Taxonomic overview of calcareous sponges (Porifera, Calcarea) in the Jan Mayen vent field at the Mohn Ridge

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Table of Contents

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Abstract

The Porifera is a very old group of organisms that has been dated back to more than 500 million years. They are important to marine benthic communities by providing protection, contributing to nutrient recycling and working as spawning grounds. The exclusively marine Calcarea is a small class of sponges characterized by having a skeleton of calcium carbonate, which separates them from the other classes. The calcareous sponges are normally found in shallow waters, however they have been found all the way down to 4000 m and even at hydrothermal vents. The Jan Mayen hydrothermal vent field is located on the Arctic Mid-Ocean Ridge (AMOR) that was discovered in 2006. From 2006 to 2013 there have been several cruises to this vent field, and sponge samples were collected in 2006, 2011 and 2012. In other habitats calcareous sponges usually represent only a small part of the total sponge fauna, but at the Jan Mayen vent field the majority of the sponge fauna was calcareous. This thesis aims to give a description of the calcareous sponges from the Jan Mayen Vent Field using morphology and molecular data (28S rRNA sequences). Five calcareous sponge species were identified, of which four were previously known from Atlantic/Arctic waters; *Brattegardia nanseni*, *Clathrina pellucida*, *Sycon* cf. *abyssale* and *Grantia* cf. *mirabilis*. The fifth species, *Grantia* sp. nov., is new to science and the most abundant species in the area. The only potentially vent-endemic species is the new species of *Grantia*, and thus the calcareous sponge fauna conforms to the general pattern of low levels of endemicity at the Jan Mayen Vent Field. Because of the harsh environment at the hydrothermal vents, it is surprising to see such an amount of calcareous sponges present.

1 Introduction

1.1 The Porifera

The phylum Porifera, commonly known as sponges, is a group of primitive animals that currently have over 8300 described species where the majority is marine (Van Soest *et al.,* 2012). It is also one of the oldest phyla recorded, and previous studies have shown that the Porifera dates back between 500 and 700 million years from various parts of the world including Australia, China and Mongolia (Müller *et al.,* 2007). Sponges are multicellular animals characterized by their aquiferous system, which pumps water through the sponge and is responsible for feeding, respiration and reproduction (Bergquist, 1978). The water current is driven by choanocytes (flagellated cells forming an internal layer) that create a unidirectional current through the sponge. Food particles and oxygen in the water are taken up by the choanocytes and other cells while the remaining water will be channeled out through an orifice called the osculum. There are three major types of sponge aquiferous systems which vary depending on the degree of complexity and folding, namely Asconoid, Syconoid and Leuconoid bodyform.

Sponges are also characterized by their extracellular matrix, the mesohyl, where many cell types are found and that show a high degree of mobility and plasticity (Brusca & Brusca, 2003). Most of the cells in the mesohyl are totipotent, which means that they can change from one type to another as required (Brusca & Brusca, 2003). This plasticity compensates for the fact that they have no tissues and organs.

The phylum Porifera is divided into four classes; Demospongiae (Siliceous sponges), Hexactinellida (glass sponges), Calcarea (calcareous sponges) and the recently reerected Homoscleromorpha (Gazave *et al*., 2012). The Demospongiae is the largest of the four classes includeing about 85% of all sponge species (Hooper & Van Soest, 2002).

Sponges can be found at all depths from the intertidal to the abyssal zones and they are an important part of their ecosystem because of their many uses for other organisms (Brusca & Brusca, 2003). They provide shelter and are used as spawning grounds for fish, and in some cases, like with *Lissodendoryx colombiensis* they form a mutualistic partnership with seagrass

where the sponge protects it from being grazed on by seastars (Wulff, 2008). They can occur in large quantities in vast areas termed “sponge gardens”, and these gardens supply and provide other organisms with the nutrients and protection they need. This can be due to anything from nutrient recycling to bioerosion (Bell, 2008). It is also shown that sponges can transfer dissolved organic matter to higher trophic levels by expelling their filter cells as detritus (De Goeij, 2013).

In many cases the sponges have bacteria living inside their mesohyl layer, either as a symbiont or a parasite (Bavestrello *et al*., 2000). The majority are symbionts, and in some cases they play a role in the metabolism of the host (Liu *et al*., 2012). To date there are at least 25 bacterial phyla associated with sponges (Webster & Taylor, 2012), for example cyanobacteria has been shown to be present in at least 26 Demospongiae families and 17 Calcarea families (Hentschel *et al*., 2006)

1.2 Calcarea

The class Calcarea, or calcareous sponges, is together with Hexactinellida the smallest classes in the Porifera, consisting of roughly 7.5% of sponge diversity each (Hooper & Van Soest, 2002). Calcarea is an exclusively marine class and all are viviparous (Bowerbank, 1864; Rapp, 2013). Calcarea can be further divided into the subclasses Calcinea and Calcaronea (Bergquist, 1978). The spicules of Calcarea differ from other sponges by being made of calcium carbonate ($CaCO_3$), in contrast to the siliceous spicules of the other three classes (Brusca & Brusca, 2003). The spicules are composed of a form of calcium carbonate called calcite and usually only have megascleres, and not mega- and microscleres like the other classes (Bergquist, 1978, Brusca & Brusca, 2003). The primary spicules types for calcareous sponges are monactines, diactines, triactines and tetractines, with a high morphological diversity in all of these types (Sethmann & Wörheide, 2008). Sponge classification is mainly based on the characteristics of the skeleton and the size and shape of the spicules. Especially the spicules are a vital part of describing species within the Calcarea.

The spicules of the subclass Calcinea are normally regular triactines and/or a tetractines, and together with free spicules there may also be a non-spicular basal system. The Calcaronea has spicules composed of diactines and sagittal triactines and tetractines (Bidder, 1898;

Borojevic *et al*., 2000). Among the Calcinea you find the families Clathrinidae and Leucascidae and within the Calcaronea you find the families Leucosoleniidae, Sycettidae, Grantiidae, Jenkinidae, Heteropiidae and Baeriidae.

1.3 Physical properties of hydrothermal vents

Hydrothermal vents are areas where geothermally heated water comes out from the seafloor, commonly around mid-oceanic ridges (Van Dover, 2000). The characteristic chimneys of hydrothermal vents are formed when hot water with an abundance of dissolved minerals from the earth's crust comes in contact with the cold ocean water and the minerals precipitate (Van Dover, 2000).

The vents can be categorized into two different types; black and white smokers. The black smokers have a higher temperature (350^{o}C – 400^{o}C) plume than the white smokers (250^{o}C – 300^{o}C; Van Dover, 2000). The water of black smokers is dark because the dissolved metals react with sulfur in the seawater to form metal sulfide minerals that are characteristically black in color (Van Dover, 2000). While seawater has a pH value ranging from 7.5 to 8.4, the black smoker fluids have a pH as low as 3 to 5 and are rich in sulfide, hydrogen, methane, manganese and other transition metals (Van Dover, 2000). The reason the black smokers emit so much metals and sulfur is because they have a higher solubility when the temperature gets close to 350^{o}C which results in their mobility from the hard rocks to liquid fluids (Seyfried *et al.*, 1988). The white smokers have a slower flow, which allow the black metal sulfides to precipitate out before reaching the surface. The white color is due to the presence of the mineral anhydrite and the plume also contains barium, calcium and silicon (Van Dover, 2000).

Hydrothermal vents can also be divided into two categories depending on where they are found, either on a fast spreading ridge or a slow spreading ridge. These two different ridge types are defined by their spreading speed, so while the fast ridges can have a speed of 55-80 mm/yr (intermediate) or >140 mm/yr (hyperfast) the slow ridges have an average speed between 20-55 mm/yr or <20 mm/yr (ultra slow) (Baker & German, 2004). The Arctic Mid Ocean Ridge is an example of an ultra slow ridge (Engen *et al.*, 2003; Schander *et al.,* 2010; Pedersen *et al*., 2010).

Hydrothermal vents are located all around the world along the borders of the continental plates. There are still many undiscovered vent systems although new vent systems are continuously being discovered (e.g. Van Dover *et al.,* 2001). The Jan Mayen Vent Field (JMVF) was discovered in 2008 (Schander *et al.,* 2010), which makes it a very young system in terms of discovery. Vent fields are found all along the world's oceanic ridges, and vent fields in different oceans have different communities, depending on the geographical distance between the vent fields and environmental properties like for example depth (Tarasov *et al*., 2005).

1.4 Vent fauna

Hydrothermal vent communities are sustained by energy derived from bacterial chemosynthesis, where bacteria use hydrogen sulfide to produce organic material (Van Dover, 2000). Chemosynthetic bacteria are found in the water and on hard surfaces (see Figure 1), but also as symbionts of invertebrate animals (Tunnicliffe, 1991). As a symbiont the bacteria then live within a host tissue, and through chemosynthesis it provides the host with energy and nutrients (Van Dover & Fry 1989, Dubilier *et al.,* 2008, Sweetman *et al.,* 2013). Another way to get a hold of nutrition derived by chemosynthesis is by grazing or filtering bacteria growing on the deposit or staying in the water column, like copepods and

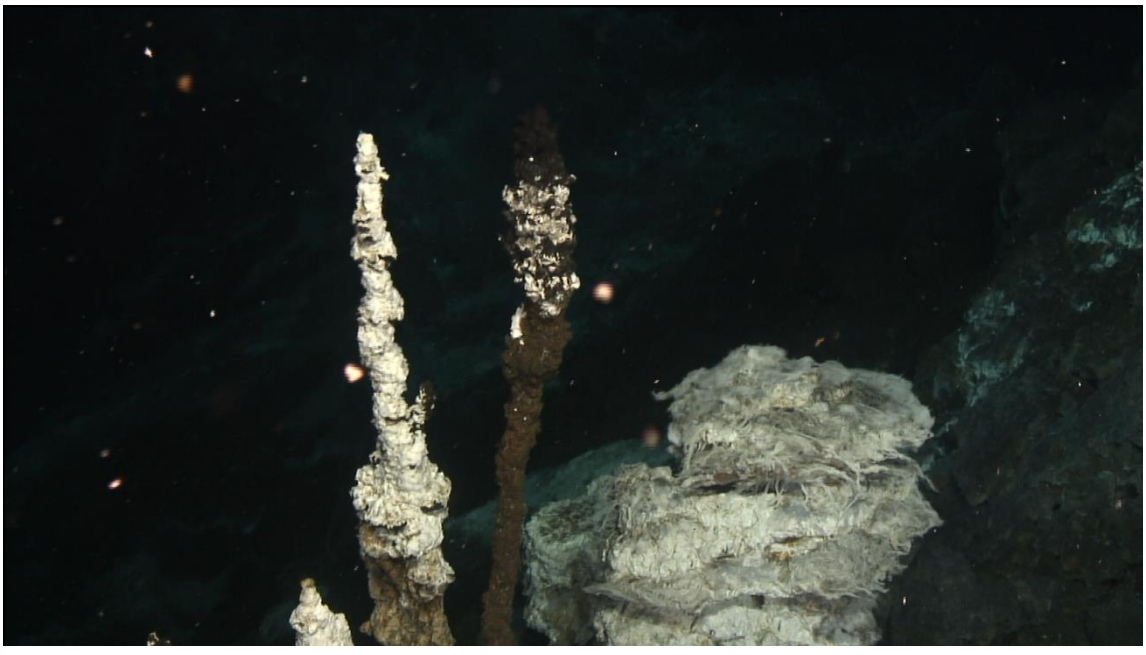

Figure 1. White smoker from Jan Mayen with bacterial mats covering the surface. Photo: Centre for Geobiology.

amphipods (Van Dover & Fry, 1994, Colaço *et al.,* 2002, Kongsrud & Rapp, 2012, Sweetman *et al.,* 2013). Further up the trophic level you will find predators like snails, tubeworms, crabs and fish among others (Van Dover *et al.,* 2001).

Sweetman et al. (2013) showed that a majority of the organisms living around the vent systems get their nutrition from photosynthetic production while a smaller portion possessed light isotopes which suggested heterotrophic assimilation of chemosynthetic production.

The fauna found around the Atlantic vent systems are very different from the Pacific systems (Tunnicliffe *et al*., 1998). Communities in the Pacific are primarily dominated by tubeworms while the deep-water vent fields on the Mid Atlantic Ridge have swarms of shrimps that cluster around the chimneys and shallower vents tend to be abundant in mussel beds (Colaço *et al*., 2002). The way these organisms disperse is not by moving adults, but rather in the larval stage, and it has been hypothesized that vent organisms can use cold seeps and organic falls (wood/whale falls) as stepping stones for dispersal (Tandberg *et. al*., 2013).

1.5 Vent fauna and physical properties around Jan Mayen

The JMVF is relatively diverse for a vent community with 180 identified species, and the most abundant taxa are crustaceans, sponges, molluscs, and annelids (Schander *et al*., 2010; see Figure 2). Of these groups the crustaceans are the most abundant, both in species richness and biomass, but the vast majority of the species found have been reported from surrounding waters, which indicate that they are not endemic to the hydrothermal vent systems (Schander *et al*., 2010). It has been shown that the smokers at JMVF are expelling fluids with H_2S values up to 6 mmol/kg, which is in the range in which endemic vent fauna can thrive, but yet it appears that the vents are dominated by non-specialized fauna (Schander *et al.*, 2010). One reason for the low levels of endemic fauna at the Southern Mohn Ridge could be the cold surrounding water temperature (around -0.1 to -0.2^{o}C) together with the shallow depths of the vents which could result in competition with background fauna (Schander *et al.,* 2010, Sweetman *et al.,* 2013).

Figure 2. Fauna around the vents including, nudibranchs, anemonies, polychetaes and sponges. Photo: Centre for Geobiology.

Sponges of small sizes have been found from around 500 m depth at the Jan Mayen Vent Field, mostly from Demospongia and Calcarea (Schander *et al*., 2010). A previous report from north of Iceland by Fricke et al. (1989) showed that shallow vents (around 106 m) were dominated by the calcareous sponge *Sycon quadrangulatum*. Carnivorous sponges were also found feeding on crustaceans in the vicinity of the vents (Schander *et al.,* 2010).

1.6 Objectives

The main aim of this thesis is to give a morphological description of the calcareous sponges found in the Jan Mayen vent field.

Secondly it was aimed to provide molecular data (28S) to supplement the species descriptions and to test the phylogenetic position of the species where no such data has been available until present.

2 Materials and methods

2.1 Study area

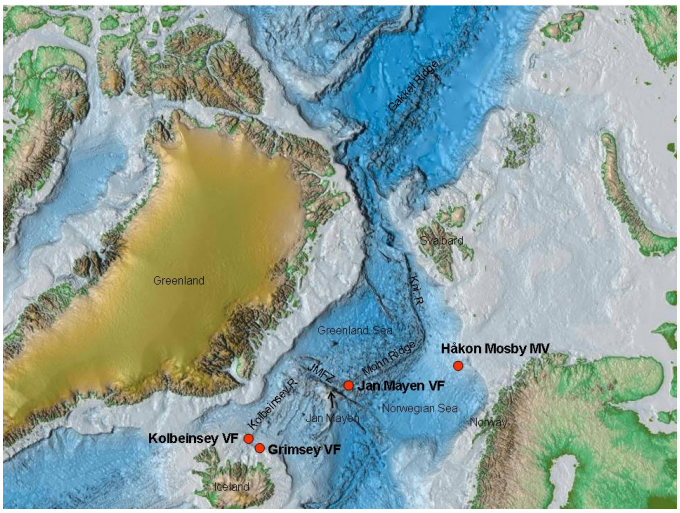

Figure 3. Jan Mayen vent field indicated with a red circle

Illustration: Centre for Geobiology.

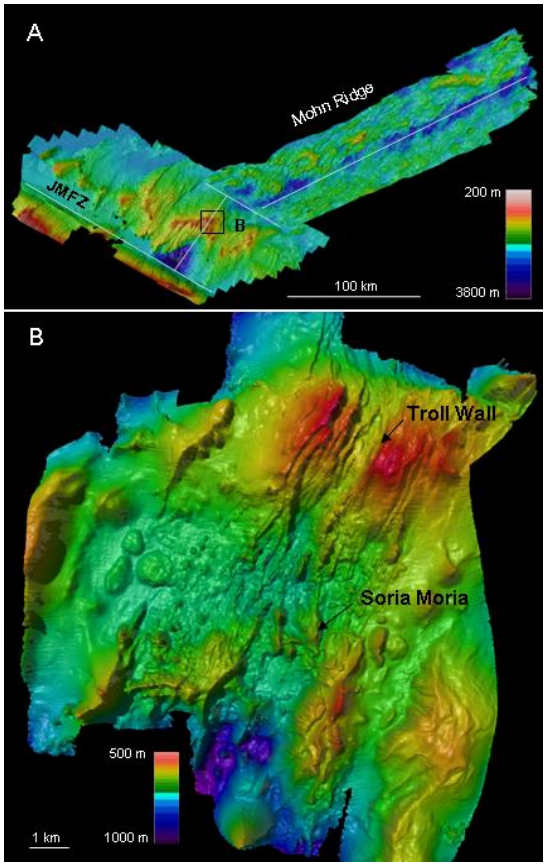

Figure 4. Location of sampling sites Trollveggen and Soria Moria. Illustration: Centre for Geobiology.

The Jan Mayen Vent Field is located north east of Iceland and east of Greenland in the Arctic Ocean (Figure 3). Three vent locations at the Jan Mayen Vent Field were sampled; Gallionella Garden, Trollveggen and Soria Moria, where the two latter are located roughly 5 km apart (Pedersen *et al.*, 2005; see Figure 4). Trollveggen is the shallower of the two sites with a depth of roughly 500 m while Soria Moria is located around 700 m deep. There were a total of 14 dives during the cruise, of which two brought back calcareous sponge material.

2.2 Sampling gear

The sampling was done from the research vessel G.O. Sars and the gear that was used to sample was a Remotely Operated Vehicle (ROV) named “Bathysaurus XL” from Argus Remote Systems, which is a submersible that can be equipped with different tools depending on what you want to sample. For this purpose it was equipped with a suction sampler with a collection container attached to the ROV. Chunks of rock were also sampled with an electrical controlled arm on the ROV, which collected the material and put it into

small chambers in the ROV for further analysis on deck. The samples were taken in the near and direct vicinity of the vents.

A CTD was used to collect water samples for the environmental data. Several dissolved gases were measured, for example oxygen, and also temperature, salinity, conductivity and depth. The CTD was lowered to the sampling depth, which varied from 500 to 700 meters.

2.3 Preservation, identification and sorting

The material that was sampled was sorted under a stereo-microscope on the research vessel. The samples were mainly sorted into subclass, but some specimens were identified down to genus level. The majority was put on 96% ethanol, while a small fraction was put on 8% formaldehyde and a smaller amount was stored using the shock freezing technique with liquid nitrogen. The samples stored on formaldehyde and the frozen samples were suspected to be similar to the ones stored on ethanol for possible further analysis. All of the samples were labeled with the station number and cruise year.

2.4 Morphological methods

Species identification was mainly done by measuring spicules, describing the specimens overall (size, shape, color etc.) and the architecture of the skeleton. The identity of the species was established after comparing it to habitus from other similar species from other reports and comparing to Arctic species.

2.4.1 Skeleton architecture

The skeleton architecture of calcareous sponges is normally categorized in three different types; the leuconoid, asconoid, and syconoid (Boury-Esnault & Rützler, 1997; Figure 5). Determining the skeleton architecture of the specimens is a key element to identifying the species you are looking at. The choanosomal layer normally consists of parasagittal triactines that stack on each other forming a solid structure for the canal system. The skeleton architecture was identified using the description by Boury-Esnault and Rützler (1997).

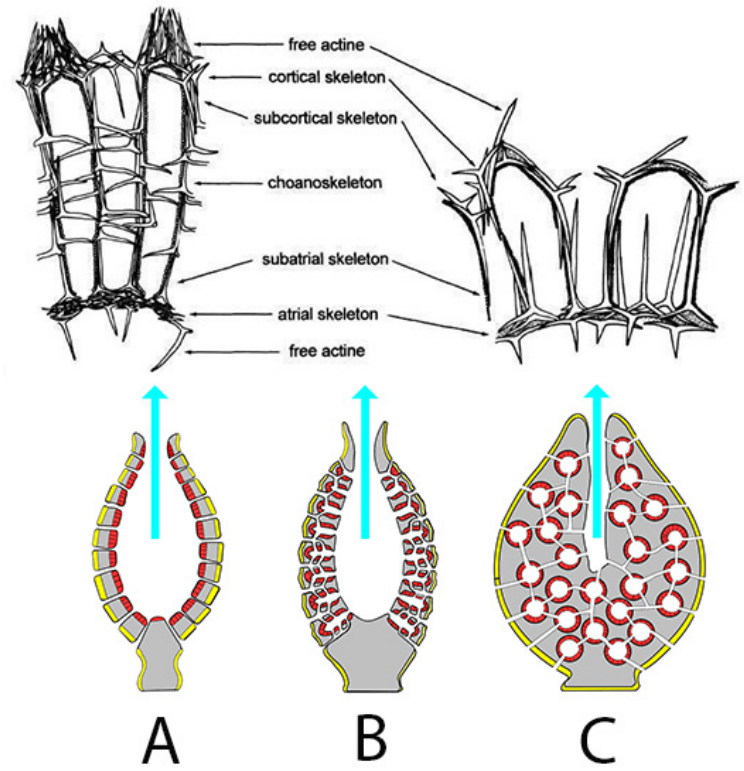

Figure 5. Top: Structural layout of a syconoid aquiferous system. Figure from Boury-Esnault & Rützler (1997); Lower: A simplified model of the three most common aquiferous systems; A) Asconoid, B) Syconoid, C) Leuconoid. Figure from Ruppert *et al*. (2004).

2.4.2 Spicules

The general spicule diversity in calcareous sponges are di- tri- and tetractines where the –actine ending refers to the number of rays or points. Among the tri- and tetractines you have different shapes that are categorized as regular, sagittal, parasagittal and pseudosagittal that can also have a basal triradiate system. The diactines may also vary in the shape although they are generally straight, they can have slight curves and have an angled end. The spicules that are mentioned in this study are listed below and shown in Figure 6. The descriptions and terms as well as the illustrations are adapted from Boury-Esnault and Rützler, 1997.

A) Regular spicule: Triactine or tetractine spicule with basal rays of equal length, and with equal angles (120^{o}) between them, when projected into a plane perpendicular to the optic axis.
B) Basal triradiate system: The three rays of a tetractine that correspond to those of a triactine.

C) Sagittal spicule: Triactine or tetractine with two equal angles (paired angles) and one dissimilar angle (unpaired angle) at the center, when projected into a plane perpendicular to the optic axis.

D) Pseudosagittal spicule: A subcortical triactine essentially sagittal, but having unequally long and differently curved rays on each side of the unpaired angle.

E) Parasagittal spicule: Bilaterally symmetrical triactine or tetractine with unequal actines, aving equal angles (120^{o}) between the basal rays when projected into a plane perpendicular to the optic axis.

F) Diactine: A spicule composed of two actines.

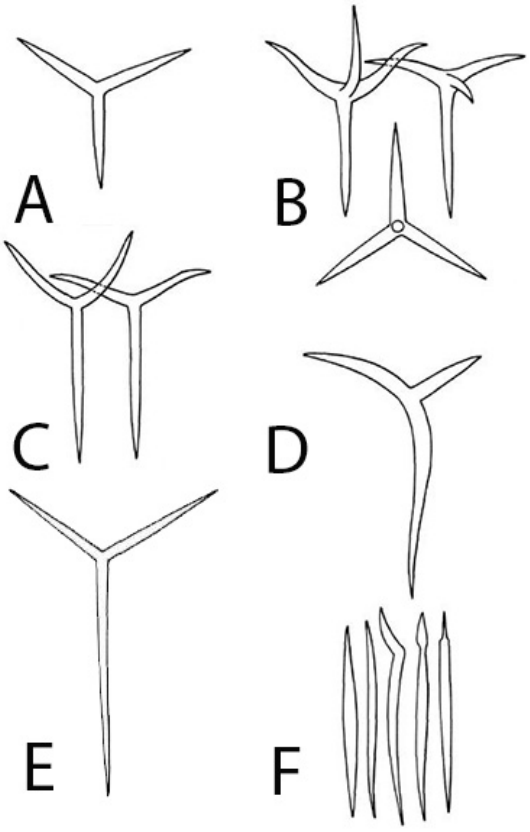

Figure 6. Spicule types. A, Regular spicule. B, Basal triradiate system. C, Sagittal spicule, D, Pseudosagittal spicule. E, Parasagittal spicule. F, Diactine. Figure from Boury-Esnault and Rützler (1997).

2.4.3 Histological slide preparations

For some of the species (*Grantia* sp. nov. and *Grantia* cf. *mirabilis*) the skeletal organization and architecture was examined under a light microscope from a histology section, which was prepared by Elena Gerasimova. The sponges were embedded in epoxy resin following the protocols described by Boury-Esnault and Bézac (2007). The histology sections were cut with a diamond saw.

2.4.4 Spicule slide preparations

Spicule slide preparations were prepared according to the procedures by Boury-Esnault and Rützler (1997). Since each specimen is very small the preparations were done directly on the slide. The material was identified as far as possible and details around the species are provided. This was done by measuring a minimum of 30 spicules for each spicule slide using a micrometric ocular. For the permanent slides a small piece of each specimen was put in household chlorine for approximately 30 minutes, after this most of the chlorine was extracted and replaced with distilled fresh water and the sample left for 30 minutes. This is repeated 2 times until the chlorine was washed out. After the final dilution 96% ethanol is added and the sample is left for 30 minutes. A slide is then preheated on low heat and a drop of the spicule sample is added to it. After the ethanol had evaporated a mounting medium was applied, which in this case was Euparal. A cover slide is placed on top of the mounted spicules and is heated for 48 hours at 50^{o}C to cure the mounting medium.

2.4.5 Scanning Electron Microscope preparation

Using a scanning electron microscope (SEM), high definition close-ups of the samples were taken, along with spicule photographs to get a better view of the spicules of the species. The spicules were placed on a double-sided carbon tape on SEM stubs which were then coated in a thin layer of gold-palladium using a Sputter coater.

2.5 Molecular methods

2.5.1 DNA extraction

A small piece of about 1 mm^2 was taken from two individuals from sample G1 (*Grantia* sp. nov.) and G2 (*Grantia* cf. *mirabilis*). One of the individuals in sample G1 was so small so the whole specimen was used. Each piece was placed in a 1.5 mL Eppendorf tube in 96% ethanol. Prior to the extraction, the ethanol was removed from the tube and replaced with double distilled water (ddH_2O) and left for approximately 3 hours. The samples were then dried either on a petri dish or in the cap of the Eppendorf tube to remove any remaning ethanol before the extraction process.

DNA was extracted using Qiagen Blood and Tissue Kit following the manufacturers spin column protocol (see Appendix 1 for description of the procedure).

2.5.2 DNA amplification

28S rRNA was amplified in three non-overlapping fragments using six primers developed by Morrow et al. (2012; see Table 1).

Table 1. Primers used for sequencing of 28S rRNA (from Morrow et al., 2012).

Marker	Name	Sequence 5'-3'
28S D1-D2	Por28S-15F	GCG AGA TCA CCY GCT GAA T
	Por28S-878R	CAC TCC TTG GTC CGT GTT TC
28S D3-D5	Por28S-830F	CAT CCG ACC CGT CTT GAA
	Por28S-1520R	GCT AGT TGA TTC GGC AGG TG
28S D6-D8	Por28S-1490F	AAC TCA CCT GCC GAA TCA AC
	Por28S-2170R	CCA ATC CTT TTC CCA ARG TT

The PCR reaction had a total volume of 25 µL with 16.35 µL of double distilled water (ddH_2O), 2.5 µL buffer 10x (TaKaRa), 2 µL dNTPS, 0.15µL TaKaRa Ex Taq DNA Polymerase, 1 µL of each of the primers and 2 µL of extracted DNA. The solutions were kept on ice during preparation.

Each run included a positive control to confirm the success of the amplification and a negative control to check for contamination. PCR thermal cycles were run with an initial

denaturation of 5 min at 95° C followed by 35 cycles with denaturation of 45 s at 94° C, annealing for 30 s at 54° C, and extension for 1 min at 72° C. Final extension was achieved by 10 min at 72° C.

The PCR product was visualized on a 1% agarose gel based on TAE buffer and containing the staining agent GelRed. 1 µL of Ficoll loading dye was mixed with 4 µL of DNA product on a piece of parafilm. All of the 5 µL of total solution was inserted into the wells and was run at 80V for 40-50 minutes before being analyzed under UV light in a UV cabinet, using GeneSnap software for capturing images.

2.5.3 Sequencing

The PCR products were purified with Exonuclease 1 (EXO, 10 U/µl) and Shrimp Alkaline Phosphatase (SAP, 10 U/µl, USB®), in 10 µl reactions (EXO = 0.1 µl, SAP = 1 µl, ddH_20 = 0.9 µl, PCR product = 8 µl). Samples were incubated at 37° C for 15 min followed by an inactivation step at 80° C for 15 min.

The three fragments were sequenced in both directions using the primers in Table 1. The sequencing reaction contained 1 µl buffer, 1µl Big Dye, 1µl primer and DNA template and ddH_2O making the total reaction 10 µl. The amount of ddH_2O depended on the amount of DNA added which was determined by band quantification visualized on the gel. The sequencing reactions were run with an initial denaturation of 5 min at 96° C followed by 25 cycles with denaturation of 10 s at 96° C, annealing for 5 s at 50° C, and extension for 4 min at 60° C. Ten µL dH_2O was added to each tube before handed over to the sequencing facility (Department of Molecular Biology, UoB).

2.5.4 Sequence assembly and alignment

The sequences were delivered from the UoB facility as ABI trace files, which were put into FinchTV (Geospiza™) for quality control of the sequences. The forward and reverse strands were assembled in Geneious R7 (Biomatters Limited), and the usable sequences were then checked against GenBank using blastn to assure there was no contamination. The resulting sequences were aligned separately for each segment using Muscle with ten iterations, including one specimen of *Grantia arctica,* two specimens of *Sycon ciliatum* and an outgroup

of *Clathrina luteoculcitella* (Wörheide & Hooper, 1999). The alignments were exported as fasta-files, checked in BioEdit (Hall, 1999), trimmed to the longest *Grantia* sequence and missing data at the ends coded as ? before the three segments were concatenated.

The three segments were tested separately for a best-fit model using jmodeltest v.2.1.4 and the Akaike Information Criterion (AIC) to determine the best evolutionary model for each segment. The concatenated fasta alignment was then converted to nexus through Mesquite v.2.75 (Maddison & Maddison, 2011) before it is loaded into MrBayes v.3.2.1 (Ronquist & Huelsenbeck, 2003) for phylogenetic analysis. Analysis was run with 3 parallel runs of 5 million generations for, with sampling every 1000 generations. Convergence of runs was checked using Tracer v.1.5 (Rambaut & Drummond, 2009) and the burnin was set to 10%. A consensus tree was generated in MrBayes, annotated and converted to graphics in FigTree v1.3.1 (Morariu et al., 2008) and final adjustments were done in Adobe Illustrator CS6.

3 Results

3.1 Species description

Unless stated otherwise the scopes of the supraspecific taxa used in this thesis corresponds to Borojevic et al. (2002), Manuel et al. (2002), Klautau et al. (2013) and Boury-Esnault & Rützler (1997).

Class Calcarea Bowerbank, 1864

Exclusively marine Porifera in which the mineral skeleton is composed entirely of calcium carbonate. Spicules are diactines, triactines and tetractines. All Calcarea are viviparous.

Subclass Calcinea Bidder, 1898

Calcarea with regular (equiangular and equiradial), or exceptionally parasagittal or sagittal tractines, and/ or a basal system of tetractines. In addition to the free spicules, there may be a non-spicular basal calcareous skeleton. In terms of ontogeny, triactines are the first spicules to be secreted. Choanocytes are basinucleate with spherical nuclei. The basal body of the flagellum is not adjacent to the nucleus. Calcinea incubate calciblastula larvae.

Order Clathrinida Hartman, 1958

Calcinea with a skeleton composed exclusively of free spicules, without hypercalcified non-spicular reinforcements, spicule tracts, calcareous scales or plates.

Family Clathrinidae Minchin, 1900

Clathrinidae with an essentially tubular organization. The skeleton is formed by tangential triactines, to which tripods, tetractines and diactines may be added. A continuous choanoderm lines all the internal cavities. The water crosses the wall through pores, delimited by porocytes. The young sponges have an olynthus form that grows through longitudinal median division, budding and anastomosis of individual tubes to form the large units, called the body. There is neither a common cortex nor a well-defined inhalant or exhalant aquiferous system.

BRATTEGARDIA Klautau et al., 2013

Type species

Brattegardia nanseni (Breitfuss, 1896). Calcinea in which the body is formed by anastomosed tubes covered by a thin membranous layer, at least in young specimens. Body is massive/globular with or without a stalk. The skeleton contains regular (equiangular and equiradiate) triactines and tetractines, but parasagittal triactines may be present. Triactines are the most numerous spicules. Aquiferous system is asconoid.

3.1.1 *Brattegardia nanseni* (Breitfuss, 1896)

(Table 2, Figure 6)

Original description

Leucosolenia nanseni Breitfuss, 1896: 427-428.

Synonyms and citations

Ascandra reticulum (Haeckel, 1872; Lütken, 1875; Breitfuss, 1897; Vanhöffen, 1897, c; Brøndsted, 1914).

Brattegardia nanseni (Klautau *et al.*, 2013; Rapp, 2013).

Clathrina nanseni (Rapp *et al.*, 2001; Klautau & Valentine, 2003; Rapp, 2006).

Leucosolenia nanseni (Breitfuss, 1898a, c; Lundbeck, 1909; Brøndsted, 1914; Breitfuss, 1933; Brøndsted, 1933).

Nardoa reticulum (Schmidt, 1869 pars; 1870 pars).

Material examined

3 specimens. Jan Mayen st. 12, Gallionella Garden, 71°17.98N, 5°46.92W, 2006, 616 m (3).

Outer morphology

Brattegardia nanseni has a globular/pearshaped body. The body is made up from a network of hollow tubes, which leads up to a single osculum at the apical end. The tubes do not have

an apparent organization. The stalk is poorly developed, and consists of the same network of asconoid tubes as the main body, although more compressed. The osculum is naked. The surface is smooth and texture is fairly soft. The coloration is whitish/grey after fixation in 96% ethanol. The length of the specimen is roughly 1 cm (2 cm including the stalk). The stalk has similar spicules as the body although a little smaller in size.

Spicules

The skeleton consists of triactines and tetractines. All spicules are regular and equiangular. In the basal part one actine may be slightly longer than the others. The apical actines from the atrial tetractines have a slight upward angle surrounding the atrium.

Table 2. Spicules of *Brattegardia nanseni*

		Length (µm)				Width (µm)	
Spicule type	N	Mean	Min.	Max.	SD	Mean	SD
Atrial Tetractines							
Paired	30	115.0	104.0	132.0	±12.4	8.3	±0.5
Unpaired	30	105.0	84.0	128.0	±18.3	8.3	±1.0
Apical actine	30	38.5	6.0	120.0	±54.6	5.5	±0.6
Cortical Triactines							
Paired	30	113.7	88.0	140.0	±13.3	10.0	±1.4
Unpaired	30	130.7	108.0	188.0	±16.8	10.8	±1.8

Remarks

The shape of *B. nanseni* can vary a bit although the general shape is the one described here. It has previously been reported as belonging to the genus *Clathrina*, but after being revised by Klautau *et al.* (2013) it is now included in the new genus *Brattegardia*.

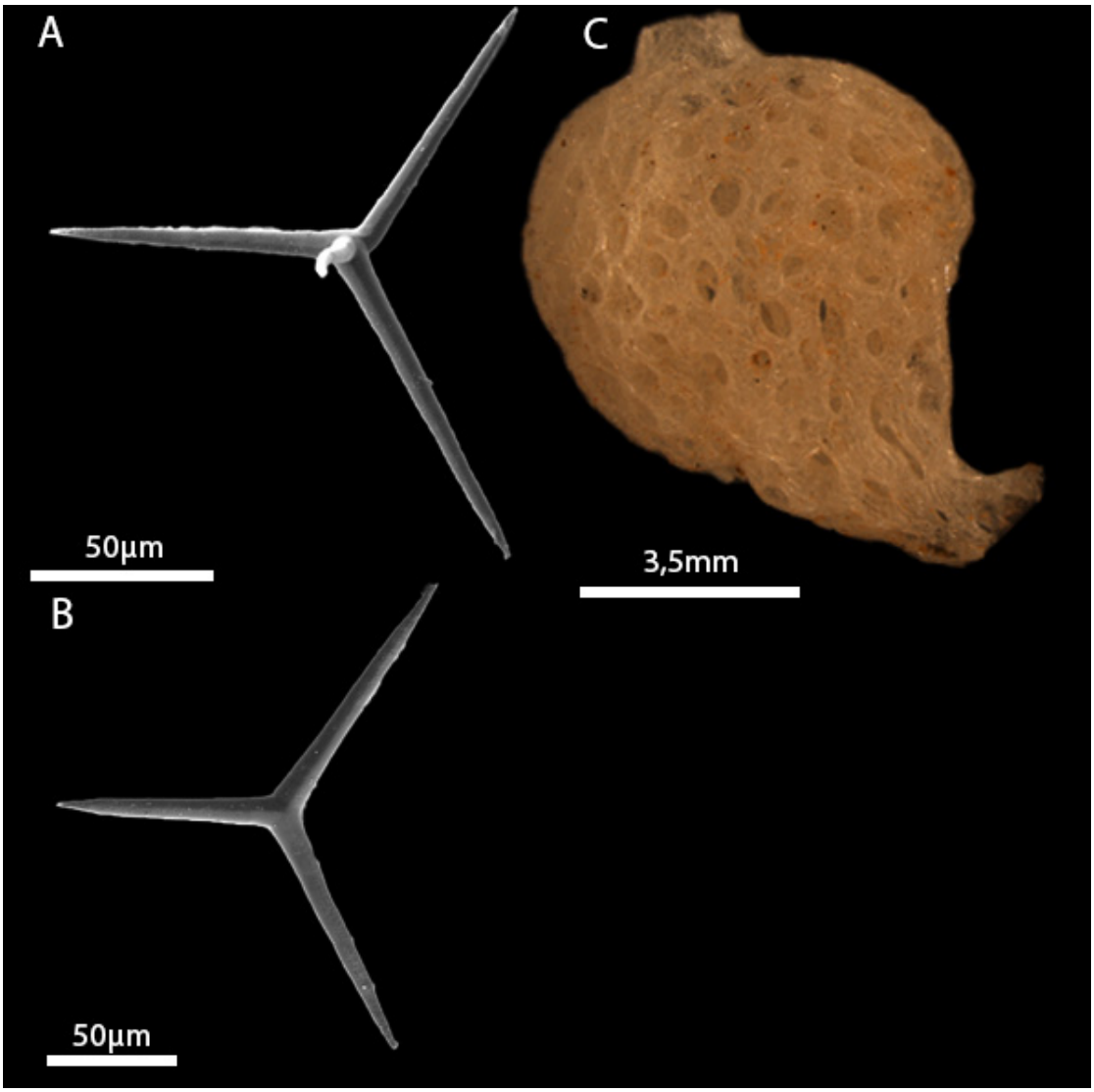

Figure 6. A: tetractine, B: triactine, C: overview of preserved specimen.

CLATHRINA Gray, 1867 emend.

Type species

Clathrina clathrus (Schmidt, 1864). Calcinea in which the cormus comprises anastomosed tubes. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) and/or parasagittal triactines, to which diactines and tripods may be added. Asconoid aquiferous system (Klautau *et al.,* 2013).

3.1.2 *Clathrina pellucida* (Rapp, 2006)

(Table 3, Figure 7)

Original description

Guancha pellucida Rapp, 2006: 357-360.

Synonyms and citations

Guancha pellucida (Rapp, 2006; Schander *et al.*, 2010; Rapp, 2013).

Material examined

3 specimens. Jan Mayen st. 12, Gallionella Gardens, 71°17.98N, 5°46.92W, 2006, 616 m (3).

Outer morphology

The length of the specimen is roughly 1 cm. It consists of highly irregular tubes that form the body. The tubes merge into a single osculum at the distal end, although the osculum is not very prominent. The color is whitish grey and yellow in 96% ethanol. The shape is very irregular and may vary, in this case its shape is an irregular droplet. The texture is very soft and fragile and the surface is smooth. If a stalk is present it is consisting of similar tubes as the body, although slightly compressed. The tubes are very thin, almost close to transparent. The general shape of the stalk is very irregular and can be highly variable in form.

Spicules

All the spicules in the specimen are regular triactines with some variation in size.

Table 3. Spicules of *Clathrina pellucida*

		Length (µm)				Width (µm)	
Spicule type	N	Mean	Min.	Max.	SD	Mean	SD
Cortical Triactines							
Paired	30	115.6	92.0	144.0	±11.2	7.9	±1.1
Unpaired	30	143.3	96.0	180.0	±22.4	8.6	±1.3

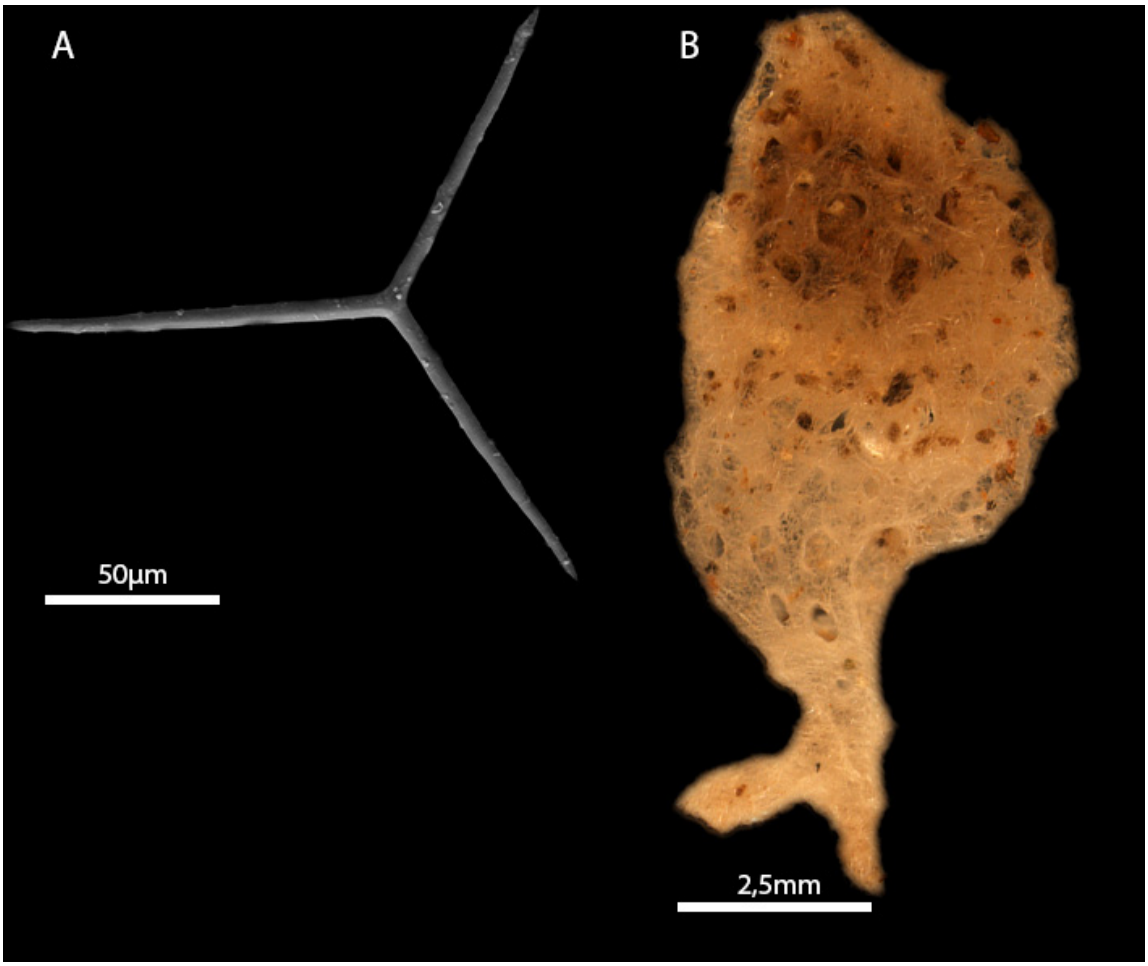

Figure 7. A: triactine, B: overview of *Clathrina pellucida.*

Skeleton

Have asconoid tubes that compose the cormus which has a thin body wall. The tubes are composed of regular triactines.

Remarks

Similar to *Brattegardia* this sponge is very variable in shape. However, a general shape is that of a reverse pear where the body is globular and narrows down closer to the stalk.

Subclass Calcaronea Bidder, 1898

Calcarea with diactines and/or sagittal triactines and tetractines, rarely also with regular spicules. In addition to free spicules there may be a non-spicular basal skeleton in which

basal spicules are cemented together or completely embedded in enveloping calcareous cement. In their ontogeny the first spicules to be produced are diactines in the settled larva. Choanocytes are apinucleate, and the basal system of the flagellum is adjacent to the apical region of the nucleus. Calcaronea incubate amphiblastula larvae.

Order Leucosolenida Hartman, 1958

Calcaronea with a skeleton composed of exclusively free spicules, without calcified non-spicular reinforcements. The aquiferous system is asconoid, syconoid, sylleibid, or leuconoid. In the latter case, radial organization around a central atrium can generally be detected by a well formed atrial skeleton tangential to the atrial wall, and/or a subatrial skeleton consisting of subatrial tri- or tetractines with the paired actines tangential to the atrial wall and the unpaired actine perpendicular to it. The post-larval development passes (presumably always) through an olynthus stage.

Family Sycettidae Dendy, 1892

Leucosolenida with a central atrial tube and perpendicular regularly arranged radial tubes lined with choanoderm. The distal cones of the radial tubes, which may be furnished with tufts of diactines, are clearly noticeable on the sponge surface. They are never covered by a cortex supported by tangential triactines and/or tetractines. The proximal skeleton of the radial tubes is composed of a row of subatrial triactines and/or tetractines which are usually followed by only a few or several rows of triactines and/or tetractines. Pseudosagittal spicules are absent. A tangential layer of triactines and/or tetractines supports the atrial wall.

SYCON Risso, 1826

Type species

Sycon humboldti Risso, 1826 (by subsequent designation; Dendy, 1892). Sycettidae with radial tubes partially or fully coalescent; the distal cones are furnished with tufts of diactines. The inhalant canals are generally well defined between the radial tubes, and are often closed

at the distal end by a membrane that is perforated by an ostium, devoid of a skeleton. There is no continuous cortex covering the distal ends of the radial tubes. Skeleton of the atrium and of the tubes composed of triactines and/or tetractines.

3.1.3 *Sycon* cf. *abyssale* Borojevic & Graat-Kleeton, 1965

(Table 4, Figure 8)

Original description

Sycon abyssale Borojevic & Graat-Kleeton, 1965: 81-85, Figure 1.

Synonyms and citations

Sycon abyssale (Tendal, 1989; Barthel & Tendal, 1993; Janussen *et al.*, 2003; Schander *et al.*, 2010; Rapp & Tendal, 2006; Rapp, 2013).

Material examined

1 specimens. Jan Mayen st. 7, Gallionella Garden, 71°17.99N, 5°46.8W, 2006, 616 m (1).

Outer morphology

Sponge is slim and elongated with a hispid surface consisting of radial chamber tufts that cover the outer surface. These tufts are diactines that decorate the chambers. Coloration is white with light yellow spots after being stored in 96% ethanol. The specimen is very small, roughly 2 mm long. Ends up in a clear stalk and has a single osculum with a small fringe surrounding it.

Have radial tubes that are fully or partially fused. The distal cones of the choanocyte chambers are decorated with tufts of diactines and there is no thin cortex covering the distal ends of the choanocyte chambers. The aquiferous system is syconoid.

Spicules

The spicules are consisting of triactines, tetractines and diactines that decorate the distal ends of the choanocyte chambers.

Table 4. Spicules of *Sycon* cf. *abyssale*

		Length (µm)				Width (µm)	
Spicule type	N	Mean	Min.	Max.	SD	Mean	SD
Diactines	6	101.2	89.0	120.0	±13.3	5.8	±1.2
Tubar Sagit. Triact.							
Paired	30	64.8	45.0	88.0	±11.6	4.8	±0.9
Unpaired	30	94.1	60.0	128.0	±19.6	4.8	±0.9
Atrial Tetractines							
Paired	12	79.7	67.2	92.8	±38.7	5.8	±2.4
Unpaired	12	171.3	140.8	211.2	±52.2	5.2	±1.8
Apical actine	12	11.6	6.4	22.4	±5.9	7.6	±2.8
Subatrial Triact.							
Paired	7	64.4	50.0	71.0	±7.0	4.7	±0.5
Unpaired	7	86.8	72.0	96.0	±8.0	5.0	±0.6

Skeleton

The choanocyte chambers are very clearly visible as you can see the ends of the chambers decorating the surface on the body.

Remarks

Because there was only one individual of this species and its size was considerably small, proper SEM spicule photographs was not possible to obtain, and the spicule measurements were incomplete.

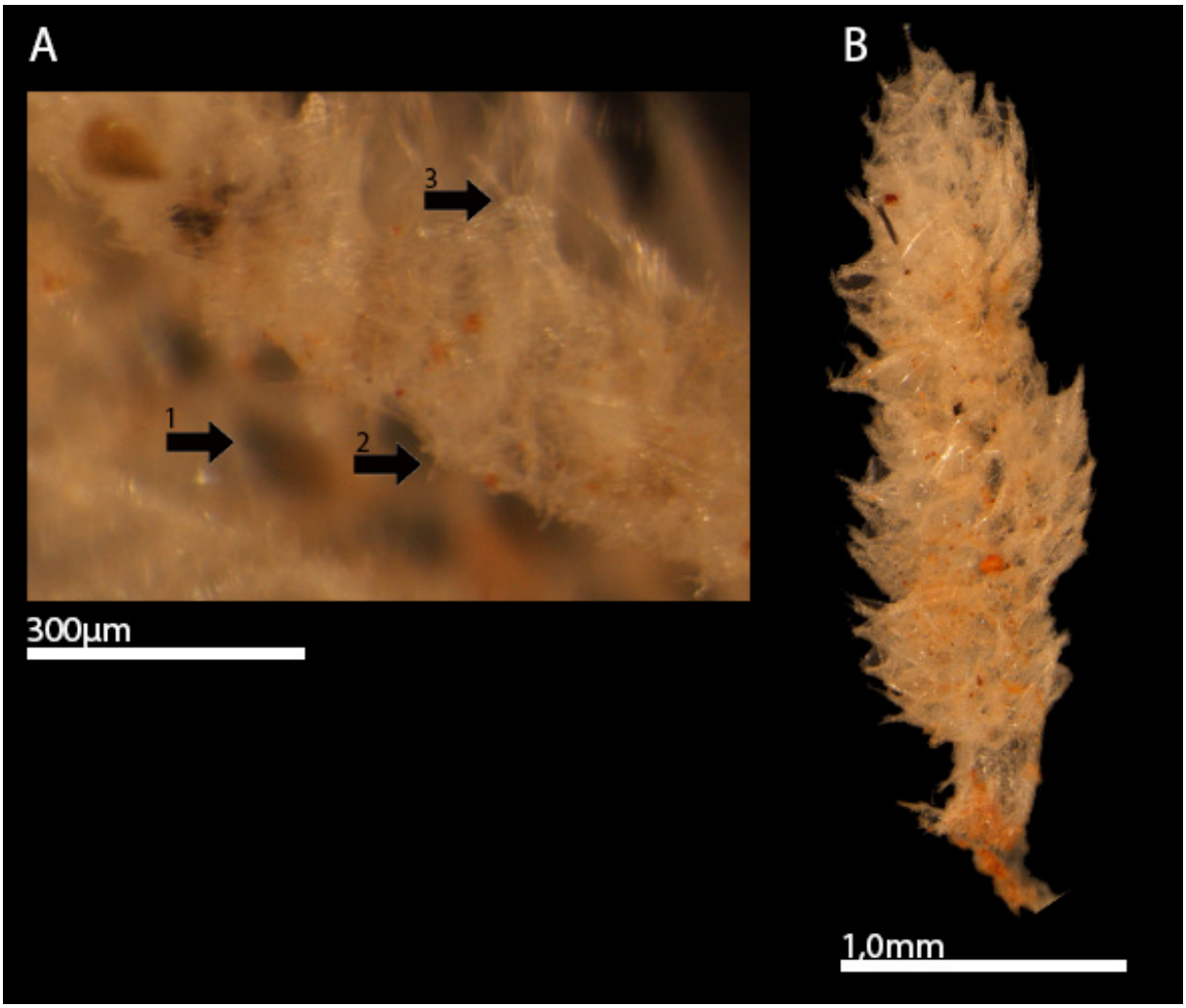

Figure 8. A1: choanocyte chambers, A2: apical actines in the atrium, A3: diactine tufts of the choanocyte chambers, B: overview of *Sycon* cf. *abyssale*.

Family Grantiidae Dendy, 1892

Leucosolenida in which there is always a cortex, supported by a skeleton of tangential spicules that can be diactines, triactines, tetractines or any combination of these. The aquiferous system is either syconoid with radial and elongate choanocyte chambers, or sylleibid or leuconoid with elongate or spherical, scattered choanocyte chambers. The inhalant and exhalant aquiferous systems are always fully developed. The choanoskeleton is always articulate, tubular in syconoid species, and contains a few to several rows of triactines and/or tetractines, or is, in leuconoid species, arranged without apparent order. In the latter case, the choanoskeleton always preserves traces of the radial organization,

particularly at the level of the subatrial triactines and/or tetractines. The atrial skeleton consisting of tangential triactines and/or tetractines is well developed.

GRANTIA Fleming, 1828

Type species

Spongia compressa Fabricius, 1780 (by original description). Grantiidae with a syconoid organization. The cortex is composed of tangential triactines and/or tetractines, occasionally with small perpendicular diactines. Longitudinal diactines, if present, are not found exclusively in the cortex, but cross obliquely, at least through a part of the choanosome and protrude from the external surface.

3.1.4 *Grantia* sp. nov.

(Table 5, Figure 9)

Materials examined

23 specimens. Jan Mayen st. 4, Trollveggen, 71°17.791N, 5°46.465W, 28.07.2012, 501 m (6). St. 14, Trollveggen, 71°17.8631'N, 5°46.2787'W, 01.08.2012, 506 m (17).

Outer morphology

The sponge is small and egg-shaped and ends in a singular apical osculum (Figure 9). It is covered in large diactines around its body which makes it very villose. The apical osculum is surrounded by a fringe of thin and long diactines, which are roughly 0.5 mm long. The fringe is supported by larger, thicker diactines that are roughly 1 mm long. The full size of the sponge varies between 0.2 – 0.8 cm in height and between 0.1 – 0.2 cm in width. The colour is greyish white with dark brown material covering most of its surface diactines. In ethanol the colour is more of a yellowish grey than grey and it has a firm texture. There is a thin, even cortex surrounding the radial chambers which is covered in dense tufts of diactines at the distal end of the radial chambers. The large diactines protruding from the radial chambers are between 0.2 mm – 0.3 mm long.

Skeleton

The radial chambers are six-sided and the incurrent canals between them are close to triangular (Figure 9). There are visible choanocytes attached to the edges of the radial chambers.

The atrium is circular and the wall consists of primarily tetractines which have their apical actines directed towards the atrium opening. The apical actines are long and thin and are between 57.4 – 82 µm long which makes the wall fairly echinated (Figure 9). They are also slightly angled towards the apical osculum. The wall itself is roughly 28.8 µm thick.

The large diactines from the end of the radial chambers protrude through the outer cortex layer, roughly 0.2 – 0.3 mm.

The skeleton of the radial chambers are made up by T-shaped triactines where the paired actines are of fairly similar length. The subatrial region is consisting of triactines which are slimmer and have a more defined angle between the paired actines than the larger choanosomal triactines. Smaller and thinner diactines make out the osculum fringe with the larger diactines working as a support on the exterior of the fringe.

The choanocyte chambers are cylindrical and end in a tuft of large diactines. Tufts are largely covered in a mineral, most likely to be iron oxides. The ends/openings of the incurrent chambers have smaller triactines with one distorted actine which serve as protection of the chamber from invading organisms.

Spicules

The sponge consists of three main types of spicules; Diactines, triactines and tetractines. The triactines are found in different shapes and sizes depending on the area of origin. Not too much variety in tetractines as the majority is found in the atrial wall. The diactines are mostly dominated by the larger types, many specimens were broken or deformed but there was such an abundance of diactines so it was not an issue getting enough measurements. These large diactines are found along the surface partially protruding through the cortex all throughout the body. Irregular triactines are found at the ends of the radial tufts working as a cover for the incurrent canals so unwanted particles are not entering the chambers and

blocking the aquiferous system. Sagittal triactines are found in the subatrial area of the sponge, which varies in size.

Table 5. Spicules of *Grantia* sp. nov.

		Length(µm)				Width(µm)	
Spicule type	N	Mean	Min.	Max.	SD	Mean	SD
Diactines	30	767.7	328.0	1264.8	±270.5	20.7	±38.9
Tubar Triactines							
Paired	30	130.3	57.6	163.2	±28.0	9.8	±1.6
Unpaired	30	139.9	54.4	246.4	±41.3	9.7	±1.4
Atrial Tetractines							
Paired	30	147.2	86.4	188.8	±25.0	11.5	±1.7
Unpaired	30	326.7	108.8	459.2	±90.5	10.1	±1.3
Apical actine	30	46.8	12.8	166.4	±27.6	8.9	±1.8
Cortical Triactines							
Paired	30	134.1	89.6	156.8	±19.3	11.5	±2.1
Unpaired	30	103.6	51.2	137.6	±24.6	10.8	±1.5
Subatrial Triactines							
Paired	30	108.3	57.6	185.6	±24.6	8.4	±1.9
Unpaired	30	213.4	118.4	533.0	±77.1	7.1	±1.6

Remarks

Compared to *Grantia arctica* (Haeckel, 1872), the shape and skeleton seems very similar. There is however a slight difference in the apical actines in the atrial wall. These are much longer than the ones seen in *G. arctica*. The sponge itself is also considerably smaller than the previous reports on *G. arctica*. Another species that resembles *Grantia* sp. nov. is *Grantia mirabilis*. *G. mirabilis* has a similar description to *G.* sp. nov. with a few differences. *G. mirabilis* has a smooth atrial surface in the atrium, while in *G.* sp. nov. it is echinated due to the apical actines of atrial tetractines. They both have clear fringes around the osculum and they also have diactines protruding the from the choanocyte chambers, but *G.* sp. nov. has a longer fringe than *G. mirabilis*. The spicules found in *G.* sp. nov. are not coinciding with

the spicule composition from *G*. *mirabilis*. *G. mirabilis* has a cortical skeleton comprised of various shapes of triactines, *G*. sp. nov. have more irregular ones. *G*. sp. nov. has an egg-shaped body while *G. mirabilis* is more oval and elongated than round.

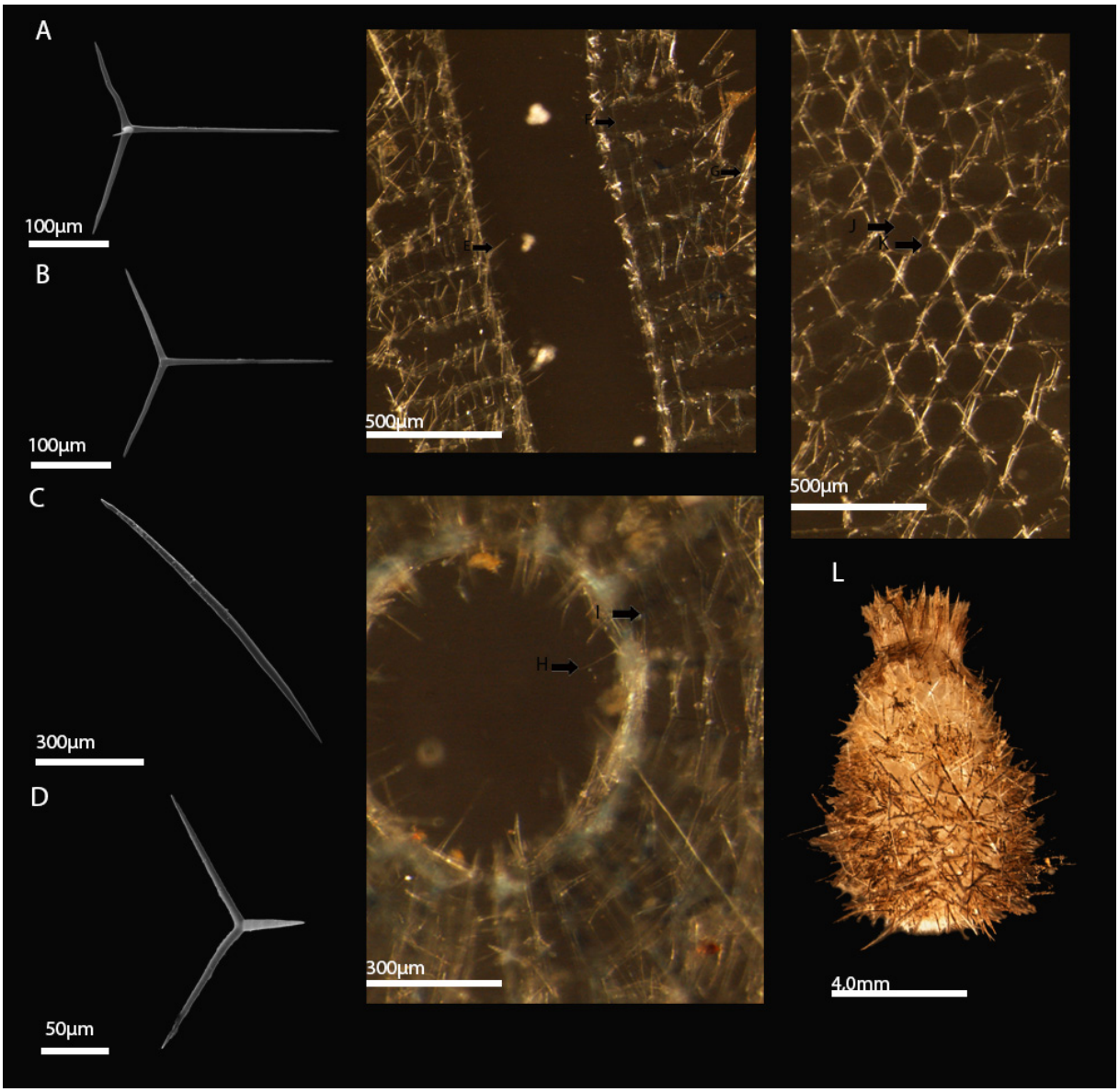

Figure 9. A: tetractine, B: triactine, C: diactine, D: subatrial triactine, E: echinated atrial wall, F: choanocyte chamber, G: diactine tuft, H: long atrial apical actines, I: cortical triactines, J: choanocytes in the six sided chambers, K: triangular incurrent canals, L: overview photo of *Grantia* sp. nov.

3.1.5 *Grantia* cf. *mirabilis* (Fristedt, 1887)

(Table 6, Figure 10)

Original description

Ascandra mirabilis Fristedt, 1887: 104-108, plate 22 figs 3-13, plate 26 figs 1-2.

Synonyms and citations

Ascandra mirabilis (Breitfuss, 1898b).

Grantia mirabilis (Lundbeck, 1909; Brøndsted, 1914; Breitfuss, 1933; Burton, 1934).

Materials examined

5 specimens. Jan Mayen st. 4, Trollveggen, 71°17.791N, 5°46.465W, 28.07.2012, 501 m (2). St. 14, Trollveggen, 71°17.8631'N, 5°46.2787'W, 01.08.2012, 506 m (3).

Outer morphology

The body is elongated with a very prominent fringe of large free diactines. The body itself is covered with large diactines which are irregularly angled. Each of the radial chambers has a tuft of diactines in the apical end. The base gets thinner the further down you get as well as the radial chambers will decrease in length.

The coloration is transparent/greyish with orange like particles attached to the free diactines protruding through the outer surface.

Spicules

The different spicule types found are long, thick diactines as well as long thin diactines, the latter being the one surrounding the fringe. There are choanosomal triactines and also a variety of "microscleres" (diactines, triactines and tetractines). The thick diactines are roughly 1.8 mm long and 22 µm thick while the thin diactines are 1.4 mm long and 2 µm thick.

Table 6. Spicules of *Grantia* cf. *mirabilis*

		Length (µm)				Width (µm)	
Spicule type	N	Mean	Min.	Max.	SD	Mean	SD
Diactines	30	264.4	124.8	483.8	±96.7	7.1	±3.0
Tubar Triact.							
Paired	30	127.7	80.0	156.8	±19.4	10.1	±1.7
Unpaired	30	164.4	99.2	230.4	±32.6	9.6	±1.2
Atrial Tetractines							
Paired	30	147.2	86.4	188.8	±25.0	11.5	±1.7
Unpaired	30	226.7	98.7	459.2	±90.5	10.1	±1.3
Apical actine	30	46.8	12.8	166.4	±27.6	8.9	±1.8
Cortical Triactines							
Paired	30	134.1	89.6	156.8	±19.3	11.5	±2.1
Unpaired	30	103.6	51.2	137.6	±24.6	10.8	±1.5
Microdiactines	30	53.0	38.4	70.4	±7.3	3.2	±0.4

Remarks

Normally for calcareans you would not distinguish between micro and megascleres although the diactines that I have named as “micro” are between 48 and 54 µm long and 3 µm thick which seem to qualify as a microsclere. And although this seems similar to the first *Grantia* sp. nov., it is longer and slimmer than the previously described species with fairly similar body composition. It has some similarities with *G.capillosa* as well in terms of physical appearance; they have similar oval shape and both have the well-developed fringe. But the presence of “microdiactines” makes this more likely to be *G. mirabilis* than *G.capillosa*.

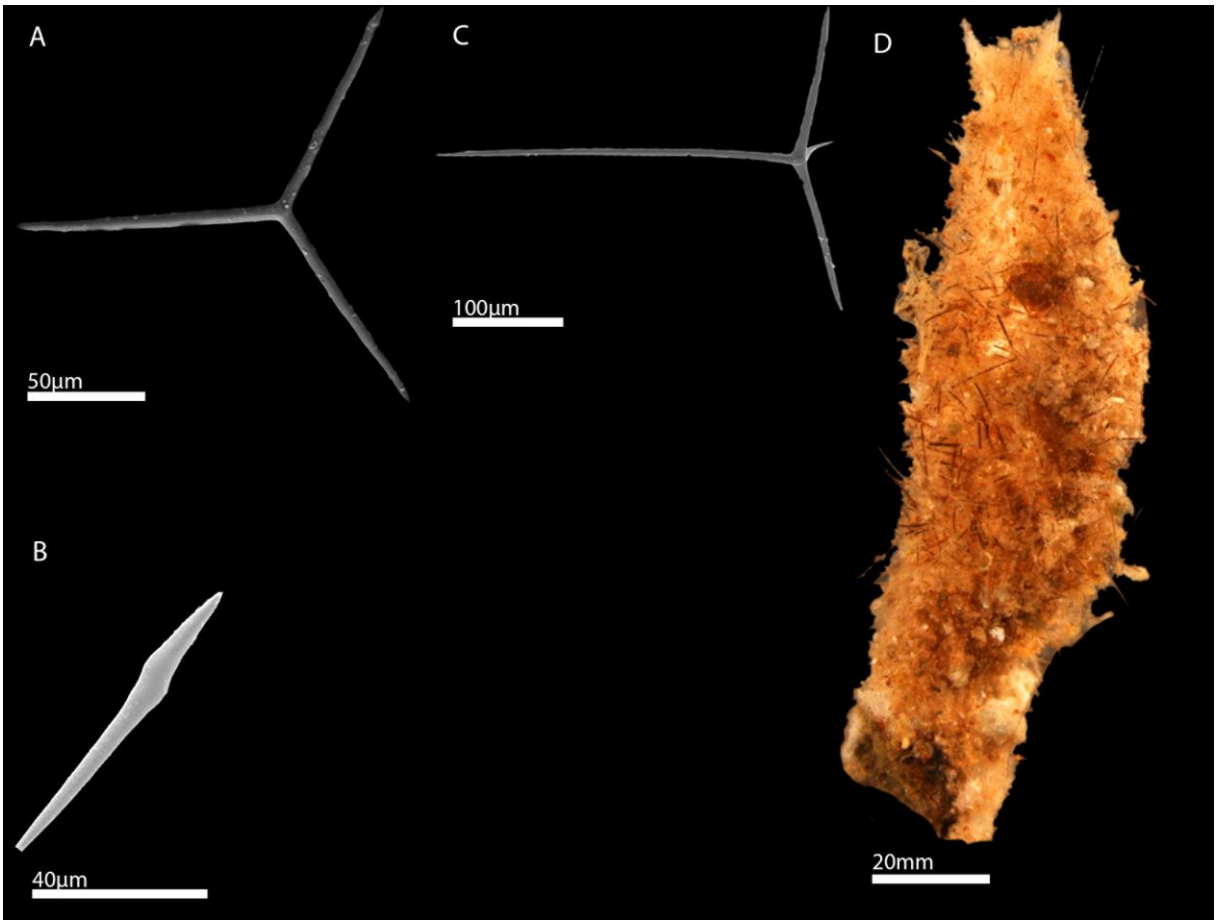

Figure 10. A: triactine, B: microdiactine, C: tetractine, D: overview of *Grantia* cf. *mirabilis*.

3.2 Molecular results

All PCR reactions were successful and gave bright bands on the electrophoresis gel (Figure 11). The ABI files all showed good peaks for both specimens of *Grantia* sp. nov. (Figure 12) and *Grantia* cf. *mirabilis,* which made them good for blasting using FinchTV. The blast results showed that specimens from the JMVF belong to the Calcarea, with blast hits representing calcareous species from different ecosystems. In the phylogenetic analysis *Sycon ciliatum* and *Grantia arctica* were included, and the calcinean sponge *Clathrina luteoculcitella* was used as outgroup. The phylogenetic tree (Figure 14) supports that the *Grantia* specimens investigated here belong to two different species, different from the supposedly closest relative, the typical *Grantia arctica*.

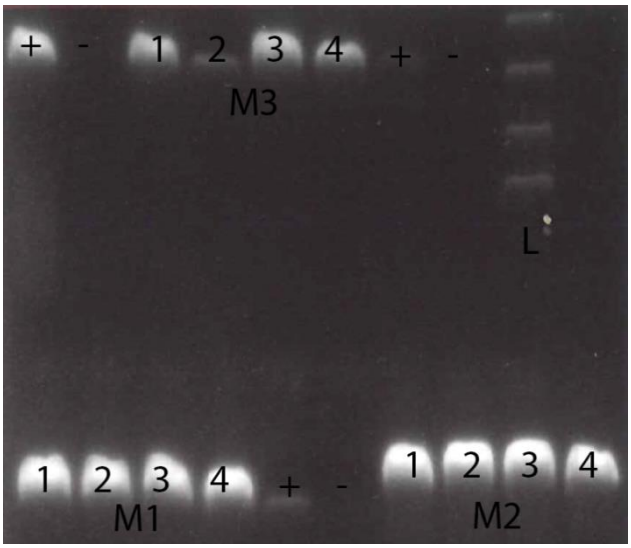

Figure 11. Electrophoresis gel with M1, M2 and M3 indicating the three primersets that were used and 1,2,3,4 indicating the four samples that were used, where 1 and 2 are *Grantia* sp. nov and 3 and 4 are *Grantia* cf. *mirabilis*. The L indicates the ladder that was used.

Section of the trace ABI files:

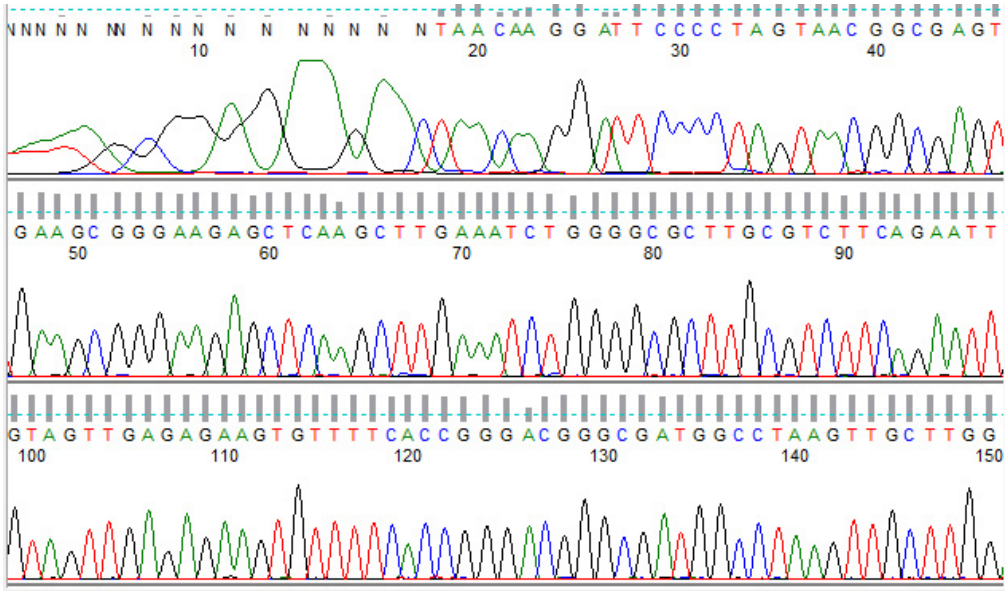

Figure 12. Part of the ABI file which shows good peaks from *Grantia* sp. nov.

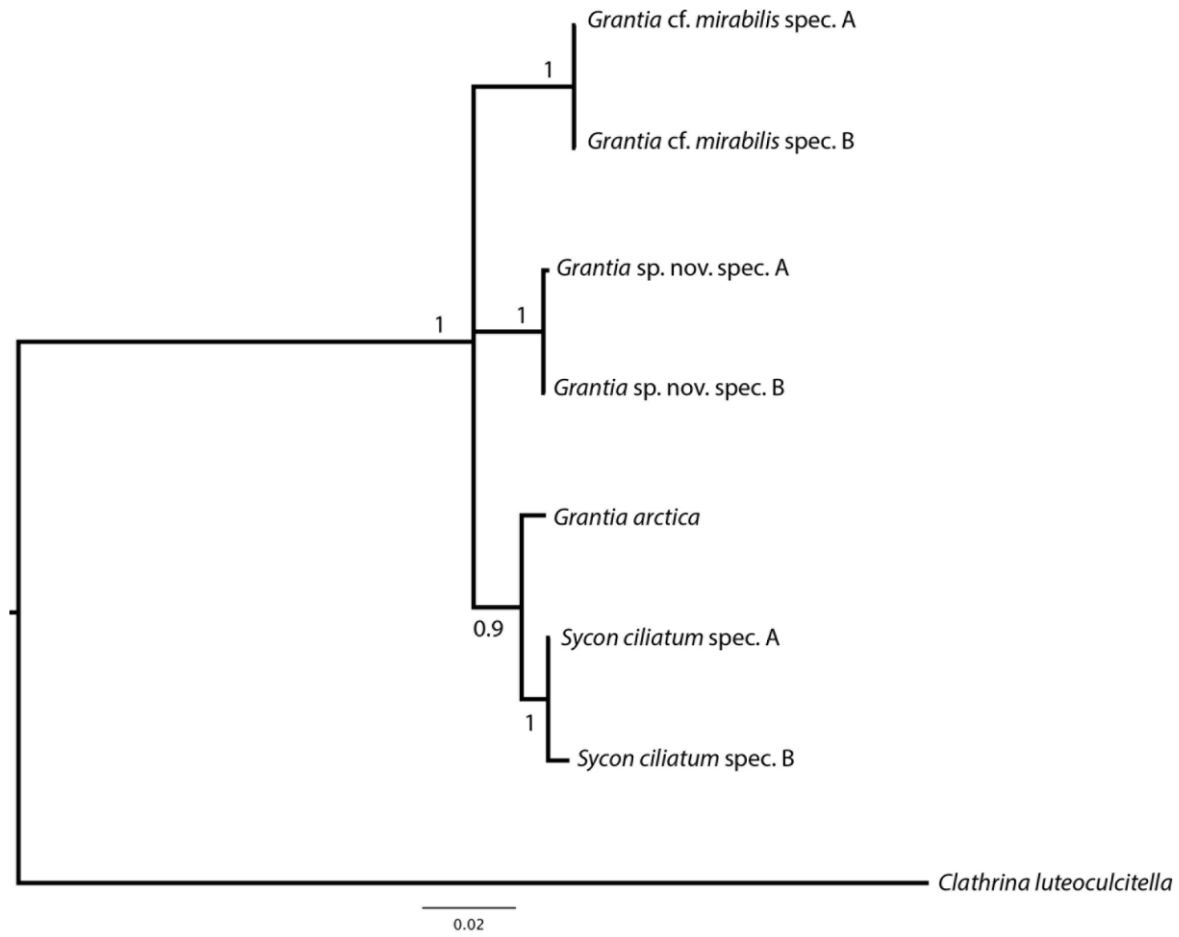

Figure 13. Consensus tree from phylogenetic analysis in MrBayes (see Methods for settings) of 28S rRNA. Branch labels represent posterior probabilities.

4 Discussion

It is established that there is a high diversity of organisms living around hydrothermal vents, and that these organisms are adapted to live in such an environment. With the lack of light at such depths organisms must find other ways of getting energy, and what happens at these depths is the reaction of chemosynthesis (Tunnicliffe & Fowler, 1996). The environment at these sites is very extreme considering the physical and chemical properties. In calcareous sponges there is an enzyme which produces calcium carbonate called carbonic anhydrase (CA), which is used in spicule formation (Müller *et al*., 2013). It is not known how this enzyme reacts to the extreme conditions when the optimal deposition figures lie at a pH around 7.5/8 and a temperature at 52^{o}C (Müller *et al*., 2013). But the calcareous sponges seem to have adapted somehow, regardless of the optimal state of formation.

Also one measure is not enough to establish a solid foundation for a good representation for physical properties at these vent systems.

In general sponges are found in several vent systems around the world, spanning geographically from the Arctic (Schander *et al*., 2010), the Mediterranean (Pansini *et al*., 2000) and the Pacific (Vacelet, 2006). The community of calcareous sponges at the Jan Mayen Vent Field is surprisingly rich, but it is mainly comprised of species that are found in the surrounding waters (Rapp, 2006, Schander *et al*., 2010, Rapp, 2013). The only exception is *Grantia* sp. nov., the most abundant calcareous sponge in this vent system. These sponges were found in the enrichment zone some meters away from the active chimneys and a total of 35 individuals were collected during three different cruises spanning from 2006 up to 2012.

The phylogenetic reconstructions based on the partial 28S gene fragment support the interpretations made based on morphology and show that the two species of *Grantia* identified here are closely related to *Grantia arctica*. The reason the *Clathrina* was used as an outgroup is because of the lack of good calcaronean sequences available.

As only a small part of the Jan Mayen vent field has been sampled thoroughly it should be noted that this work most likely underestimates the diversity of calcareous sponges in the area.

5 Concluding remarks

The knowledge about calcareous sponges in deep-sea systems in is very limited, and for hydrothermal vents in deeper waters the Jan Mayen vent field is the only one hosting calcareous sponges. It can be concluded that:

- The calcareous sponge community at the Jan Mayen Vent Field comprises of at least five species.
- Four of the species described here were previously known from the Atlantic/Arctic waters, and are not considered to be endemic for hydrothermal vents.
- One species is considered to be new to science (*Grantia* sp. nov.), and a redescription of the poorly known *Grantia mirabilis* was made.

This thesis will serve as a good background for further studies of diversity and ecology of calcareous sponges on hydrothermal vents. The presence and high abundance of calcareous sponges in an acidic and CO_2-rich vent system rise many interesting questions for future research. The Jan Mayen vents may in that respect serve as a natural laboratory for studying special adaptations to ocean acidification, especially for organisms building calcareous skeletons in such an inhospitable environment.

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Appendix 1 – Extraction protocol

DNeasy Blood & Tissue Kit, Qiagen, Hilden

The samples were put into a new 1.5 mL Eppendorf tube with 180 µL ATL buffer and 20 µL proteinase K and incubated at 56^{o}C overnight.

After incubation the samples were vortexed for 2 x 30 seconds because of the spicule density and then 200µL of AL buffer was added and the sample vortexed again.

To remove the spicules, which may clog the spin column, the mix was transferred to a clean 1.5 mL tube to get a clean as possible sample. The residue at the bottom of the tube was discarded.

200µL of 96% ethanol was added and mixed thoroughly.

The sample was then transferred to a DNeasy spin column which was placed in a 2 mL collection tube and centrifuged at 8000 rpm for 1 minute. The residue at the bottom of the 2 mL tube was discarded.

The DNeasy spin column filter was then transferred to a new 2 mL tube and 500 µL of AW1 buffer was added.

The column was centrifuged at 8000 rpm for 1 minute. The residue at the bottom of the 2 mL tube was discarded.

The DNeasy spin column filter was transferred to a new 2 mL tube and 500 µL of AW2 buffer was added. The column was then centrifuged at 14000 rpm for 3 minutes. The residue at the bottom of the 2 mL tube was discarded.

The DNeasy spin column filter was transferred to a 1.5 mL Eppendorf tube and 200 µL of AE buffer was added.

The column was incubated at room temperature for approximately 5 minutes before it was centrifuged at 8000 rpm for 1 minute.

Finally the filter was removed and the extracted DNA was stored at 4^{o}C.