This material is composed of 71 specimens, 36 of which belong to the Order Poecilosclerida. Isodictyidae is the most abundant family, due to the presence of the species *Isodictya grandis* (Ridley & Dendy, 1886), *Isodictya kerguelenensis* (Ridley & Dendy, 1886), *Isodictya setifer* (Topsent, 1901) and *Isodictya erinacea* (Topsent, 1916). Other species recorded belong to genera *Myxilla* Gray, 1867; *Guitarra* Carter, 1874; *Mycale* Schmidt, 1862; *Iophon* Gray, 1867; *Tedania* Gray, 1867; *Ophlitaspongia* Bowerbank, 1866 and *Artemisina* Vosmaer, 1885.

Sponges have been found in 23,8 % of the stations, the most effective method being the rock dredge.

SPONGES ON CORAL REEFS: A COMMUNITY SHAPED BY COMPETITIVE COOPERATION

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Sponges are benthic sessile invertebrates, intimately associated with other animals and with a plethora of plants and microbes. They are specialized filter feeders and require solid substrate without excessive sediment exposure to flourish. Tropical coral reefs and associated communities offer an abundance of supporting structures, from coral rock to mangrove stilt roots, but this opportunity comes with the cost of a fierce struggle for ecological balance among members of this ecological system. With the advent of scientific diving some 50 years ago, numerous studies have shown and quantified the impact sponges have on reefs, owing to high diversity, large biomass, complex physiology and chemistry, and long evolutionary history. Important ecological conditions and processes generated by sponges and their endosymbionts relate to space competition, habitat provision, predation, and chemical defense; primary production, nutrients cycling, nitrification, and food chains; and bioerosion, mineralization, and cementation. Despite these realizations, most reef ecologists not specializing in Porifera, as well as resource managers and conservationists barely acknowledge the important role of sponges in their work. Three reasons are to blame: sponges remain an enigmatic group because they are difficult to identify and to maintain under laboratory conditions; sponge scientists are few and highly specialized and despite sporadic and local efforts were not successful in producing authoritative, well illustrated field manuals for large geographic areas; and all that is known about sponges on tropical reefs comes from studies of hundreds of different reefs worldwide and lacks the focus of a comprehensive study at one site. There is a lot of new evidence that coral reefs are rapidly deteriorating because of habitat destruction, pollution, water warming, and overexploitation. Certain sponges seem to be able to benefit from the stressing of coral but the vital support function of the reef will cease as soon as forces of bioerosion and water dynamics exceed the constructive processes of calcium carbonate secretion and cementation, leading to the demise of reef sponge communities. The present forum is ideally suited to discuss and evaluate this bleak outlook and recommend ways to stop and possibly reverse the negative trend.

A PROBLEM IN TAXONOMY: THE SPONGE FAMILY LATRUNCULIIDAE TOPSENT (1922)

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Latrunculiidae Topsent (1922) is characterized primarily by the possession of a dense accumulation of acanthose dischorhabds or "chessman" spicules, disposed in a perpendicular palisade in the outer ectosome, areolate porefields, and short fistular oscules on the sponge surface. Prior to this revision, the Latrunculiidae was considered to exhibit substantial heterogeneity and comprised nine nominal genera, *incertae sedis* within the Class Demospongiae. Revisionary work has resulted in the retention of three valid genera within the Latrunculiidae, i.e. *Latrunculia* du Bocage, *Sceptrella* Schmidt, and *Strongylodesma* Lévi, and a proposal for transfer of Latrunculiidae to the Order Poecilosclerida (Class Demospongiae, Subclass Ceractinomorpha). Following Kelly-Borges and Vacelet (1995), *Sigmosceptrella*, *Diacarnus*, *Negombata*, *Podospongia* and synonym *Alcyospongia* are separate from Latrunculiidae, and Family Podospongiidae de Laubenfels resurrected from inclusion in the Latrunculiidae to receive these genera. Other atypical genera, previously classified within Latrunculiidae, were also transferred to other families within the Class Demospongiae. This paper reviews and redefines the specific diagnostic characters of the family Latrunculiidae and provides a framework for the recognition and establishment of new genera and species.

MOLECULAR SYSTEMATIC SURVEY OF SPONGE-DERIVED MARINE MICROBES

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Marine sponges are known to host a wide taxonomic spectrum of microbes. The Harbor Branch collection of marine microbes, developed over 17 years, contains >14,000 eubacterial and fungal isolates of which 9,500 were derived from deep-water (> 120 fsw) marine sponges and other invertebrates. The invertebrate hosts, collected primarily in the Atlantic and Caribbean regions, encompass at least 80 genera of deep-water sponges with some identified to the species level. Primary tools for morphological description of the microbes include colony morphology, cell morphology and Gram staining, while molecular systematics analysis relies on PCR amplification of isolate and

PHYSICAL AND CHEMICAL ANALYSIS OF THE GLASSY SKELETONS IN SIX MARINE SPONGES REPRESENTING TWO SPONGE GROUPS (DEMOSPONGIAE AND HEXACTINELLIDA)

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The glassy skeletons of six different hexactinellids and demosponges were examined and compared using a series of physical and chemical tests. The sponges were two hermit crab sponges (Class Demospongiae, family Suberitidae), one from Scotland, *Suberites domuncula* and the other, *Pseudospongosorites suberitoides*, from the Gulf of Mexico, and four hexactinellids, *Hyalonema* sp., *Euplectella aspergillum*, *Rhabdocalyptus dawsoni*, and *Aphrocallistes vastus*. The operating hypothesis was that testing might reveal noteworthy differences in the nature of the amorphous hydrated silica skeletal material for Demosponges and Hexactinellids, that could prove taxonomically useful. Physical properties and structure were studied as follows: SEM, glass density, thermal analyses to determine glass transition temperatures (Tg) and water content, and FTIR spectra. Chemical determinations of SiO_2 and trace elements were made by both inductively coupled plasma optimal emission spectroscopy (ICP-OES) and energy dispersive X-ray fluorescence (EDXRF).

Results showed a great similarity in the physical and chemical composition of the glassy skeletal material in all six sponges studied. In all the spicular skeletal material is deposited in successive layers around the axial canal. Density was closely similar in all sponges (range 2.05 – 2.24 g/cc) and similar to the density of pure SiO_2 at 2.205 g/cc. Thermal analyses showed a Tg event from 425-545° C for some sponges, and all showed a dramatic "exothermic event" occurring from 905-1010° C, possibly associated with the occurrence of a partial crystallization at this temperature. IR spectra using two different methods (direct transmission vs. pellet technique) were similar with prominent absorption bands at 460-470, 800, and 1090-1100 cm^{-1} (due to different vibrational modes of Si-O-Si linkages) and at 1650 cm^{-1} (due to water). The skeletons of all 6 sponges each showed similar spectra to that of silica gel and opal. Water comprised 10-14 % of the skeleton by weight.

Chemical studies showed that the % Si in all sponges was similar and amounts did not differ significantly between the demosponges and the hexactinellids (Mann-Whitney test, P = 0.93, ns). Analyses of SiO_2 and trace elements with EDXRF showed that all 6 sponges had skeletons that were closely similar in the amount of SiO_2 content (94.8-97.1 %, not including water) and seven trace elements (Ca, S, Mg, K, Na, Al, and Cl, together approx. 3 % by weight). Water, SiO_2, and these 7 trace elements comprised 99.5 % or more of spicule chemical content.

A LINNAEUS II CD-ROM FOR IDENTIFICATION OF THE COMMONEST DEMOSPONGIAE OF THE SÃO SEBASTIÃO CHANNEL AREA, SOUTHWESTERN ATLANTIC

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The publication of results originating from biodiversity surveys, in a form that best meets the needs of science and society, is one of the missions of the Systematics Agenda 2000. Multimedia systems, such as CD-ROMs and Internet sites are greatly adjusted to such goals, by allowing the propagation of great amounts of information at low cost. Created for the management of data in multimedia, the software Linnaeus II (ETI, University of Amsterdam/UNESCO) has being used here for the elaboration of a CD-ROM with two main objectives: 1) aid in basic education concerning the Phylum Porifera (high school and university: the portuguese language was chosen as more appropriate for a product of regional interest only), and 2) tool for the identification of Porifera, which will be presented here. The area has been chosen in view of: 1) São Paulo State's over 50 % share of all research conducted in Brazil, 2) location in the Channel of Brazil's largest tankers facility and associated intermittent chemical stress, 3) urban development pressure, and 4) marine biological laboratory facilities (a rarity in Brazil). Twenty-five species amongst the most conspicuous occuring in the São Sebastião Channel were selected: *Amphimedon viridis, Aplysina caissara, A. fulva, Axinella corrugata, Chondrilla* aff. *nucula, Chondrosia* aff. *reniformis, Cinachyrella alloclada, Clathria campechea, Cliona* aff. *celata, Dragmacidon reticulatus, Geodia corticostylifera, Halichondria cebimarensis, Haliclona melana, Hymeniacidon heliophila, Laxosuberites aurantiaca, Mycale* aff. *americana, M. angulosa, M. laxissima, M. magnirhaphidifera, M. microsigmatosa, Petromica citrina, Polymastia janeirensis, Scopalina ruetzleri, Tedania ignis* and *Tetilla radiata*. For each species, the following data were supplied: a description of the external and internal morphology, its ecology, literature, list of specimens in the collection of Museu Nacional/UFRJ and distribution in the São Sebastião Channel, in Brazil and in the world. The Identify-IT file associated to a glossary of technical terms, allows a moderately unexperienced user to select from a series of characters which ones he is more familiar with, starting thus the identification process. The program attaches a probability to likely identifications, which are then analysed in detail by returning of the full species description, prior to considering a sample actually identified.

SPONGE PECULIARITIES AND THEIR IMPACT ON GENERAL BIOLOGY AT THE THRESHOLD OF 2000

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To understand the present status of sponge research at the beginning of a new millennium, also in relation to general biology, could be useful to give a retrospect of its historical course. An overview of publications on living sponges since 1870 till 2000 shows a turning point around 1970. The years around this date show the beginning of an expansion characterized by a growing number of papers and the rise of new research fields and methodologies. It occurs together with an impressive increase, especially after 1990, of papers of applied biochemistry in the flourishing sector of the chemistry of natural products. On one hand there is the splitting of the research fields in more specialized subdivisions but on the other the blending of different sectors in multifarious research objectives with different methodologies, reflecting general tendencies in recent biology. Sponge research has acquired a major impact on general biology after the recent acknowledgement based on sound molecular data that sponges evolved early from a common ancestor with all other Metazoa and not as a separate branch. Sponge peculiarities, as the lack of definite tissues and organs and of well defined nervous and muscle systems, represent a crucial problem of animal evolution. Furthermore, the primitive organization of sponges has allowed them an extraordinary organizational and developmental freedom and plasticity and winning ecological strategies. Then, sponges represent the broadest possible element of comparison to understand animal organization. Sponges show outstanding potentialities in regeneration and reproduction, the presence of a primitive type of a conductive and contractile system, the occurrence of collagen and then of pluricellularity and that of advanced immunological properties, an extraordinary biochemical versatility, the physiological and ecological impact of harbouring large populations of bacterial endosymbionts, an intimate relationship with the aquatic and substrate environments, a wide extent of morphological variability and plasticity, also in relation to the species problem. These and other peculiarities are of great relevance in biology. A general question is also to understand how and why sponges were able to maintain their primitive organization from their appearance, 580 mya, to now, even if, especially in mesozoic, they underwent some evolutionary changes. As regards the future directions of sponge research it may be foreseen that molecular genetic research, till now little developed in relation to the advanced animal models, will considerably increase, to clarify biological problems that regard sponges as near to the ancestor of the animal kingdom. But it is also important to remember that any advanced research requires the basic naturalistic knowledge coming from taxonomy and ecology, in sponges as in other groups. What we know on taxonomy and ecology of sponges, in spite of the amount of study till now performed, is always too little and many exciting discoveries are waiting us. Unfortunately, "the crop is large and the workers are few": however, in spite of the present heavy shortage of positions and funds for basic research, we need to be optimistic for the future.

OPEN WATER MARICULTURE WITH MARINE SPONGES IN LIMSKJ CANAL (ROVINJ, CROATIA, NORTHERN ADRIATIC SEA)

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The Northern Adriatic Sea in particular the Rovinj area with the Limskj Canal is well known for its richness of sponge species. In the Limskj Canal, an 11 Km long fjord north to Rovinj (Croatia, Northern Adriatic Sea), a mariculture platform was installed to deploy several cultivation units on which different sponge species were transplanted to different substrates.

The mariculture platform provides space for all the technical installations necessary and for scuba divers working on the different cultivation units in the sublittoral area below the platform. The platform, made of the modular JETFLOAT® system, was fixed by three anchor ropes about 15 meters away from the southern coastline within the protected area of the Limskj Canal.

Four species of marine sponges have been tested with different transplantation techniques onto three different hard substrates: *Axinella polypoides*, *Aplysina aerophoba*, *Chondrilla nucula* and *Dysidea avara*.

From 37 specimens, collected at different depths in the area surrounding the platform, a total of 127 transplants were obtained and fixed onto the cultivation units using different methods (wires, plastic strings, boreholes, etc.). The cultivation units are of different size, ranging from 16,0 cm^2 to 2,0 m^2. The units were distributed between 10,0 and 18,0 m water depth and fixed to the sea bottom. Smaller cultivation units (16,0 cm^2) carry single transplants and were grouped on wooden frames. The larger units (0,6 m^2, 1,0 m^2, 2,0 m^2) are of single character and carry several transplants.

The different substrates for transplantation were either taken from the sublittoral of the Limskj Canal (pieces of limestone) or from quarries nearby Rovinj (preshaped blocks of limestone). A third, artificial hard substrate was produced by the ARCON® technology. Survival and growth rates of the transplants from the four target species in relation to transplantation techniques and substrates are presented and discussed.

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BIODIVERSITY OF DEEP SEA SPONGE COMMUNITIES ON SEAMOUNTS: "SPOT ENDEMISM" AND RARITY AS PREVALENT COMPONENTS OF SPATIAL HETEROGENEITY

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Perhaps the most pervasive characteristics of our understanding of deep-sea ecosystems is a paradox: although the deep-sea environment compromises the single largest ecological unit of the globe, spanning 3 x 10^8 km^2, it is also the most poorly known and explored. Within the deep sea, seamounts are frequently seen as "island systems" of extraordinary biomass and richness. Equally high levels of biodiversity but restricted species ranges may accompany this localised enrichment of benthic communities. Here we investigated spatial patterns of species richness in the sponge fauna of South Pacific seamounts along the Norfolk Ridge (New Caledonia), incorporating a quantification of species' occurrence in relation to community turnover and spatial heterogeneity. Sponge assemblages of the 10 seamounts were sampled over a depth range that straddles the subtidal–bathyal transition (236-583 m) with a Beam trawl and a "Dredge Warren". Average sampling time was 2.25 hrs per seamount, yielding a rich fauna of 114 species belonging to 40 families, 17 orders and 2 classes. "Spot endemics" dominated the fauna, with 69 % of recorded species being restricted to a single site and thus rare. Species richness per individual seamount varied widely from 6 to 47 species. Equally, species turnover (beta diversity) among habitats was high and independent of spatial scale. Adjacent seamounts did on average not share significantly more species than site pairs further apart. Multivariate analysis (clustering & MDS) of community composition using records at the order level of taxonomic identity indicated four assemblages: (1) the southern slope of the main island of Grand Terre, (2) two deeper seamounts in the southern section of the ridge system ("Eponge" & "Introuvable"), (3) a single seamount in the centre of the ridge ("Stylaster"), and (4) a cluster of seamounts in the central part plus a single southern seamount. Depth appears to be the dominant factor in controlling community composition, while bottom type or latitude had no consistent relationship with biological patterns. At a family level, evenness of the distribution of taxa (VarTD) on seamounts was high, with no site departing significantly from the overall regional species pool. By contrast, clear differences in average taxonomic distance (AvTD) were found between seamounts: AvTD was high on "Stylaster" (a seamount with many Hexactinellids), but low on "Introuvable" and "Eponge" (2 seamounts with many Lithistids). The spatially disjunct mosaic of biodiversity on the seamounts coupled with a prevalence of rarity in the form of spot endemism, parallels patterns in shallow water systems (Schlacher et al 1998). While both carry similar implications for conservation strategies, the isolation of deep sea seamounts may make them good candidates to study evolutionary trajectories and speciation in the deep sea.

POPULATION DYNAMICS OF SPONGES OF STETSON BANK, NORTHWESTERN GULF OF MEXICO, DERIVED FROM ANNUAL PHOTOGRAPHIC MONITORING

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Aspects of the population dynamics of three species of sponges were investigated at Stetson Bank in the northwestern Gulf of Mexico (28° 09.985' N, 94° 17.842' W). Annual repetitive photographs of 44 permanently marked stations, taken between 1993 and 2002, 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 these 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 10-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. *I. 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. *C. 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. *A. 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.

NEW ADVANCES IN SPONGE SCIENCE – APPLICATION OF MICROSENSORS

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To date microsensors have mainly been employed to study conditions in sediments, microbial and algal mats, and boundary layer properties. Nevertheless, microsensors can also be used on macrozooic organisms such as live invertebrates. As an example, they have been successfully applied to investigate calcification in scleractinian corals. Drawn-out sensor tips of a diameter of 10 µm enable very localised positioning, without significantly disturbing the microenvironment. Various parameters can be measured on a microscale and gradients can be studied in 1 µm steps. Bioeroding sponges were used to test the suitability of this technique on sponges. Sensors for calcium, pH and oxygen were constructed to investigate decalcification by bioeroding sponges and photosynthesis by their symbiotic zooxanthellae. Preliminary results are presented.

PIONE LAMPA, A BIOERODING SPONGE LIVING IN UNSTABLE ENVIRONMENTS

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A bioeroding sponge was found in a sabellariid worm reef in Florida, USA and was identified as the clionid *Pione lampa* (Laubenfels, 1950). It contained numerous gemmules, which were attached to erosion chamber walls. They were subspherical to lentil-shaped and had an aspicular, unstructured, smooth and rigid coat. It enclosed dense cell material and various spicule types. Gemmules are likely to ensure survival under adverse conditions such as smothering, exposure to air and high temperatures. Gemmules from this site might occasionally be freed and scattered, since the Florida reef can suffer severe damage during periods of heavy wave activity such as that created during hurricane season. Bioerosion activity of the sponge increases the chance to free gemmules, as the sponge not only etches into calcareous particles cemented into the matrix produced by the worms, but also into the matrix itself. This ability enables the sponge to utilise the reef as substrate.

Within the Clionidae, *Cliona annulifera* and three species of the genus *Pione* are the only species known to produce typical gemmules. Possible reasons are 1) reproduction of bioeroding sponges is understudied and asexual bodies may have been overlooked in other species, and 2) clionid gemmules are an adaptation to survive life in risky environments. Sponges of the genus *Pione* are comparatively successful in environments, in which they are close to their physical limits or in potentially unstable or mobile substrates.

REPRODUCTIVE ASPECTS OF *CHONDRILLA NUCULA* IN THE MEDITERRANEAN SEA

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Chondrilla nucula Schmidt, 1892 is known to be gonochoristic and oviparous; the sexual reproduction, in the Mediterranean Sea, takes place in summer-autumn. We investigated some morphological aspects related to egg and sperm production. Specimens were collected in Portofino (Ligurian Sea) during August and September 2001. Some were fixed (Glutaraldehyd 2.5 %) and embedded in paraffin for histology, while some others were reared in conditioned aquaria. Hystological sections showed the presence of either oocytes or spermiocysts in almost all female and male individuals. A reorganisation of the aquiferous system was also evident. Specimens transferred into the aquaria released eggs, then were fixed in Glutaraldehyd 2.5 % for light and transmission electron microscopy or placed in pots to follow their development. The first stages of cellular division were observed and photographed *in vivo* under the light microscope, proving that *C. nucula* undergoes internal fertilisation.

As a control, samples of *C. nucula* were collected in non-reproductive periods (late autumn-winter); in most of them the presence of few spermiocysts was still recorded but oocysts were never observed.

From the macro-morphological point of view, specimens in reproduction present a rough surface. In females, the presence of a darker, grey mesohyl layer, under the cortex, indicates the presence of a continous carpet of mature eggs.

REVISION OF WESTERN ATLANTIC *GEODIA* LAMARCK, 1815 (ASTROPHORIDA, GEODIIDAE): I. BRAZILIAN SPECIES, INCLUDING A NEW RECORD

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The genus *Geodia* has Tethyan distribution, with about seventeen species registered for the Western Atlantic Region. The literature has described seven species belonging to this genus in the Brazilian coast before 1998: *Geodia gibberosa* Lamarck, 1815, *G. glariosa* (Sollas, 1886 as *Cydonium glariosus*), *G. vosmaeri* (Sollas, 1886 as *Synops vosmaeri*), *G. neptuni* (Sollas, 1886 as *S. neptuni*), *G. tylastra* Boury-Esnault, 1973, *G. papyracea* Hechtel, 1976 and *G. corticostylifera* Hajdu et al., 1992. Three new species were described from bathyal depths off Rio Grande do Sul State coast by Silva & Mothes (2000): *G. australis*, *G. splendida* and *G. riograndensis*. The examined material was collected by Scuba diving from 0 to 25 m (36 samples) or dredged from 33 to 634m (18 samples), carried out under the auspices of Projeto Talude, Fundação Universidade do Rio Grande (FURG), Brazil; Projeto dos Recursos Vivos da Zona Econômica Exclusiva (REVIZEE/SE), Brazil; R/V "Almirante Saldanha", Brazil; "National Oceanographic and Atmospheric Administration Oregon II" Cruise, USA; "Calypso" Expedition, France and H.M.S. "Challenger" Expedition, England. Dissociated spicule mounts, thick sections and SEM stubs preparation has followed the methodology described by Mothes (1996) and Mothes & Silva (2002). The comparative analysis detected for the first time the presence of *G. magellani* (Sollas, 1888 as *C. magellani*) in Brazilian waters. In this way, this Magellan species has now its known northern limit at the coast of Rio Grande do Sul State (31°20' S/49°52' W, 128 m). This study has also detected and increased the geographic distribution area along the Brazilian coast of seven species, in comparison to previous researches: *G. papyracea* (Fernando de Noronha Archipelago, Alagoas and São Paulo States), *G. gibberosa* (Alagoas and Bahia States), *G. glariosa* (Pernambuco, Espirito Santo and São Paulo States), *G. tylastra* (Fernando de Noronha Archipelago), *G. neptuni* (Rio de Janeiro State), *G. corticostylifera* (Bahia and São Paulo States) and *G. splendida* (Santa Catarina State). Our data confirm the conspecificity of *G. neptuni* and *G. vosmaeri*, proposed by Hajdu et al. (1992). A key for Brazilian *Geodia* species is presented and a new opening arrangement and spicule set based approach to the genus at a SEM level detailing is suggested.

IMPORTANT STEPS FOR STARTING-UP A SPONGE-CELL CULTURE

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Sponge cell culture could be the most simple and efficient way for the production of sponge biomass and its natural products because cell cultures can easily be controlled, maintained and scaled-up with present expertise of animal cell cultivation. However, sponge-cell culture is still in its infancy, because a continuous cell line has not been established. A recurring problem with sponge cell culture is the possible infection of the cell culture by unicellular eukaryotes. Many of these organisms are present on sponges and are not so easily removed. Since a lot of these eukaryotic contaminants are not so easily distinguished from sponge cells, a method should be developed for unambiguous determination of the origin of a growing cell culture. We have developed a genetic identification method for sponge cells. As an example we showed the technique for *Dysidea avara*. We used the sequence of the 18S ribosomal unit to test the origin of cells from a cell culture. The 18S sequence of the cells was compared with the sequence of the 18S sequence of the sponge. A second step in starting-up a sponge-cell culture, is physical separation of sponge cells from other eukaryotic cells to start a cell culture without any contaminants. Currently, we are working on a method to select sponge cells from a mixture of eukaryotic cells.

BIODIVERSITY AND COMPETITIVE INTERACTIONS OF CAVE SPONGES IN THE BAHAMAS

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The extensive marine cave systems of the Bahamas represent one of the last faunistic frontiers of the region, and certainly one of the least studied. Caves serve as refuges for "living fossils" with origins in the deep sea, as well as recent immigrants from the surrounding reefs and deep forereef slopes. Sponges represent one of the most conspicuous members of coral reef communities, but little is known about the biodiversity and ecology of cave sponges. To date we have determined that approximately a third of the sponge species within 7 Bahamas cave systems are probably species that are unique to the cave ecosystem (i.e., they are not conspicuous members of the nearby reefs), while the rest represent immigrants from the surrounding reef-flats. There was a high degree of variability in the individual cave sponge faunas; only one species, *Plakortis* sp., was common to 5 of 7 caves. The remaining 28 species collected typically were found in a single cave, even though the entrances to distinct cave systems could be less than 50m distance. The caves also exhibit a zonation pattern of facultative to obligate species as one moves from the cave mouth backwards. Cave obligates were competitively dominant to the facultative species, and bioassays provided evidence that these interactions are mediated by chemical defenses. Nonetheless, the high degree of system-specific "endemism" within these caves, and the high potential for anthropogenic disturbance suggests that these cave obligate sponges are an incredibly fragile resource.

MODULATORS OF POLYPEPTIDE GALNAC TRANSFERASE FROM AN AXINELLID SPONGE

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2-N-Acetyl galactosamine (GalNAc) residues are found in O-linked glycoproteins that adorn cell membranes and mediate important cellular events including progression of cell fertilization, cell recognition and disease states such as metastasis of cancer and chronic inflammation. UDP-GalNAc is produced by an epimerase that isomerizes UDP-GlcNAc, and is subsequently incorporated into polysaccharide chains of mucin-type glycoproteins by . The enzyme polypeptide GalNAc transferase (ppGalNAcT). Both the epimerase and transferase are important targets, but a cell-permeant ppGalNAcT inhibitor may be a most useful tool to study glycopeptide assembly. Bioassay-guided screening identified an extracts from an Axinellid sponge collected in Western Australia which showed inhibition of ppGalNAcT (EC50 40 µg/mL). We describe here isolation and structural studies of compounds from this extract.

SPONGES OF THE SULTANATE OF OMAN

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The Sultanate of Oman harbours rich populations of sponges, especially in the four coral reef areas (Musandam Peninsula, Muscat coast & Daymaniyat Islands, coasts of Masirah Island, and the Khuriya Muriya Islands). Apart from a few incidental samples recorded by e.g. Carter (1869) and Sara & Bavestrello (1995), the sponges of Oman were known up till now only from 5 dredge stations made by the John Murray Expedition off the south coasts of Oman, near the Khuriya Muriya Islands, between 13.5 and 1415 m. To complement these dredge samples, we recently obtained 65 samples belonging to 50 species by SCUBA and shore collecting, mostly from the northern part of Oman (project EU MAS3 CT97-0144). Combined with the earlier records, approximately 150 nominal species are now known from Oman, but the earlier collections are in need of revision. This preliminary list of sponges includes 48 species so far known only from the Sultanate of Oman, but 24 of these are not (yet) identified to species level and will be the subject of ongoing taxonomic work of our group with several new taxa to be described. Predictably, the remaining 100 species belong to sponges also occurring in adjacent regions such as the Seychelles (66 species shared), India (59), and the Red Sea (26). Each of these Western Indian Ocean regions shares a number of species (up to 17) exclusively with Oman, indicating "overlapping" interregional areas of endemism, e.g. separate Insular-Arabian, African-Arabian and Indian-Arabian regions. Less expected is the relatively low similarity with sponges from nearby Yemen (20 species shared); this is probably due to the fact that only a few dredge samples from the Gulf of Aden are available so far (also by the John Murray Expedition). The similarity with the sponges of East Africa is also relatively low (26 species shared). A minority of species recorded from Oman are shared with far off locations such as the north and west coasts of Australia, Japan and the Philippines, etc. These sponges are invariably widespread over the whole Indo-West Pacific.

PHOTOSYNTHETIC ACTIVITY OF INTERTIDAL AND SUBTIDAL TROPICAL SPONGES

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The frequency of marine sponges that harbour photosynthetic organisms was examined in a tropical area of the Western Indian Ocean. Out of 77 species from five different habitats in Zanzibar, 55 were found to have photosynthetic activity as assayed by *in situ* pulse amplitude modulated (PAM) fluorometry and later validated in the laboratory by measurements of chlorophyll content. A significantly highet percentage of intertidal (85%, n=27) than of subtidal (64%, n=50) species was found to be photosynthetically active (X^2=3.85, $p<0.05$ (df=1)). The fact that each habitat contained its own characteristic sponge species (only 8 sponge species were found in more than one habitat) suggests that the generally high presence of photosymbionts in all the various intertidal communities may be an important component to the successful adaptation of those species to life in the intertidal. We propose that such intertidal sponges may be more dependent on autotrophic symbionts to meet their energetic needs (even in environments relatively rich in nutrients such as mangrove stands) because they are limited in their filtering capacity during exposure to air at low tide.

THE LONG-TERM RECOVERY OF SPONGE POPULATIONS IN FLORIDA KEYS, USA FOLLOWING A WIDESPREAD SPONGE MORTALITY

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During 1992 and 1993, widespread sponge mortalities significantly impacted sponge populations in the Florida Keys, USA. The extent of the impacted areas was estimated to be approximately 1000 km². The cause of the mortalities was attributed to cyanobacteria blooms. It was hypothesized that the sponge mortality resulted from clogging of the sponges' filter feeding mechanism, bloom toxicity, or perhaps lowered dissolved oxygen levels. However, the exact cause has not yet been documented.

The work described here was initiated in response to concerns regarding the ecological and fishery impacts resulting from increased commercial sponge (sponges of the genera *Hippospongia* and *Spongia*) harvesting effort in the late 1980s and early 1990s. Beginning in 1994, the work entered a second phase: documentation of the sponge mortality impact on sponge community biomass, and long-term evaluation of sponge community recovery. Data on the recovery of sponge populations in two areas has been collected on an annual basis from 1993 through 2002.

Project data documented a highly significant decline in sponge numerical abundance, with an even more significant reduction (up to 90%) in sponge community volumetric biomass. However, the severity of the mortality varied significantly over the affected area. Sponges of the genera *Ircinia, Hippospongia* and *Spongia* appeared to be the most susceptible to the mortality. *Spheciospongia vesparia* appeared to be more resistant than many other species, but was completely eliminated throughout extensive areas. One species, *Cinachyra* sp., appeared to be particularly resistant.

As work has progressed a more comprehensive description of the sponge fauna in the study area has been undertaken. Data are now collected for 30 sponge taxa and we have a reasonable complete description of the sponge fauna and relative abundance throughout the study area.

Data have documented a highly significant recovery of certain species in recent years (1998-2002) of the genera *Hippospongia, Spongia* and *Ircinia*. However, the extent of recovery of these species was not uniform throughout the study area. Two species of the genus *Ircinia* (*I. strobilina* and *Ircinia* sp.) have recovered to their former abundance. In contrast, *Ircinia campana*, formerly a particularly abundant species, has shown no indications of any recovery.

The most conspicuous sponge, *Spheciospongia vesparia*, in terms of size and abundance, has shown limited signs of recovery. Two species of sponges, *Spheciospongia vesparia* and *Ircinia campana*, accounted for approximately 70 % of the sponge community biomass prior to the sponge mortalities. To date, there has been limited or no recovery of these species even though ten years have passed since the sponge mortalities.

As the project evolves into a truly long-term evaluation of sponge community recover, data are being collected that indicate that there are several sponge species that have exhibited rather dramatic fluctuations in abundance over the past ten years. These data may indicate that certain sponge species (*Halichondria melanoforia, Adocia* sp., *Hystrios* sp., *Cinachyra* sp.) may undergo significant natural fluctuations in abundance.

The survey work is expected to continue. Future data will document and evaluate the long-term recovery of the sponge community throughout the area affected by the sponge mortality. These data will also assist in monitoring environmental conditions and modeling food webs. Furthermore, such long-term analysis may provide insights into differences in the life histories and ecology of certain sponge species.

SPONGE BIOMASS ESTIMATES IN THE UPPER AND MIDDLE FLORIDA KEYS, U.S.A

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The work described here was initiated in response to concerns regarding ecological and fishery impacts resulting from increased sponge harvesting effort in the late 1980's and early 1990's. The objective of the initial phase of the work was to document and quantify the contribution of commercial sponges (genera *Hippospongia* and *Spongia*) to total sponge community biomass.

During 1991 and 1992 a total of 15 areas were sampled. The total area surveyed was 34620 m². Sampling methodology consisted of counting all sponges found with twelve 100 m x 2 m transects at each area. Numerical abundance data was recorded only for commercial species (*Hippospongia lachne*, *Spongia barbara*, *Spongia graminea*) and largest most common species (*Spheciospongia vesparia*, *Ircinia campana*, *Ircinia strobilina*, and *Ircinia* sp.). All other sponges were lumped into a miscellaneous unidentified category. In addition to numerical counts, data on volumetric biomass of the different sponge species and sampling categories were collected. This methodology consisted of estimating sponge specimen volume by measuring the volume of water displaced when the sponge was placed in a bucket fitted with an overflow spout.

The mean abundance for all sponges was 7250/hectare and for commercial sponges was 106/hectare. The mean volumetric biomass of all sponges was 364 ml/m². Both methods employed to estimate sponge biomass indicated that the contribution of commercial sponge biomass to the total sponge community biomass was relatively small (1.4 % based on numerical counts, 2.4 % based on volumetric estimates). Two species of sponges, *Spheciospongia vesparia* and *Ircinia campana* represented 69 % of the total sponge community biomass based on volumetric estimates.

During completion of the study, a widespread sponge mortality occurred over a 1000 km² area. Consequently, a new phase of the study was initiated to document the impacts of the mortality on sponge community biomass and evaluate the long-term recovery of sponge populations.

A NEW SPECIES AND SUBSPECIES OF *FIELDINGIA* AND FORMATION OF A NEW FAMILY AND ORDER OF HEXACTINELLID, HEXASTEROPHORA

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Reinvestigation of *Fieldingia* collected off the Indonesian Archipelago and described by Schulze (1887) as *F. lagettoides* and finding of a new similar specimen from the South China Sea allow to consider Schultze's specimens to be a doubtless *Fieldingia*. A new species *F. valentini* and a new subspecies *F. valentini tizardi* are described. The presence of complete sets of spicules allows to settle the problems with both dictional framework construction and loose spicule specification of the poorly known genus. These data give grounds to distinguish Fieldingidae, a new family with a single recent genus. The unic construction of dictional choanosomal skeleton together with some other less important characters of loose spicules are features of a level of an order in Hexactinellida and a new order Fieldingida is established.

THE NEW SPECIMENS OF RARE EURETIDAE: *LEFROYELLA DECORA, GYMNODICTYUM VARIOLOSUM, TRETOCHONE DUPLICATA* AND *PLEUROCHORIUM ANNANDALEI* (PORIFERA, HEXACTINELLIDA) PROVIDE THE FAMILY REORGANISATION WITH FORMATION OF A NEW SUBFAMILY

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Finding of a well-preserved flexible stage specimen of *Lefroyella decora* allow to settle the question of the subfamilies allocation of this genus. The wall of this sponge is a result of a plication process with amararhyses formation and it is not composed with longitudinal tubes formed by the dichotomous branching. So *Lefroyella* must be considered as a doubtless representative of Euretidae-Chonelasmatinae. Its diagnosis is corrected. Investigations of new specimens of *Gymnodictyum variolosum* (elder synonym of *Endorete pertusum*) and *Tretochone duplicata* allow to supplement data on their morphology. *Pleurochorium annandalei* known previously from the N Indian ocean is turned to be widely distributed in the W Indian ocean as well. Two new subspecies of *P. annandalei* are described. A finding of a more complete, branching specimen and examination of its spicule content showed that *Pleurochorium* has much more complex body form then it was considered bifore. The type of tubular branching observed in this genus may not be attributed to dichotomous or to any of its variations but it shoud be considered as a regular emission of tubular branches from the side of the wall, hence the genus should be conserved within Chonelasmatinae. A scheme of phylogenetic relations of the genera of Euretidae is suggested. A new subfamily with a single representative is suggested.

A NEW GENUS AND SPECIES REPRESENTATIVE OF AULOCALYCIDAE WITH UNIQUE BODY FORM (PORIFERA, HEXACTINELLIDA)

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Description and diagnosis are given of a new hexactinellid sponge, (family Aulocalycidae) collected S of New Zealand. The rigid skeleton of this sponge seems to be similar to a body form erroneously supposed for *Mylinsia callocyathus* (family Eutretidae) by Reid (1964) and described as a form with "short irregularly expanding lateral outgrowths, whose margins unite locally so that lateral views show a network of anastomosing margins". Later revision by Reiswig and Wheeler (in press) showed that this interpretation of the body form of *M. callocyathus* is erroneous. The skeleton of the new sponge is similar to the form suggested by Reid for *M. callocyathus*.

NEW SPECIMENS OF RARE EURETIDAE: *LEFROYELLA DECORA*, *GYMNODICTYUM VARIOLOSUM* AND *TRETOCHONE DUPLICATA* PROVIDE THE FAMILY REORGANISATION WITH FORMATION OF A NEW HEXACTINELLID SUBFAMILY

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Discovery of a well-preserved specimen of *Lefroyella decora* in its early flexible stage enabled settlement of subfamily allocation of its genus. The wall of this sponge results from a plication process with amararhyses formation; it is not composed of longitudinal tubes formed by dichotomous branching. Hence *Lefroyella* must be considered as a valid member of Euretidae-Chonelasmatinae. Its diagnosis is here corrected. Investigation of new specimens of *Gymnodictyum variolosum* (elder synonym of *Endorete pertusum*) and *Tretochone duplicata* enabled determination of new supplementary data on their morphology. A scheme of phylogenetic relations among the genera of Euretidae is suggested. A new subfamily with a single representative *Bathyxiphus subtilis* is proposed.

COEVOLUTION OF MICROBIAL SYMBIONTS AND *DYSIDEA* (ORDER DENDROCERATIDA, FAMILY DYSIDEIDAE)

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Although marine sponges can host a variety of cyanobacterial and bacterial symbionts, it is often not known whether these symbionts are generalists that occur in a variety of host species or specialists that occur only in certain species or populations of sponges. We observed the filamentous cyanobacterium *Oscillatoria spongeliae* in collections of the sponge *Dysidea* cf. *herbacea* and similar cyanobacteria in collections of *D.* cf. *granulosa* and *D.* cf. *avara*. We hypothesized that host-specific strains of cyanobacteria are found in each sponge species. To test this hypothesis, we extracted total genomic DNA from specimens of all three *Dysidea* species from several locations on Guam, including several color forms of *D.* cf. *herbacea*. We used sponge-specific PCR primers to amplify a 683 bp fragment of sponge nuclear ribosomal DNA, including the ITS-2 region, and we used cyanobacteria-specific PCR primers to amplify 1265 bp of cyanobacterial 16S ribosomal DNA. After sequencing these products, we constructed phylogenies for both the symbiotic cyanobacteria and the host sponges. The resulting phylograms show that a separate cyanobacterial strain is found in each sponge species. We found little sequence variation within sponge species (sequence divergence within species: 0 to 0.53 %; among species: 7.74 to 14.26 %) or cyanobacterial strains (sequence divergence within strains: 0 to 1.44 %; among strains: 1.67 to 3.02 %), with no genetic differentiation of sponges or cyanobacteria among locations or among sponge color forms. Log-det distance matrices from each group were significantly positively correlated, indicating that more closely related sponges host more closely related cyanobacteria. The coevolution of photosynthetic symbioses between sponges and cyanobacteria may parallel those of green plants and red algae.

CHEMICAL DESIGN OF SPONGE CELL MEDIUM

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Sponges are the most primitive multicellular organisms within the animal Kingdom. A wide variety of interesting, new compounds has been isolated from marine sponges and several of them have shown a wide variety of biological activities. Unfortunately only few of these natural products have reached the stage of commercial production because of the limited availability of starting material (marine sponges). Biotechnological production under well-defined conditions of sponge biomass is a necessary step towards commercial phase of these potentially interesting products.

It was hypothesized that cells of marine sponges can be cultured and that the cultured cells will continue to produce bioactive metabolites. Recent studies demonstrated the ability of sponge cell cultures to produce secondary metabolites. From several sponges we have obtained primary cell cultures, which have been monitored in their growth. Proliferation of cells can be improved with a well-defined design of medium that satisfies nutritional requirements of marine sponges and we have studies in progress about metabolism of sponge cells to understand their better condition of growth.

In this communication the importance of the presence of some metabolites in the growth medium of sponge cells will be discussed.

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PHOTOSYNTHETIC AND RESPIRATORY ACTIVITY OF THE SYMBIOTIC ASSOCIATION BETWEEN THE CORAL REEF SPONGE *HALICLONA CYMAEFORMIS* AND THE RHODOPHYTE *CERATODICTYON SPONGIOSUM*

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Measurements of photosynthesis and respiration obtained from clumps of the symbiotic association between the sponge *Haliclona cymaeformis* and the red macroalga *Ceratodictyon spongiosum* show that this association makes a significant contribution to primary productivity on the rubble banks at One Tree Reef, fixing up to 1.2 g C m^{-2} d^{-1}. Maximum rates of photosynthesis and respiration are more than 3.5 fold higher in summer than in winter. The rates of photosynthesis and respiration are the same in the growing tips of the association as in pieces cut from near the base. Differences are observed in the photosynthetic parameters when clumps of the association are collected from different sites within One Tree Lagoon. Clumps collected from the more turbid waters in the centre of the lagoon have characteristics of clumps that have undergone adaptation to low light. Maximum photosynthesis is greater in the intact association than in unialgal cultures of *C. spongiosum*; however, there is no difference in the respiratory rates of the intact association and the isolated alga. This indicates that the sponge makes very little contribution to the total respiratory rate of the association.

SOME PECULIARITIES OF THE FRESHWATER SPONGES (PORIFERA, SPONGILLIDAE) SPREADING OVER THE TERRITORY OF UKRAINIAN POLISSYA

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In Ukraine, the border between forest and forest-steppe biogeografic zones passes about the latitude of Kiev. The territory north of this border belongs to the forest zone and is called Ukrainian Polissya ("Woodlands"). This region covers upper reaches, embouchement and all right tributaries of Pripyat River as well as part of that of Desna River.

We studied spongofauna of Ukrainian Polissya in 1987–2001. During this time, over 400 samples of sponges from 32 stations at 16 rivers and lakes were collected and identified. The collection was made chiefly by hand, often with the aid of diving equipment. For sponges identification the keys by Penny & Racek, 1968, and Rezvoy, 1936, were used. In rivers of the region, sponges represent a considerable component of the periphyton, often being dominant in it, which, in the conditions of small rivers with abundance of hard substrata, leads to domination of sponges in the ecosystem's fauna by biomass. Five species of sponges were found in the region: *Spongilla lacustris* (L.), *Eunapius fragilis* (Leidy), *Ephydatia muelleri* (Lieberkuhn), *Ephydatia fluviatilis* (L.), and *Trochospongilla horrida* (Weltner). *S. lacustris* is the most widespread; it occurs almost everywhere, predominates in spongofauna in lakes and also in river stretches with the heaviest anthropogenic pollution. Usually, *E. fragilis* is the most mass species, particularly in stretches of swift flow. Where the flow is slower, *E. muelleri* can predominate. Unlike the widespread *S. lacustris*, *E. fragilis* and *E. muelleri*, *T. horrida* is quite uncommon and occurs only in purest stretches (Ubort, Snov, headstreams of Pripyat). Extremely rarely occurs *E. fluviatilis*, though widespread over the rest of Ukraine. Trustworthy findings of this species in Ukrainian Polissya were made only in left tributaries of Desna River, whose water-catchment area is situated mostly in forest-steppe zone (Seym and Oster Rivers), and in Desna itself. Neither in right tributaries of Desna, nor in Ukrainian part of Pripyat basin *E. fluviatilis* is found. It should be noted, however, that small colonies of this species usually do not form gemmules, so that it cannot be reliably differentiated from *E. fragilis*. But in cases of abundance of mature colonies of *E. fragilis* in a water body or its surroundings and lack of colonies of *E. fluviatilis*, doubtful samples were identified as presumably belonging to *E. fragilis*.

Thus the specificity of spongofauna of Ukrainian Polissya, evidently, is based on the lack in it of *E. fluviatilis*, common in the forest-steppe zone of Ukraine. No more specific distinctions in the distribution of other freshwater sponges species mentioned were found in the region.

BECOMING AN ADULT: A CHALLENGE FOR SPONGE PROPAGULA

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In his very lucid opening conference in Brisbane (1998), Professor Claude Lévi expressed his amazement at the fact that, despite the prolific research on the embryology, postlarval, postgemular and postmetamorphic development of sponges, we knew so little on growth, true morphogenesis, and, specifically, on how a particular sponge shape is achieved. Although no empirical support has been provided, it is widely accepted that growth and morphogenesis are genetically controlled, although modulated by external factors. They may determine the paradigmatic sponge plasticity that has allowed sponges to adapt to a variety of heterogeneous habitats. To obtain empirical data allowing advance in this field, current sponge science can take advantage of modern working techniques that are widespread in other fields. Cell and molecule labeling, molecular markers, and gene cloning and expression are, among others, available tools or desirable directions for further investigations. Homeobox genes that confer positional information have been reported in both larvae and adult sponges, although how they are involved in achieving the species-specific sponge shape or skeletal arrangement remains to be explained. The classic concept of a sponge as a cluster of cells in continuous rearrangement has been supported by video and ultrastructural images and accounts for the sponge adaptation capabilities. However, the genetic basis of what is known as the "permanent embryonic stage" of the sponges has not been approached yet and requires investigation.

The episodes that encompass sponge propagula from their release to the water column until they join a sponge population as new recruits, are crucial to an understanding of sponge distributions. Post-recruitment processes: growth, feeding, defense, reproduction, mortality, potential longevity, and individual dynamics have received increasing attention in recent decades, but field data are difficult to obtain and consequently scarce. Monitoring of larval behavior or "true" dispersal capacity in the field should be combined with studies of the genetic structure of populations to understand the extent to which sexual and asexual events contribute to colonization of new habitats. Studies of post-recruitment processes have shown contrasting results depending on the species studied, and research should be extended to other sponge types to allow generalizations. The ontogeny of the chemical defenses, why and how defenses vary during the life of the sponge, is a growing study field with implications for evolution. Chemical defenses together with the possible role of chemical cues in inducing settlement and metamorphosis will contribute to the understanding of some relevant ecological aspects such as species abundance, failure at settlement, larval and settler mortality, and recruits success, among others.

THE DISTRIBUTION OF *CHONDRILLA* SPECIES (DEMOSPONGIAE) IN AUSTRALIA

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The number of species of Chondrilla and their distribution around the Australian coastline was investigated using classical taxonomy and molecular techniques. Distinguishing between Chondrilla species using classical taxonomy has been difficult as this genus has few characters for this purpose. We directly sequenced sections of the 28S rDNA and the ITS region to determine the level of correlation between the results of the sequence data from these two regions of DNA and the classical identification techniques. We found that the results of these methods were in agreement, with three species of Chondrilla found in the southern oceans of Australia, sometimes growing alongside each other. Chondrilla australiensis was found to grow in both the tropical and temperate oceans of Australia.

STUDIES ON THE CELLULAR LOCALIZATION AND ECOLOGICAL FUNCTION OF BIOACTIVE COMPOUNDS OF SOME CARIBBEAN SPONGES

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In the framework of the Symbiosponge project dealing with the biology of sponge natural products we have investigated 4 sponge species to establish the origin and role of the bioactive secondary metabolites they produce and to ascertain whether these compounds could be recovered from cell cultures.

Three species, *Agelas conifera*, *Ircinia felix* and *Xestospongia muta* were characterized by the presence of great numbers of extracellular bacteria. On the contrary, the fourth species *Haliclona vansoesti* only contained very few and small heterotrophic bacteria. Cyanobacteria typical for each species were observed in *H. vansoesti*, *I. felix* and *X. Muta*.

Cell fractions obtained by differential, Ficoll and Percoll density gradient centrifugation as well as cell cultures were analysed by HPLC for the presence of the compounds of interest. The data revealed that the major secondary metabolites of *A. conifera* and *H. vansoesti*, namely sceptrin-oroidin and (2R, 3R, 7Z)-2-aminotetradec-7-ene-1, 3-diol were associated with sponge cells (spherulous cells and archaeocytes) rather than with bacteria. In contrast, current results obtained for the other two species, *X. Muta* and *I. felix*, strongly suggested that the compounds characteristic of these species, i.e. bromopolyacetylenic acids and variabilin derivatives, were of microbial origin rather than of sponge origin and that they were likely associated with heterotrophic bacteria. The target compounds could be recovered in different amounts from short-term cultures of *A. conifera*, *H. vansoesti* and *X. Muta* pointing to the feasibility of cell culture as an alternative method to extensive collections in the field. Laboratory and field experiments indicated that sceptrin and oroidin played a role in defence mechanisms against predators and possibly in space competition while (2R, 3R, 7Z)-2-aminotetradec-7-ene-1, 3-diol was only involved in space competition. The ecological role of bromopolyacetylenic acids and variabilin derivatives remained uncertain although variabilin derivatives may act as an internal antibiotic protection.

DISTINCTION OF *AMPHIMEDON* (PORIFERA) SPECIES FROM BRAZILIAN COAST THROUGH COMBINED MORPHOLOGIC AND MOLECULAR METHODS

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The genus *Amphimedon* (Duchassaing & Michelotti, 1864) is a typical case of difficulties faced by specialists in the taxonomy of Porifera. Its species present several morphotypes what makes a certain number of species with doubtful allocation. This fact is surprising in view that *Amphimedon* is a Haplosclerida of the family Niphatidae (Chalinina) with considerable number of species, which possesses a diversified known chemistry. Atlantic Ocean is richer in *Amphimedon* than elsewhere, however there are only two registrations published for the Brazilian coast: *A. viridis* and *A. erina*. Recent collections made in several points of the eight thousand kilometers of Brazilian coast revealed the presence of some species with a difficult classification, which motivated the beginning of a taxonomic revision of the genus. Such revision is made particularly urgent due to the recent discovery of potent hemolytic action in *A. viridis* and *A.* sp. of the Brazilian coast, and the potential biochemical/pharmacological/immunological application of substances extracted from these sponges. To correctly identify these species, we applied the PCR (Polymerase Chain Reaction) linked-SSCP (Single Strand Conformation Polymorphism) method, to detect the sequence variation in the first and second internal transcribed spacers (ITS 1 and ITS 2) of the nuclear ribosomal DNA genes. Our results allowed the diagnosis of *Amphimedon* species through the combined PCR-SSCP method, showing the viability of this technique in the discrimination of sibling sponge species prior to sequence analysis.

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THE IMPACT OF A NUCLEAR POWER PLANT DISCHARGE IN A SPONGE COMMUNITY OF A TROPICAL BAY

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The Almirante Álvaro Alberto Nuclear Central (CNAAA), at Ilha Grande Bay, south coast of Rio de Janeiro state (SE Brazil), is composed by two nuclear power plants. The chlorinated discharge from the cooling water system flows into the bay, rising the water temperature and the current velocity. Sponge distribution is strongly influenced by abiotic factors, and they are therefore useful tools for environmental monitoring. Water temperature (surface and bottom, current velocity and chlorine levels were measured weekly over three months in two sites (discharge and control). Mean surface temperature value was significantly higher ($t = 5.02$, $p = 0.01$) at discharge (31.9° C) than at control (26.5° C), while the mean bottom temperature value was very similar in both areas (≅ 26° C). Mean chlorine level was 0.04 $mg.l^{-1}$ at the discharge, control samples did not show chlorine. Mean current velocity was 0.24 knots in the discharge and 0.18 knots in the control. Quantitative samples of sponge community were taken by SCUBA dive along horizontal transects (10 square meters) in two depths (surface – 1.5 m and bottom – 3.5 m) at discharge and control site. The codes of transects are: discharge surface - DS, discharge bottom - DB, control surface - CS, control bottom - CB. Nineteen spp. were recorded for this study. Only two species (*Haliclona* sp.1 and *Mycale microsigmatosa*) were found at DS while at CS were found 13 spp. Six spp. were found in DB and 13 at CB. The diversity and evenness was higher in CS ($H' = 2.01$; $J = 0.82$) and CB ($H' = 2.09$; $J = 0.78$) than at DS ($H' = 0{,}25$; $J = 0.35$) and DB ($H' = 1.20$; $J = 0.67$). The density of individuals was also higher at CS (37.3 $ind.m^{-2}$) and CB (42.2 $ind.m^{-2}$) than at DS (5.9 $ind.m^{-2}$) and DB (10.4 $ind.m^{-2}$). The most abundant species, *Tedania ignis*, *Mycale americana*, *Amphimedon viridis* and *Haliclona melana*, were found in all transects except at DS. *Mycale microsigmatosa* was also abundant, and the only species that occurred in all transects. However, its abundance was significantly lower ($KW = 21.130$, $p < 0.0001$) in DS. This species is considered tolerant towards organic pollution, and may also be considered a negative bioindicator for the impact approached in this study. *Haliclona* sp.1 showed relative abundance of 93.2 % at DS, and was found exclusively in this transect. This result suggests that this species is opportunistic and tolerant of extreme conditions, and thus may be used as a positive bioindicator. Cluster analysis (Bray-Curtis index) showed low similarity between discharge and control transects. DS showed only 2 % of similarity with DB and control transects and DB showed similarity of 36 % with the control transects. The results indicate a high impact of the CNAAA discharge on the sponge community, which, however, is local (≅ 750 m) and stronger at the upper levels.

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PHOSPHOLIPID DISTRIBUTION AND PHOSPHOLIPID FATTY ACIDS IN SOME SAUDI RED SEA SPONGES

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Sponge phospholipid fatty acids are quite unusual and possess long chain (23-34 carbon atoms) and unsaturation pattern (Δ5,9). Thus, they contrast sharply with their common counterparts where the methylene-interrupted unsaturation pattern found in nunemrous other organisms. As part of our ongoing investigations, lipids were analysed in four sponges collected by scuba diving in Saudi Red Sea, namely *Chalinula saudiensis*, *Acanthella carteri*, *Cinachyrella alloclada* and *C. kükenthali*.

Phospholipid distribution and phospholipid fatty acid composition were studied. The occurrence of phosphatidylglycerol at high levels (12-15 %) revealed the presence of bacteria in sponges such as the *Cinachyrella* species. In that case, several rare monounsaturated branched short-chain acids were identified, likely originating from associated bacteria. Typical Δ5,9 demospongic fatty acids were identified in all sponge studied, including the new 6-bromo-5,9-nonacosadienoic acid in *C. alloclada*. Other new compounds were characterized in the latter sponges, namely 17-methyltetracosanoic, 18-methyltetracosanoic, 18-methylpentacosanoic, 18-methylhexacosanoic and 18,24-dimethylhexacosanoic acids. *A. carteri* contained a quite 42 % of 5,9-hexacosadienoic acid, phytanic acid at unusual level of (20 %) and three 2-hydroxy long-chain fatty acids. In contrast with the *Cinachyrella* species, *C. saudiensis* did not contain symbionts. Thus, the fatty acids found in that sponge such as arachidonic (20 :4 n-6) and docosahexaenoic acid (DHA, 22 :6 n-3) seem originate from the sponge itself.

AN ASSESSMENT OF SPONGE MARICULTURE IN THE SPERMONDE ARCHIPELAGO, INDONESIA

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Sessile marine invertebrates are sources for a wide variety of substances with bioactive properties. Many of these substances have a potential as pharmaceuticals or as biochemical tools for a variety of useful applications. Of all marine invertebrates, sponges are the most diverse in both numbers and types of compounds. As the supply-matter is a problem, alternative ways to obtain these compounds are necessary. In most cases it is not feasible to harvest wild sponges on a commercial scale as sponge populations are often small and have a patchy distribution. A fast depletion of the natural sponge resources will be the consequence. A more viable and reliable method is to culture sponges in the open sea, with natural sponge populations acting as a stock. Several attempts to culture sponges have appeared to be successful in temperate waters with respect to growth, survival of the sponge, and yield of the target compounds.

It is assumed that sponge bioactivity is enhanced in tropical environments, such as coral reefs. As many of these reefs are under great environmental pressure caused by over-exploitation by human coastal communities, there is a growing need for methods for a more sustainable use of the coral reef resources. It is therefore expected that the inexpensive requirements of sponge aquaculture could surely benefit local communities.

A first assessment is presented of the farming potential of six sponge species at South Sulawesi, Indonesia. Experiments will be done to determine the feasibility for sponge aquaculture in the region, and characteristics of the ecological role of the compounds will be described for morphologically different sponge species. Here, we focus on the outcome of different farming techniques, sponge growth and survival. These latter parameters have been monitored for a period of six months, during which sponge explants and wild sponges have been collected at different time intervals. The extracts of these samples have been studied using bioactivity assays and HPLC to check for the presence and bioactivity of the various compounds previously detected.

THE CHEMICAL ECOLOGY OF THE ARCTIC SPONGE *HALICLONA VISCOSA*

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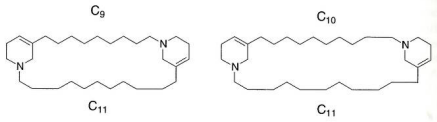
Marine animals are a source of novel natural products with diverse ecological functions. Sessile organisms often depend upon a chemical defense for protection from predators and competitors, instead of a physical mechanism like quills, scales or camouflage. Previous chemical and ecological studies have focused primarily on tropical organisms. We have begun to investigate chemically mediated interactions of organisms from arctic waters, given the abundance of soft-bodied animals that may rely on chemical deterrence. Our work focused on sponges of the family Haliclonidae, which are known to contain secondary metabolites with biological activities. Several antifungal and cytotoxic alkaloids have been isolated from sponges of the genus *Haliclona*, e.g. haliclamines and haliclonacyclamines. The sponge *Haliclona viscosa* was collected in 1999 at Königsfjord near Ny-Ålesund, Spits-bergen, and was shown to be chemically protected from predators. Ecological assays with tissue, extracts and isolated compounds showed activity against predators and fouling organisms, including the amphipod *Anonyx nugax*, the starfish *Asterias rubens*, and five bacteria isolated from the natural environment. The results of the ecological assays as well as the isolation and structure elucidation of the major secondary metabolites (e.g. X 3 and X 4) will be discussed.



X3

X4

SPONGE INTERACTIONS WITH SPATIAL COMPETITORS

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Tropical marine environments are a great source of bioactive compounds with pharmaceutical potential. Since the demand versus supply ratio for the material is a major problem, there is a need to find alternative ways to obtain these compounds. Aquaculture of bioactive sponges could be preferable above wild harvesting and other more costly methods. To be able to culture sponges and exploits their products efficiently; the ecological functions of the secondary metabolites have to be fully understood. In highly competitive environments, such as coral reefs, space is an important limiting factor; it must be gained and maintained at all times. Sponges are important spatial competitors; this can be observed by the high frequency of overgrowth of their neighbours and the necrosis or bleaching that these interactions can cause. This study describes the *in situ* effects of four known toxic sponges on their neighbours at different locations and depths in the Spermonde Archipelago, Indonesia.

The target species, *Aaptos suberitoides*, *Amphimedon paraviridis*, *Callyspongia* sp. and *Niphates olemda*, were carefully chosen on the basis of their known bioactivity, natural abundance and potential use in future aquaculture in the region. Within a circular transect with a diameter of 25 cm, the natural rates of interaction between the sponge species and eight possible competitive invertebrate groups were defined and quantified, along with the choice of substratum and the total live cover. Most interactions occurred with other sponges and corals. Coral overgrowth by the four target species caused more than 85 % necrosis, whereas this was less than 25 % in most sponge overgrowths. *Callyspongia* sp was significantly smaller in size and had a different morphology, encrusting *vs* branching, in high coral cover environments in comparison to low ones. Additionally, the encrusting growth form showed more lethal overgrowth interactions than the branching growth form.

For all four target species, one individual could at the same be lethal to one type of neighbour and not be lethal to another type in its overgrowth interaction.

These results support the hypothesis that the toxins are used in spatial competition and that the concentrations of the toxins of the target species differ both intra-individually and intra-specifically. However, future quantification of the toxins is suggested for more conclusive evidence.

SYMBIONTS IN THE SPONGE *CANDIDASPONGIA FLABELLATA* (DICTYOCERATIDA)

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Candidaspongia flabellata, is a rare Dictyoceratid sponge found on the Australian Great Barrier Reef. It is a foliose sponge up to 25 cm high and 35 cm wide and yet only 3-4.5 mm thick. A thin cortex, which consists basically of sand grains in a collagen network, occurs on both surfaces. Internally, the soft tissue is supported by a spongin network and is totally free of sand grains. Electron microscope studies of this sponge have shown that it is rich in associated microorganisms. High numbers of a cyanobacteria occur within the tissue of the central region of this sponge, amongst the choanocyte chambers and were never seen in the cortex areas. These appear to be members of the Oscillatoriaceae. Additionally, associated with all sand grains in the cortex are large numbers of bacterial cells. These are non-cyanobacterial bacteria or archaea. The distribution of these cells was strictly within the cortex. Many different morphotypes are present, and are always closely associated with the sand grains, contained within a sheath surrounding each grain. It appears that they maybe involved in the breakdown of these sand grains. Grains in all states of dissolution can be seen with bacteria penetrating deep within them. Free sand grains, taken adjacent to a sponge, were shown to also have a bacterial biofilm and similar morphotypes to those in the sponge were seen. It is therefore likely that the bacteria are acquired with the sand grains at the time of inclusion within the cellular matrix. The role of the symbionts and their distribution within the sponge will be discussed.

HUMAN IMPACTS AND THE MICROBIAL ECOLOGY OF ANTARCTIC SPONGES

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Increasing human pressure on Antarctica has highlighted the necessity for effective monitoring tools to assess the health of this fragile environment. Sponges dominate many of the shallow marine Antarctic habitats and are ideal indicator organisms for assessing benthic condition. This study utilised the microbial symbionts of Antarctic sponges as sensitive indicators for the detection of sub-lethal stress caused by human impacts in the regions surrounding Scott Base and McMurdo Station. This was achieved by comparing the microbial ecology of sponges in polluted and pristine environments. A polyphasic strategy incorporating cultivation, denaturing gradient gel electrophoresis, 16S rRNA sequencing and electron microscopy techniques was performed to monitor changes in microbial diversity with increasing levels of pollution stress for the sponge species *Homaxinella balfourensis*, *Kirkpatrickia variolosa*, *Latrunculia apicalis*, *Myxale acerata* and *Sphaerotylus antarcticus*. The level of contamination at each site was defined by heavy metal, hydrocarbon and nutrient analysis of sediments and bivalves and fecal coliform counts from seawater and sediment. Sponges have considerable potential for monitoring elevated concentrations of heavy metals and examining changes in their microbial symbionts is a novel and sensitive bioindicator for the assessment of pollution on important microbial communities.

PHYLOGENETIC SIGNIFICANCE OF SPINED RAPHIDES WITHIN *HALICLONA* (HAPLOSCLERIDA, CHALINIDAE)

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Chalinid microscleres are of a striking simplicity and consist only of sigmas, toxas, microxeas, and raphides. All these microscleres occur outside the Chalinidae as well and have no significant phylogenetic value within the family. Until now all spicule categories of marine haplosclerids were known to be entirely smooth, but recently raphides with vestigial spines were found in two *Haliclona* species from the Mediterranean Sea and the northern North Atlantic. The nature of these raphides is compared with those occurring outside the Chalinidae in order to establish the phylogenetic value of these spicules within the family.

SPONGE-INDUCED CORAL MORTALITY IN THE CARIBBEAN. A POTENTIAL NEW THREAT TO CARIBBEAN CORAL REEFS

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In the last 20 years Caribbean coral reefs have been experiencing significant deterioration due to the combination of more frequent (and intense) natural events (hurricanes, African dust, diseases, bleaching, etc.) and increasing anthropogenic intervention in the region. In the last 15 years, the thin, brown, continuous, veneer-like crust (beta stage) of one species of the taxonomically unresolved species complex *Cliona langae - C. caribbaea - C. aprica* is becoming a major problem in many Caribbean reefs due to its rapid proliferation, intrusive monopolization of substrate, high bioerosion rates, and the infestation and killing of high proportions of live colonies of many reef building corals. Since the author first observed this phenomenon in Panama in 1986, this problem has apparently extended to the north and east of the Caribbean, Florida and the Bahamas. Results from 15 years of observations around the wider Caribbean, communications from colleagues, and quantitative ecological studies in Puerto Rico over the last five years are summarized as follows: (1) the sponge has a wide geographic distribution but is limited to the northern and west Caribbean. It has been observed killing corals and monopolizing reef substrate in some 62 reef sites in Colombia, Panama, Roatan–Nicaragua, Belize, Grand Cayman, Jamaica, Dominican Republic, Florida, Bahamas and Puerto Rico. Curiously, the beta stage is not present in the eastern lesser Antilles and the south Caribbean regions (Virgin Islands, Dominica, Grenada, Barbados, Tobago and the coast and islands north of Venezuela); (2) the sponge can infect and kill up to 24 different reef building coral species and the hydrocoral *Millepora complanata*; (3) up to 20 % of the colonies of some important coral species (*Montastraea cavernosa, M. faveolata*) can be infected in a particular reef; (4) the average linear extension rates (= coral tissue mortality rates) are significantly higher (2.74-16.79 cm/year) than most specific growth rates (linear extension) of the affected coral taxa; (5) the sponge's extension rates are significantly higher in live coral colonies than in dead consolidated substrate; (6) the sponge can dissolve high amounts of calcium carbonate (bioerosion) in a short time and it can completely kill a medium size coral head in one year; (7) the sponge has a wide depth distribution range (0.2–40 m); (8) linear extension rates do not vary significantly with depth; (9) up to 20 % of the reef substrate may be covered by this sponge in some localities; (10) there is high variability in the incidence, growth, abundance and distribution of the sponge across reef habitats and geographic localities; (11) the taxonomy of the group is confusing at best, and (12) the sponge is producing high rates of coral tissue mortality in many reef sites. Very few references to Clionids killing corals exist in the literature, and nothing like what is presented here was reported prior to 1999. Some potential explanations to the phenomenon include: (a) changes in the competitive abilities of the sponge and/or the defense abilities of the corals due to habitat degradation or environmental changes (global warming?); (b) an introduction of an exotic species;

(c) a natural cycle in the life history of the particular, indigenous clionid species (i.e. successful reproductive years in combination with low mortalities, etc.); (d) absence of herbivorous pressure on the surface of the reef due to the disappearance of the black sea urchin *Diadema antillarum* in the early 80's, and overfishing, and (e) any combination of these. Ongoing research on the biology and ecology of these sponges in conjunction with ongoing efforts to clarify their taxonomy will provide more useful information in the near future.

SPONGIAL FAUNA OF PLIOCENE-QUATERNARY SEDIMENTS IN LAKE BAIKAL

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At present, sponge fauna of Lake Baikal is represented by two families: the cosmopolite Spongillidae (including 3 genera and 3 species) (Masuda, 1999) and the endemic Lubomirskiidae (4 genera and 14 species) (Efremova, 2001). Was the species composition of this sponge fauna always so? When Lubomirskiidae separated from Spongillidae, and how the development of these two sponge families was progressing? Micro-palaeontological method of study of silicon sponge spicules buried in Lake Baikal bottom sediment helps to answer this question. Some short cores were studied (ST18, ST16, ST15, STX-3, ST-2), their age is within Pleistocene; we studied as well a part of the deep-drilling core BDP -96/1, its age range is 3.1-2.1 My, and the lowermost part (50 cm) of the deep-drilling core BDP - 98, its age is supposed to be Upper Miocene (7.5 M.Y.B.P.). All the cores were sampled from the top of underwater Akademichesky Ridge. This ridge separates Central and Northern Baikal basins and is an ideal site for sampling used for palaeolimnological studies as it is isolated by large depths from direct income of coarse sediments from the coastal zone, as well as from influence of riverine outflow. It guarantees a quiet, undisturbed type of sedimentation.

Studies of short cores have shown that the distribution of sponge spicules in them occurs according to the same regularity that the distribution of other micro-fossils and palaeo-markers (diatom algae, vegetation pollen, biogenic silica). Spicule content is the highest in diatom silts corresponding to warm interglacial periods, and it is lower in clayey intervals corresponding to cold glacial periods. Species composition of the sponge fauna is close to the present one.

In the sediments from the core BDP-96/1 we found the spicules of all sponge species known by nowadays. Besides spicules which could be related to modern species there were ones which differed from them by morphological features. We call them further "fossil" spicules: 8 spicules types of them were related to the family Spongillidae, and 19 types of "fossil" spicules to the family Lubomirskiidae. The analysis of species composition of the sponge fauna in the studied part of the core BDP-96 has shown that until the boundary of 2.9 M.Y.B.P., the composition of fossil sponge fauna considerably differed from present one. Part of spicules of the family Spongillidae (up to 60 %) and of spicules of "fossil" spicules was significant. During the period of 2.9 -2.5 M.Y.B.P., a dramatic change in species composition and spicule abundance in the sediments occurred due to climate change. The amount of spicule of the warm-loving family Spongillidae considerably decreased and many "fossil" spicule disappeared; evidently, they also belonged to warm-loving species. Since 2.5 My BP, a graduate reconstitution of abundance and species composition of

the Baikal sponge fauna began; however, this fauna never reached such prosperity as before the cooling period. In general, species composition of the sponge fauna became close to the present one.

Studies of species composition of the sponge fauna from lower intervals of the core BDP-98 showed that during that period (7.5 M.Y.B.P.) two quite separated sponge families existed: the cosmopolite Spongillidae and the endemic Lubomirskiidae represented by the genera *Lubomirskia, Baikalospongia, Swartschewskia.*

INTERACTION BETWEEN THE BAIKALIAN SPONGES AND THEIR INHABITANTS

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Our study was aiming at the determination of the composition of invertebrates inhabiting Baikalian sponges and elucidation of the interactions between sponges and their inhabitants. Main part of material was obtained in August 2001 in Kharin-Irgi Bay (Middle Baikal). Besides, the samples obtained in 2000 in the region of Ushkany Islands and at Bolshie Koty (Southern Baikal) were used. Sampling has been performed by divers according to procedure by Kamaltynov et al. (1993). Six species of Baikalian sponges (*Lubomirskia baicalensis, L. incrustans, Baikalospongia intermedia, B. bacillifera, Swartschewskia papyracea, Rezinkovia echinata*) were studied. Abundance and biomass of invertebrates were normalized by a sponge volume which was determined as the volume of water substituted by the sponge in a measuring flask (according to Dr. Y. Masuda, Kawasaki Medical School, Japan) Fatty acids composition (as methyl ethers) was analyzed according to Keits (1975) on gas/liquid chromatographer Shimadzu GC-9A.

All sponges examined were inhabited by invertebrates. Their number varied from 80 to 280 individuals, the biomass was found to be from 200 to 1020 mg per 100 cm^3 of sponge. All quantitative characteristics tend to increase with the increase of sponge body size irrespective of sponge species identity. Crustaceans always dominate the community. In total 17 species of amphipods, 9 species of copepods and 2 species of isopods were found. There are two distinctive sets of species, within the sponge inhabiting crustaceans, which we designate as "specific" and "non-specific" ones. The latter indifferent in sponges sampled in different parts of the lake. Usually it consists of planctic (*Cyclops kolensis*) and benthic invertebrates widespread on the shallow water platform of the lake at the place of sampling. The specific set consists of species which are typical for all species of sponges sampled from different parts of the lake. Among the crustaceans there are: *Brandtia (Spinacanthus) parasitica* and *B. latissima* (Amphipoda), *Acanthocyclops incolotaenia* and *A.intermedius* (Copepoda). Besides, two crustaceans occur on the sponge *Lubomirskia baicalensis* everywhere: amphipod *Eulimnogammarus violaceus* and copepod *Acanthocyclops spongicola.*

Interaction between the sponges and invertebrates inhabiting them includes topic and trophic links. One of the characteristics of the topic links is the morphological adaptation of the invertebrates from the "specific" species set. All of them besides *E. violaceus*, which lives in holes gnawed in sponge bodies, have spines on body and especially on appendages which help them to stick to the sponge surface and use it

efficiently as a substrate. Trophic links are marked by "demospongic" acids synthesized by sponges. Composition of fatty acids from two amphipod species - *Brandtia. parasitica* and *B. latissima* - were analised. High level of poly-unsaturated fatty acids (48 %) and acids of bacterial and plant origin have been found. Marker "demospongic" acids were not found. Previous studies (Dembitsly et al, 1994) found only small amounts of "demospongic" acids in *B. parasitica* which means that the sponge does not represent the main source of food for them. Basing on these data we may conclude that trophic links like host-parasite, are highly unlikely. The study of feeding preferences of *B. parasitica* (Mekhanikova, 2001) resulted in the same conclusion. The Author had found that food lump of this species consists of remainders of plant and animal origin, which most likely originate from other organisms dwelling on the sponge surface, as well as of planctic algae. High abundance of fatty acids of bacterial and plant origin suggests that bacteria inhabiting the sponge may play a major role in feeding of *B. parasitica*. Therefore host invertebrates clean the sponge by scavenging epiphytic organism from its surface. Most likely, for most of sponge-dwelling invertebrates we may define their interaction with sponge as proto-cooperation (non-obligate co-existence), and as mutualism (obligate co-existence) for some species like *B.parasiticus* and *A.spongicola*. But we still cannot deny the existence of more complicated interactions in the community of invertebrates and sponges, because there are no studies of the impact of the former on sponges.

MECHANICAL PROPERTIES OF THE COLLAGENOUS MESOHYL OF *CHONDROSIA RENIFORMIS*: EVIDENCE OF PHYSIOLOGICAL CONTROL

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Chondrosia reniformis shows a form of asexual reproduction in which portions of the parent body de-stiffen and eventually break away completely. In addition, intact sponges stiffen in response to mechanical stimulation. These phenomena suggest that the mechanical properties of the collagenous mesohyl, which is the dominant structural material of *C. reniformis*, may be under physiological control. Evidence supporting this view was obtained by examining the effects of various agents on the flexural stiffness of beam-shaped samples of ectosome and choanosome tissue. For example, elevation of [Ca2+] stiffened and reduction of [Ca2+] de-stiffened these samples; treatments that cause cell lysis, including the detergents Triton X-100 and saponin, distilled water and freeze-thawing, had an extreme and irreversible stiffening effect. The effects of the different treatments were checked in the samples at histological and ultrastructural level with particular reference to the possible structural changes of the cellular components of the mesohyl. On the basis of our results it is hypothesised that cells in the mesohyl synthesise and secrete a molecule that influences, directly or indirectly, interactions between the collagen fibrils of the extracellular matrix.

ULTRASTRUCTURE OF SPERMATOGENESIS AND STORAGE CELLS IN THE CARIBBEAN SCLEROSPONGE *GOREAUIELLA AURICULATA* (CERATOPORELLIDAE: PORIFERA)

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Fine ultrastructural analysis of the living tissue of the coralline sponge (sclerosponge) *Goreauiella auriculata* collected in the vicinity of Discovery Bay, Jamaica, between 1984 and 2002, was carried out using transmission and scanning electron microscopy (TEM and SEM). Two new features of the biology of *Goreauiella* are reported.

Spermatogenesis is described for the first time in a coralline sponge of the family Ceratoporellidae. Storage cells with numerous inclusions are located at the base of digitations of the living tissue inserted within the calcareous skeleton. These storage cells are compared to similar structures observed in other coralline sponges.

IN VITRO GENE EXPRESSION IN MARINE SPONGE CELLS

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The marine sponge *Axinella corrugata* is a model for cell culture development and is known to respond to phytohemagglutinin (PHA) stimulation in vitro. This report documents changes in gene expression related to PHA treatment of sponge primary cell cultures. The research employed a cross-species technique in which marine sponge cDNA was hybridized to commercially available microarrays of human gene sequences. A panel of potentially regulated genes was derived through basic statistical methods. Results indicate that PHA effects proliferative and anti-apoptotic molecular changes in marine sponge cells.

NEW SPECIES AQUACULTURE OF TROPICAL DICTYOCERATIDS FOR PRODUCTION OF COMMERCIAL SPONGES IN THE GREAT BARRIER REEF REGION

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Demand for commercial sponges currently exceeds supply worldwide. To develop a successful sponge aquaculture venture, survival must be maximised. The concept of growing sponges by *in situ* aquaculture is not new. Tropical aquaculture in particular, however, is in its infancy.

Several dictyoceratid species, including *Rhopaloeides odorabile* have been grown near Townsville under a variety of conditions, including distance from shore, different depths and using various rope and bag materials. Where possible, both the explants and the wild seed stock were regularly monitored for survivorship and growth. None of the original *Rhopaloeides* seedstock died, but replaced the removed biomass only slowly. Mean survivorship of the explants from this species varied between 0 and 75 % depending on treatment category, and volumetric growth-means over 21 months were up to 1200 %. After harvest, analyses included elasticity measurements of cleared skeletons to establish a commercial grading and to optimise growing conditions. There was a very distinct correlation between certain treatments and elasticity.

The results of further work including an as yet unnamed *Ircinia* species will be presented for comparison.

NESTED CLADE ANALYSIS AND PHYLOGEOGRAPHY OF WESTERN PACIFIC *LEUCETTA "CHAGOSENSIS"* (PORIFERA: CALCAREA): CLUES FOR CONSERVATION OF THE GREAT BARRIER REEF WORLD HERITAGE AREA (AUSTRALIA)

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Phylogeography investigates the geographical distribution of genealogical lineages, including those at the intraspecific level. While phylogeographic relationships of terrestrial taxa have been quite well studied during the last years, marine phylogeography is still in its infancies. In the present study we explore phylogeographic relationships of the widespread calcareous sponge *Leucetta "chagosensis"*, occurring in shaded habitats of Indo-Pacific coral reefs. It provides a good model system to investigate marine phylogeographic relationships due to its allegedly limited dispersal capabilities. Maximum parsimony analysis of 19 ribosomal sequencetypes from 28 locations in the western Pacific revealed phylogeographic structuring into 4 major clades, corresponding to the northern/central GBR with Guam and Taiwan, the southern GBR and subtropical regions south to Brisbane, Vanuatu, and Indonesia. Subsequent nested clade analysis confirmed this structure with a probability of >95 %. A pattern of range expansion from the internal Indonesian clade was inferred at the total cladogram level, supporting the "Centre of Origin" hypothesis. Two distinct clades were found on the GBR, which narrowly overlap geographically in a line approximately from the Whitsunday Islands to the northern Swain Reefs. At various clade levels, the northern GBR clade was influenced by past fragmentation and contiguous range expansion events, presumably during/after sea level low stands in the Pleistocene, after which the northern GBR might have been recolonised from the Queensland Plateau in the Coral Sea. The southern GBR clade is most closely related to subtropical *L. "chagosensis"*, and we infer that the southern GBR was recolonized from there after sea level low stands. Our results have important implications for conservation and management of the GBR, as they highlight the importance of marginal transition zones in the generation and maintenance of species rich zones, such as the Great Barrier Reef World Heritage Area.

HOW MUCH CAN COMPETITION AND PREDATION INFLUENCE SPONGE COMMUNITY COMPOSITION?

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Over 170 sponge species inhabit mangrove roots in two sets of cays on the Belize Barrier Reef. Overlap in species between the faunas of the Pelican Cays and Twin Cays is remarkably small, less than 30 % of the species. Experiments in which individuals of the 6 most common sponge species in each set of cays were transferred between sites and also placed in different situations with respect to competition and predation within each site strongly implicate competition and predation in determining which sponge species are found in each set of cays. Put most simply, spongivores prevent sponges typical of Twin Cays from inhabiting the Pelican Cays, and competition from rapidly growing species quicky eliminates sponges typical of the Pelican Cays from Twin Cays. Some species are so strongly influenced by, or resistant to, either competition or predation that their presence or absence can be used as a clear environmental indicator. However, one intriguing result is that many of the sponge species do not divide dichotomously into "edible" vs. "inedible" and "good competitors" vs. "poor competitors", but instead display a complex mix of intermediate possibilities.

HISTORICAL RECORDS OF THE PORIFERA FAUNA IN THE AZORES. THE CONTRIBUTION OF PRINCE ALBERT I OF MONACO SCIENTIFIC CAMPAIGNS

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The first scientific works on Porifera fauna in the Azores were done by Topsent (1892, 1904) on material collected in a series of 6 scientific campaigns ordered by Prince Albert I of Monaco on board the yachts Hirondelle and Princesse Alice.

The output of the zoological exploration enterprised on board Hirondelle in three regions of the North Atlantic (Gascogne Gulf, New Foundland, Açores) resulted in a list of 167 sponge species, 58 of which are new records for science.

In 1904 the Prince Albert published the 2nd series of the scientific campaigns results dedicating a volume to the Azorean sponges. Entitled "Spongiaires des Açores" the monography by Emile Topsent mentions briefly or in detail 243 species.

It was to the deep-water Porifera fauna that the Prince Albert expeditions mostly contributed. In fact, most sampling operations (dredging and trawling) were made at 800-2000 m, and some ranged to 5005 m depth.

These campaigns are therefore considered to be of important historical and scientific value and are definitely a milestone to Porifera taxonomy in the region.

SPONGES AS BYCATCH OF LONG-LINE FISHING PRELIMINARY RESULTS ON A PROGRAM OF COOPERATION WITH LOCAL FISHERMEN

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Considering that the Azorean Islands lack a continental shelf, a great number of non-coastal sponge species is yet to be known. As a matter of fact deep-water sponges from the Azores have received no attention since the beginning of the 20th century, when the Prince Albert I of Monaco scientific campaigns took place.

The present lack of knowledge of much of the deep-water fauna is related to the high costs and specificity of sampling effort needed to obtain specimens from depth under 100 m. However, sponges constitute a considerable part of traditional long-line fisheries by-catch in the Azores, and therefore a program of cooperation with a local fishermen association was started in order to obtain biological material.

In this work we present the preliminary results of this program where fishermen play an important role in sampling.

FEEDING ECOLOGY OF *SERICOLOPHUS HAWAIICUS*: A FIRST LOOK AT HEXACTINELLID DIET AND WATER PROCESSING.

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Sericolophus hawaiicus is the dominant megafauna of the slope of the Maua Lona volcano at a depth of 350-450 m. These sponges form a dense bed of a variety of size classes from new recruits to dead stalks. We quantified sponge diet and rates of water processing *in situ* using the Pisces V deep submergence vehicle. Water samples were simultaneously collected from the exhalent current of the sponge and upstream of the sponge using a "Sucker". The Sucker is pitchfork shaped device with a syringe on each end. One syringe was positioned within the exhalent current of the sponge and the other took an ambient water sample 20 cm upstream from the sponge. Water samples were analysed by flow cytometry to quantify changes in ultraplankton abundance between ambient and exhalent current samples. We also measured sponge water processing rates by video analysis of fluorescence dye. Dye was released on the incurrent aspect of the sponge. Water processing rates were determined as a function of the velocity of the visualised exhalent current and diameter of the oscula. Overall, this bed of *S. hawaiicus* has the potential to flux a large amount of material from the overlying water column into the benthos of the deep sea.

COMPARATIVE CHEMICAL AND ECOLOGICAL STUDIES OF SPONGES FROM THE ORKNEY AND SHETLAND ISLANDS (NORTH SEA, GREAT BRITAIN)

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Despite their sessile existence and their lack of morphological defensive structures, most marine sponge species appear to be minimally affected by predators, competitors or fouling organisms. This could be possibly due to a chemical defence of the sponges. Marine sponges produce a diversity of chemical compounds, but the ecological functions of these metabolites remain largely unknown. The aim of this study was the isolation and identification of the main secondary metabolites of some sponge species. Furthermore, the ecological functions of these compounds were studied.

Seventeen sponge specimens, containing Demosponges as well as some Calcarea species, were collected from different subtidal habitats off the Orkney and Shetland Islands (Great Britain) during July 2001. Sampling was performed by scuba diving. After the collection the sponges were frozen and freeze dried, followed by extraction with organic solvents of the whole sponge tissue. The crude extracts were chemically investigated by different chromatographic methods (e.g. LH-20 and preparative RP-HPLC). The structure elucidation was carried out by NMR spectroscopy and mass spectrometry.

To study the ecological functions (toxic and deterrent properties) of the sponge extracts, bio-assays were carried out. Antifouling is one possible defensive function of marine natural products isolated from sponges. To evaluate this assumption crude organic extracts of sponges were added to stable gels and exposed to seawater for several weeks. Thereafter the fouling ratio was determined and the results were compared between the different 17 sponge species. The extracts were also tested for antilarval activity. Therefore, ascidian larvae of species coexisting with the collected sponges were added to seawater containing sponge extract. The feeding deterrency was tested with predatory starfish which were fed with artificial food pellets containing the sponge crude extracts. The results of the chemical studies on the isolation and structure elucidation of the major metabolites as well as the results of the ecological assays will be discussed in detail.

TOWARDS THE TAXONOMIC RESOLUTION OF THE CARIBBEAN EXCAVATING SPONGE SPECIES COMPLEX *CLIONA CARIBBAEA-C. LANGAE-C. APRICA* (PORIFERA, HADROMERIDA, CLIONIDAE)

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There are sponges that both excavate and encrust the surface of the substratum. They excavate and advance mostly in the lateral direction. Upon encounter, they often kill coral tissues. In the Caribbean Sea, those having a dark brown to brown-black color are variably reported as *Cliona caribbaea*, *C. langae* or *C. aprica*. While ecological studies of their impact on reef corals were being made, the need to solve the taxonomic status of the species became evident. For this, detailed observations and sampling was carried out in several areas of the South (continental coast of Colombia) and Southwestern (San Andrés and Old Providence Archipelago, Colombia) Caribbean Sea. Visits to other areas (Belize, Bahamas, Puerto Rico, Venezuela) allowed further material for comparisons. Fragments of sponges and their substrata were obtained by hammer and chisel and fixed. Clean spicule mounts were made from nitric acid digestion of previously decalcified samples. Thick sections for microscopical observation of the internal tissues and excavations were obtained by cutting fragments with a circular diamond saw. These were then embedded in epoxy resin, cut and mounted in slides, and ground and polished with graded series of diamond coated grinding paper. Field observations of external morphology and color revealed the existence of only two distinct species in most localities. One species, *C. langae* Pang, 1973, is fully encrusting, maroon to dark brown, and may reach several square meters in size. Even as young, it starts to grow in the beta, fully encrusting stage. As it excavates the substratum laterally, its edges are usually smooth and continuous. Once it cannot advance more, its tissues thicken and the dermis darkens, and often the surface is invaded by epibiotic zoanthids. This species can become secondarily papillated where the tissues have been removed by grazers or smothered by sediments, filamentous or crustose algae, or when excavating tissue filaments reach the surface after crossing beneath an elevated ridge. The other species, *C. aprica* Pang, 1973, is very dark brown, almost black. It often grows as closely packed inhalant and oscular papillae, which may have a variable degree of fusion. Oscular papillae often have a grayish oscular rim. Interestingly, only at the San Andrés and Old Providence Archipelago, this species most often reaches the fully encrusting, beta stage, when growing on flat surfaces or thick or massive corals. However, it always leaves scattered bits of substratum free of tissues, where tufts of filamentous algae grow; the growing edges are often papillated, but can also be smooth. It does not grow as large a *C. langae*, but when lateral growth is not possible, it may also thicken and become invaded by epibiotic zoanthids. When growing in rubble, it seldom becomes encrusting, and tissue bridges bind and stabilize the substratum, forming small bioherms. Detailed examination of the

spicule characteristics showed a perplexing deal of variation within and between localities within a species, and a strong overlap between the two species. No single spicular characteristic could be used to diagnose each species throughout the full range of sampled localities. However, when spicules of specimens of the two species which had been growing near or side by side were examined, subtle but clear differences were found in most localities. The internal disposition of spicules and tissues depended more on the growth stage and the type of substratum than on the species. Comparisons of internal structures in paired specimens from the same locality and substratum are under way. It also remains to be determined which of the above mentioned species fall into junior synonymy of *C. caribbaea* Carter, 1882.

OCEAN BIOGEOGRAPHIC INFORMATION SYSTEM AND THE DEVELOPMENT OF SPONGE INFORMATICS

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Ocean Biogeographic Information System (OBIS) is an on-line, open-access, globally-distributed network of systematic, ecological, and environmental information systems. Collectively, these systems operate as a dynamic, global digital atlas to communicate biological information about the ocean and serve as a platform for further study of biogeographic relationships in the marine environment. Emphasis is on accurately-identified, species-level, geo-referenced abundance data. OBIS will build coalitions with national and international data systems to energize regional, national, and international scale development of ocean biogeographic and systematic databases and foster collaboration and interoperability by promoting standards and protocols. In OBIS development, globally distributed data nodes (functional modules) are first established for experts to store, manage, version and quality- control data in their specialty fields. A portal server is then developed to provide one-stop data shopping for end users and operate system-wide tools and models. OBIS researchers have agreed upon a set of communication protocols, query interface, data exchange format, and common vocabulary for communication within the distributed network. As a result, the world's principal databases on fish, octopus, squid, anemones, corals, zooplankton, and seamounts are for the first time integrated. Through one single Web-based user interface, the end user can now do one-stop data shopping for more than 400000 occurrence. As OBIS is a federation of heterogeneous data systems, its functional modules follow different development paradigms. We give a general review of these paradigms and as an example the development of History of Marine Animal Populations (HMAP) and OBIS/HMAP connectivity are discussed in detail. We further analyze some sample sponge datasets and address the issues of data gathering, modeling, system and on-line presence development. Alternate development paths are presented and their pros and cons are discussed.

MARINE SPONGES FROM CHINA OCEANS: DISTRIBUTION, DIVERSITY AND BIOACTIVE COMPOUNDS

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This report is intended to provide a first thorough overview of marine sponges from China ocean territories: their distribution, diversity and bioactive compounds. It is expected to serve as an entry point for understanding Chinese sponges and for furthering R&D on their bioactive compounds for new drug development.

China oceans cover a total area of 4750000 km^2, straddling the Indo-Pacific, and extending across warm-temperate, subtropical and tropical zones. The depth of the sea ranges from 2 to 5567 meters and the salinity ranges between 30 and 34. With a great diversity of ecosystems, it offers a prolific marine environment for diversified sponge species and the isolation of novel natural products. Chinese sponge taxonomists estimate a few thousands sponge species in China oceans. Currently, there are more than 200 identified species of sponges that have been classified into 3 Classes (Demospongiae, Hexactinellida and Calcarea), 17 Orders and 38 Families. However, sponge taxonomic study in China has progressed very slowly, due to the lack of funding for developing advanced tools for collection and the traditionally ignorance of non aqua-cultured marine species.

A detailed survey of 17 international and Chinese Journals on Chinese sponges and their natural products has been carried out in the period from 1980 to 2001. There was very scarce investigation of Chinese sponges before 1980. During the past two decades, less than 90 research papers or reports have been published with only a few papers published during the first one. Since 1990, a sudden increase in R&D activity on Chinese sponges has been seen, with an annual average number of publications more than 7. This trend coincides with the worldwide rapidly growing exploitation of marine sponges in the search for new anticancer and antiviral drugs in the 1990's. In addition, it also indicates that the significance of R&D on sponge bioactive compounds has been recognized recently by scientists and funding agencies in China.

In the search for new bioactive entities, sponges from Chinese waters that have been investigated are classified into 1 Class (Demospongiae), 8 Orders, 13 Families and 18 species (excluding 7 unidentified sponge species). All sponge species investigated were collected from the South China Sea except one from the China Yellow Sea. With this very limited number of sponge species studied, a variety of natural products including alkaloids, terpenoids, sterols, lipids have been isolated, which are indeed similar to the spectra of compounds discovered in sponges worldwide. In addition, several unusual structures have also been isolated. However, the bioactivity assays and pharmacological studies fall far behind the isolation and elucidation of new chemical structures from Chinese sponges. Only a few studies have dealt with the preliminary bioactivity screening. The early studies used

extractant mixtures and later purified compounds. The bioactivities tested include antitumoral activity, neuro-toxicity, cyto-toxicity and anti-cardiac disease properties. The lack of bioactivity screening studies hindered the R&D on sponge natural products in China.

In conclusion, the China oceans remain as one of the largest untapped water bodies for sponge taxonomy and the discovery of novel bioactive entities. R&D on sponges and their natural products in China will result in fruitful achievements in the coming decade.

FUNCTIONAL EFFECTS DOWNSTREAM OF THE TEMPERATURE-SIGNALING CASCADE IN SPONGES INCLUDE SHORT- AND LONG-TERM EFFECTS ON RESPIRATION AND FILTRATION RATES

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ADP-ribosyl cyclase activity is expressed along the phylogenetic tree from unicellular protists (Masuda *et al.*, 1997) to mammals (Lee, 1997). It converts NAD^+ to cyclic ADP-ribose (cADPR) (Lee, 1994), an intracellular calcium mobilizer which is being increasingly recognized as a pivotal signaling molecule, involved in such diverse functions as cell cycle regulation (Masuda *et al.*, 1997) (protists), oocyte fertilization (Lee, 1996) (invertebrates), insulin secretion (Takasawa *et al.*, 1993) and cell proliferation (Zocchi *et al.*, 1998) (mammals). The peculiar position of Porifera in the phylogenetic tree (they are the oldest known Metazoa, sharing a common ancestor with all multicellular animals (Rodrigo *et al.*, 1994)) prompted us to investigate the presence, and the functional role, of the ADP-ribosyl cyclase/cADPR system in marine sponges.

ADP-ribosyl cyclase is present in cell lysates obtained from several different sponge genera, including demospongiae and calcispongiae (Zocchi *et al.*, 2001). *Axinella polypoides* (Demospongiae, Axinellidae), an arborescent sponge living on coralligenous or detritic bottoms (Ligurian Sea), expresses by far the highest ADP-ribosyl cyclase activity among the species tested. In *A. polypoides*, ADP-ribosyl cyclase is activated by temperature increases via an abscisic acid (ABA)-induced, protein kinase A-dependent mechanism. Recently, we elucidated the complete thermosensing pathway in *A. polypoides*, which includes: i) a cation channel thermoreceptor, sensitive to heat, mechanical stress, phosphorylation and anesthetics; ii) the phytohormone abscisic acid (ABA) and cADPR as its second messenger (Zocchi *et al.*, 2001).

Here we investigated the functional effects downstream of this signal transduction pathway, which included short-term stimulation and long-term depression of sponge oxygen consumption and of the filtration rate. These effects were induced by transient heat stress or ABA administration.

An 80 % and 70 % increase of the sponge respiration and filtration rate, respectively, were observed after exposure of the animal to a transient temperature increase (8° C for 30 min) or to micromolar ABA. The short-term stimulation of both respiration and filtration, which lasted for 60-120 min, was inhibited by the

targeted interruption of the above described signaling pathway. Specifically, the heat-induced increase in oxygen consumption and filtration rate was prevented by pre-treatment of the sponge with the cation channel inhibitors bupivacaine and Gd^{3+} while both the temperature- and the ABA-induced effects were prevented by the intracellular calcium chelator EGTA-AM and by the membrane-permeant cADPR antagonist 8-Br-cADPR. Thus, these results unequivocally establish a causal relationship between the cADPR-induced calcium mobilization and the increase of sponge functional activities.

The short-term stimulation of respiration and filtration rate was followed by a long-term decrease of both functions, which was observed over a period of 6-24 hours after exposure to heat-stress or ABA. These effects could also be prevented by pre-treatment of the animal with EGTA-AM or 8-Br-cADPR prior to heat-stress or ABA exposure: thus, they are likely due to the profound derangement of the intracellular calcium homeostasis which follows the cADPR-induced calcium mobilization and is caused by extracellular calcium influx (Zocchi *et al.*, 2001).

These results may be of interest for their evolutionary and ecological implications: this is the first observation of functional effects exerted on Metazoa by the phytohormone ABA, which is involved in drought-stress signaling in plants: conservation of the ABA/cADPR stress signaling cascade suggests its ancient evolutionary origin in a common precursor of modern Metazoa and Metaphyta; the long-term depression of sponge functional activities induced by heat-stress may be responsible for the recently observed mass mortality episodes attributed to sudden sea water temperature increases in the Ligurian Sea (Cerrano *et al.*, 2000).

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