Carnivorous sponges of the Atlantic and Arctic Oceans

Phylogeny, taxonomy, distribution and microbial associations of the Cladorhizidae (Demospongiae, Poecilosclerida)

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Scientific environment

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Acknowledgements

I have, initially through my master's thesis and now during these four years of my PhD, in all been involved with carnivorous sponges for some six years. Trying to look back and somehow summarizing my experience with this work a certain realization springs to mind:

It took some time before I understood my luck.

My first in-depth exposure to sponges was in undergraduate zoology, and I especially remember watching "The Shape of Life", an American PBS-produced documentary series focusing on the different animal phyla, with an enthusiastic Dr. Cristina Diaz representing sponge science (I am happy that I now have had the opportunity to have her former student, Adriana, as a colleague; it is a small sponge world).

Looking for a master's thesis a sponge-based project was by no means certain, having also considered other projects including mollusks and herring population structure. Doubtlessly, those would have been fascinating subjects, but almost by chance I ended up in Hans Tore's office discussing a, I was told, very special sponge family, the Cladorhizidae. Not really understanding the significance of sponge carnivory at that time (but lured by the promise of joining the R/V "G.O. Sars" in the Arctic), I was soon starting out with my first spicule preps and one thing led to another (the master's thesis left a lot of unanswered questions, and I had the opportunity to rejoin the Marine biodiversity research group at the University of Bergen a couple of years after my MSc).

Being able to spend several years to pursue a PhD degree is a privilege. I am grateful for the opportunity not just to work with a group of organisms that I consider utterly fascinating, but also having a methodically diverse project that includes morphological taxonomy, molecular phylogeny, biogeography and even some microbial ecology. The deep sea is the least investigated habitat on Earth, and perhaps in some ways still retains a bit of the wonder of discovery associated with biological investigation from an earlier time. Part of it can probably be attributed to

advances in unmanned vehicles, allowing high definition *in-situ* records of habitats that were only indirectly imagined using traditional collection gear. Together with the rapid advance of computational and molecular methods and data, I am grateful for being part of marine biological science at this very exciting time.

I am also fortunate having been surrounded during my project by a positive working environment made up of great, supportive, and really smart people at the University of Bergen, Marine biodiversity group: Hans Tore Rapp, my main supervisor, one of the kindest people I know, his office door always open (a cliché, but in this case very literally; my office was right across the hall making for frequent trips); Henrik Glenner, with his excellent analytical skills in terms of taxonomy and phylogenetics; Joana Xavier, who joined the group a little time after the start of my PhD, and whose skill and good spirits quickly made her an indispensable part of the group; my gang of fellow PhD students: Adriana, Alexander, Bernt, Carrie, Christof, Francisca and Mari; other people in the marine biodiversity group: David, Elena, Kenneth, Kjersti, Solveig, and (honorary member) Louise; as well as Håkon Dahle and Steffen Jørgensen at the Centre for Geobiology, whose microbiology background made paper V possible.

I am completely indebted to my collaborators from other institutions, without whose generosity and readiness to cooperate this project would have been impossible in its current form. I feel extremely lucky to have had the opportunity to collaborate with Jean Vacelet and Nicole Boury-Esnault who, together with Michelle Kelly, provided indispensable parts of the material for papers I and IV. I was also fortunate to get the opportunity to have as additional co-authors Gabrielle Tompkins-MacDonald, Javier Cristobo, Pilar Ríos, Shirley Pomponi, Carole Borchiellini and Maïa Fourt, who each brought additional vital data and expertise to various parts of this project.

I also want to thank others who have helped by sending specimens, providing access to museum collections, or other assistance: Ole Tendal and Emma Sherlock, my hosts at the Natural History collections in Copenhagen and London respectively; Eric Lazo-Wasem and Klaus Rützler, who agreed to lend specimens from the Yale

Peabody and Smithsonian collections; Rob van Soest, who organized sending specimen subsamples from the Naturalis Biodiversity Center; Ellen Kenchington, Megan Best and Javier Murillo for continually supporting my collaboration with Fisheries and Oceans Canada; Paco Cárdenas, for various assistance; and Dorte Janussen, for access to Senckenberg material.

Thus, looking back at the project at this stage, not only have I been able to work with a high-profile group of sponges that represent a strange and incredible evolutionary modification of the poriferan bauplan; I have had the opportunity to do so at a time where molecular tools and recent discoveries have made possible a new systematic review of the Cladorhizidae. Furthermore, everyone I have been in contact with, without fail, have been accommodating, friendly and ready to provide assistance in helping to make this project a reality. To reiterate: It took some time before I understood my luck.

Finally, I want to thank my parents, Einar and Øyunn, who always cultivated in me an interest for academia both in the humanities and natural sciences; Ms. Judy Jones, my biology teacher during my one year of US high school, who kindled my specific interest in biology; my wife, Kjersti, whose support and love mean the world to me; and my children, Runar and Liv, who make everything worthwhile.

Dubrovnik, Croatia, July 2016

Jon

Abstract

The sponges (phylum Porifera) are defined by the presence of an aquiferous system in which choanoflagellate cells create a current and filter water flowing through the sponge body. The carnivorous sponges represent the only known exception to filter feeding within the phylum, and instead are able to capture prey including small crustaceans and larval plankton, using a combination of an adhesive surface and numerous filaments suitable for entangling prey. Mobile cells are able to slowly cover prey entangled on the surface of the sponge, and prey items are encapsulated and digested in a process that can last several days. The aquiferous system is either strongly reduced or entirely absent in the carnivorous sponges, which typically have an erect pennate, branching or stipitate pedunculate morphology. Carnivory is usually considered an adaptation to deep-sea conditions, where filter feeding is less efficient due to a lower density of suspended particulate matter. An exceptional evolutionary innovation within the phylum, sponge carnivory was not properly known to science until 1995. Interest in carnivorous sponges have been high in recent years, and over 150 species are currently considered valid, up from some 90 known species at the turn of the millennium.

Carnivorous sponges are found within the demosponge order Poecilosclerida, defined by the presence of skeletal chela microsclere spicules. Almost all carnivorous sponges have traditionally been assigned to Cladorhizidae, with a few species assigned to Guitarridae (*Eucheliplum*a) and Esperiopsidae (five *Esperiopsis* spp.). As spicule morphology is the main diagnostic character in sponge systematics, the large diversity of chela forms found within the genera assigned to Cladorhizidae implies the possibility that the family is polyphyletic, and that carnivory has evolved in several independent poecilosclerid lineages. On the other hand, recent molecular studies have shown that spicule morphology is often more plastic and intricate than previously believed. Thus the question of whether carnivorous sponges represent a monophyletic group is currently unanswered.

Recent studies have greatly expanded the number and known distribution of carnivorous sponges, which are now known to be present at a variety of depths worldwide. Still, as deep-sea sponges, records are comparably sparse. The greatest number of records is from the North Atlantic. However, records are scattered, and species descriptions frequently lacking in detail. In other areas of the Atlantic and worldwide, species are typically known only from a few or even one collection event.

As they have an affinity to the deep sea, carnivorous sponges are often reported in the vicinity of vent and seep sites. In one particular instance, chemoautotrophic symbiosis has been reported between the carnivorous sponge *Cladorhiza methanophila* and methanotrophic prokaryotes from the Barbados Accretionary Prism. The extent of this type of symbiosis within the group is unknown, however, and though general sponge microbiome data is increasingly published as NGS studies have become more prevalent, almost no such data is currently published for carnivorous sponges.

Answering a number of current questions connected to carnivorous sponges, the aims of this thesis include (1) elucidating the systematic relationships of the carnivorous sponges using molecular data, (2) presenting a taxonomic inventory of carnivorous sponges focusing on Atlantic species, and (3) conducting a comparative study of the microbial community of several cladorhizid species including *C. methanophila* using mainly 16S rRNA Ion Torrent data.

The work presented here provides a comprehensive phylogenetic analysis containing representatives of almost all carnivorous sponge groups, including species not traditionally included in Cladorhizidae, as well as an outgroup sampling of non-carnivorous relatives. The phylogenetic study is able to show that carnivorous sponges represent a monophyletic group, strengthening the hypothesis that carnivory only has evolved once within the sponges, and assigning all carnivorous sponges to Cladorhizidae. Furthermore, this work shows the position of Cladorhizidae relative to other poecilosclerid families, and is able to reconstruct cladorhizid relationships at the genus and subgenus level in most cases. Using an integrated taxonomical approach,

molecular data and morphological characters are combined to create an updated classification for all known carnivorous sponges.

The thesis adds to, and includes an overview of the known cladorhizid species diversity in different regions of the Atlantic Ocean. It offers a comprehensive overview of the cladorhizid fauna of the boreal North Atlantic and Arctic, including descriptions of 25 species and an overview of their known distributions, and explores the cladorhizid fauna of the abyssal Atlantic and Caribbean and adjacent areas respectively. A summary of known carnivorous sponges for the Atlantic Ocean in general, with a discussion on the relationships of the regional Atlantic faunas, is also presented, as well as observations on the depth preference of different species.

Finally, this thesis also presents a comparative examination of 16S rRNA microbiome and isotope data from several carnivorous sponge species including *Cladorhiza methanophila*. Results show that cladorhizid sponges have rich microbial communities, which partially overlap between species. No further evidence of major chemoautotrophic symbiosis was found in species other than *C. methanophila*, where methanotrophic bacteria were abundant, suggesting that this species is likely an exception within carnivorous sponges in general.

There is currently a high degree of interest in carnivorous sponges. As more morphological, molecular and biogeographic data is published, refinements to both the systematics, taxonomical diversity, function and ecology of this group are expected, further building on the results presented in this thesis and giving a more complete picture of the known diversity, evolutionary history and biogeography of the Cladorhizidae.

List of publications

I. Hestetun JT, Fourt M, Vacelet J, Boury-Esnault N, Rapp HT (2015). Cladorhizidae (Porifera, Demospongiae, Poecilosclerida) of the deep Atlantic collected during Ifremer cruises, with a biogeographic overview of the Atlantic species. Journal of the Marine Biological Association of the United Kingdom 95(7), 1311-1342.

II. Hestetun JT, Pomponi S, Rapp HT. The cladorhizid fauna (Porifera, Poecilosclerida) of the Caribbean and adjacent waters (accepted manuscript). *Zootaxa*.

III. Hestetun JT, Tompkins-MacDonald G, Rapp HT. A review of carnivorous sponges (Porifera: Cladorhizidae) from the boreal North Atlantic and Arctic (submitted manuscript).

IV. Hestetun JT, Vacelet J, Boury-Esnault N, Borchiellini C, Kelly M, Ríos P, Cristobo J, Rapp HT (2016). The systematics of carnivorous sponges. *Molecular Phylogenetics and Evolution* 94, 327-345.

V. Hestetun JT, Dahle H, Jørgensen SL, Olsen BR, Rapp HT. The microbiome and occurrence of methanotrophy in carnivorous sponges (submitted manuscript).

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1 Introduction

1.1 Phylum Porifera: the sponges

The sponges, or Porifera, is one of the major animal phyla and are represented worldwide with over 8,700 described species. The vast majority of sponges are marine, with only some 200 freshwater species (van Soest *et al.*, 2016). Sponges are present in almost all marine benthic habitats around the world, are important organisms in many aquatic systems, can act as ecosystem engineers in habitats such as sponge grounds, and include numerous examples of keystone species in a variety of habitats ranging from shallow tropical seas to polar areas and the deep sea (Maldonado *et al.*, 2016; van Soest *et al.*, 2012). Morphologically, they range from massive species such as the giant barrel sponge *Xestospongia muta* to branching or encrusting forms. Four extant classes, representing the major divisions within the phylum, are currently recognized: Demospongiae, silicate sponges containing the vast majority of species; Calcarea, containing sponges with a calcareous skeleton; Hexactinellida, the glass sponges; and more recently, Homoscleromorpha, which was raised to class rank from Demospongiae chiefly based on molecular evidence (Gazave *et al.*, 2012; Hooper & van Soest, 2002a).

While the sponges comprise a diverse phylum, nearly all sponges share some defining characteristics. The most important functional character that more than any other defines the sponges is the presence of an aquiferous system: a system of pores and canals allowing water to pass through the sponge (Fig. 1). Functionally, this arrangement allows water to pass through any number of inhalant pores into channels named ostia set with chambers lined with flagellated choanocytes, which beat their flagella creating a current through the sponge. By way of mucous collar structures, they are also able to trap food particles from the water, which is expelled through one or several larger openings called oscula (Bergquist, 1978).

Sponges have no true tissues or body symmetry, and lack structures found in the vast majority of animals such as a digestive, nervous or circulatory system. They have a

simple organization consisting of a single cell outer surface layer of pinacocytes called the pinacoderm, while the interior surface is covered by a combination of choanocytes or porocytes. Between these two layers, the bulk of the sponge consists of a gelatinous extracellular matrix called the mesohyl. A combination of skeletal spicules (silicate or calcareous) and collagen fibers creates the structural skeleton of the sponge. Specialized cells move through the mesohyl, including totipotent amoeba-like archaeocytes that are able to change into any of a set of more specialized cells involved in secreting spicules, collagen or other structural elements, or in other functions such as reproduction or defense. The size, types and morphology of the skeletal spicules, in many groups divided into larger megascleres and smaller microscleres are, in addition to the organization of the skeleton, possibly the single most important type of morphological character for sponge systematics and taxonomy (Bergquist, 1978; Hooper & van Soest, 2002a).

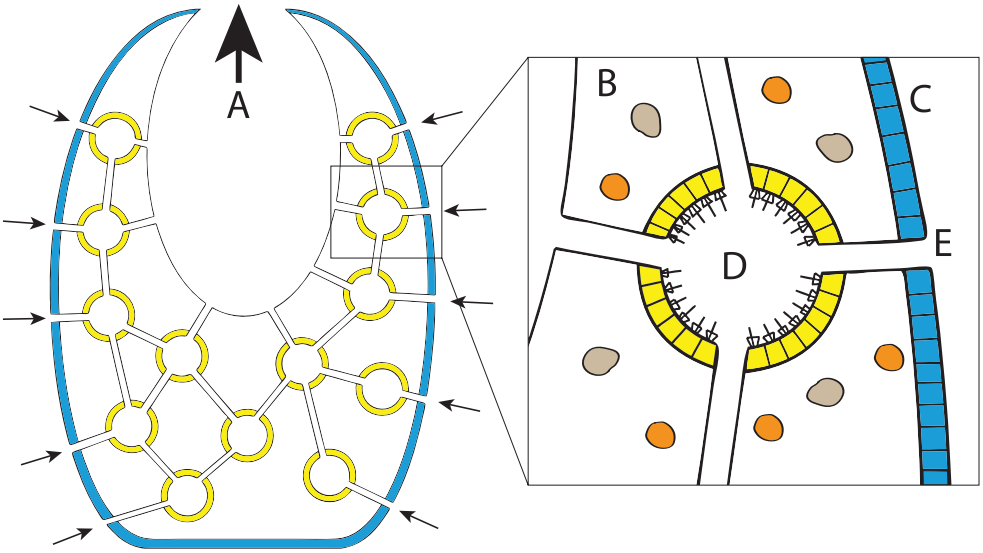

Figure 1. A diagram showing the structure of a filter-feeding sponge with a leuconoid aquiferous system. A) the osculum, an exhalant structure, B) the mesohyl, C) the pinacoderm cell layer, D) a chamber lined with choanocytes, E) an inhalant pore.

Sponges can reproduce either asexually through fragmentation or budding, or sexually, and sponges can be either gonochoristic or hermaphroditic. Choanocytes

create spermatic cysts within the mesohyl, producing sperm, while eggs are usually produced from archaeocytes. Sperm are expelled through the aquiferous system and when in contact with a sponge of the same species, they are absorbed through capture by choanocytes of the new sponge. Eggs are either released in oviparous species, or kept after fertilization in viviparous species, with eggs or larvae being released into the water using the aquiferous system (Bergquist, 1978; Maldonado & Riesgo, 2008).

Most sponges also contain a large number of microorganisms, with the extent, importance and specificity of the sponge microbial component variable among different sponge groups. The microorganism component of a sponge is usually referred to as the sponge microbiome, and often consist of dense and diverse communities of bacteria, archaea, as well as eukaryote single-cell organisms in concentrations several orders of magnitude higher than in surrounding sea water (Hentschel *et al.*, 2012; Taylor *et al.*, 2007).

Symbiotic microorganisms are found in the mesohyl, and the host sponge is able to differentiate between symbiont organisms and food particles, which are also digested here. In many cases, intracellular symbionts are also present (Hentschel et al., 2012). The composition of the sponge microbiome has been compared to that of the gut microbiome of higher animals, filling a vital role in the metabolism and general function of the sponge (Hentschel *et al.*, 2002; Hentschel *et al.*, 2012; Hoffmann *et al.*, 2009). Photoautotrophic symbionts are found in some sponges, where they can constitute a large percentage of total sponge biomass. Investigating the diversity of the microbiomes of different sponges is a work that has been greatly facilitated by recent advances in next-generation sequencing (NGS) platforms, with several recent studies greatly expanding current knowledge (e.g. Kennedy *et al.*, 2014; Thomas *et al.*, 2016).

1.1.1 Systematics and evolution

Sponges branch off very early from the rest of the animals and have traditionally been considered the most basal clade within Metazoa (e.g. Bergquist, 1978). Evidence of early sponges is mainly in the form of fossils, traces of sponge-specific biomarkers or

through the use of molecular clock models. The fossil record clearly shows sponges as present during the Cambrian (535 Mya), but much of the Precambrian fossil record is problematic (see Antcliffe *et al.*, 2014 for a review). However, a recent study has published a more well-preserved fossil specimen some 60 My into the Precambrian (Yin *et al.*, 2015). Biomarker compounds represent an alternative approach but have its own problems (dos Reis *et al.*, 2015). Still, though the exact date is uncertain, most authors place the origin of the sponges well into the Precambrian, with most molecular clock models supporting this hypothesis (dos Reis *et al.*, 2015).

In the last 15-20 years molecular analyses have challenged two assumptions regarding sponge origin: (1) that phylum Porifera, i.e. the sponges, is monophyletic, and (2) that it represents the most basal branching event within Metazoa. Several studies (e.g. Borchiellini *et al.*, 2001; Sperling *et al.*, 2007) have recovered phylogenies where Porifera is paraphyletic, with combinations of the sponge classes Demospongiae, Homoscleromorpha, Calcarea and Hexactinellida as separate branching events, a finding which if true strongly suggest a sponge-like ancestor for all extant metazoans. However, other studies have recovered the sponges as a monophyletic clade supporting the traditional interpretation (e.g. Philippe *et al.*, 2009; Wörheide *et al.*, 2012). Secondarily, based on several molecular studies, phylum Ctenophora (comb jellies) has been suggested as the most basal branching event within Metazoa, predating the sponges (Dunn *et al.*, 2008; Halanych, 2015; Hejnol *et al.*, 2009; Whelan *et al.*, 2015), though others disagree (Nosenko *et al.*, 2013; Pisani *et al.*, 2015). If true, this would have major consequences for the interpretation of the evolution (and reduction) of early metazoan traits. Both these cases illustrate the difficulty of basal metazoan phylogeny, and neither question is settled; though in the case of sponge monophyly the weight of the evidence currently seems to favor the conservative interpretation (i.e. the sponges represent a monophyletic clade) (Fig. 2).

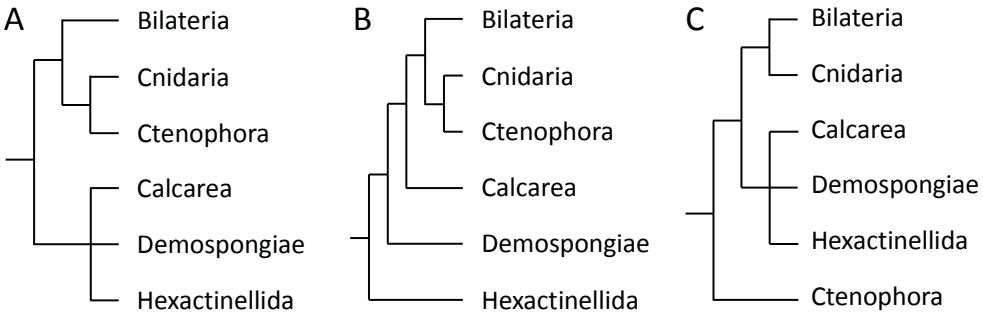

Figure 2. Hypotheses regarding early metazoan radiation. A) The traditional view, B) a variant of a sponge paraphyly hypothesis, C) Ctenophora as the earliest branching event hypothesis.

While the precise arrangement of early metazoan radiation is a contentious issue, it is clear that ancestral sponges still are part of the very early radiation within the Metazoa, which make extant sponges invaluable in comparative evolutionary and developmental (Evo-Devo) investigations and studies of gene expression (e.g. Adamska *et al.*, 2011; Dunn *et al.*, 2015; Fortunato *et al.*, 2012; Lanna, 2015). Comparative genomic analyses of basal metazoans such as the sponges and more derived phyla allow for the reconstruction of early animal evolution, the appearance of structural traits at the genomic level, and the rise of the metazoan "genetic toolkit".

Sponge systematics has a well-earned reputation for being difficult, with ambiguous and often obscure diagnostic characters owing to the frequently amorphous and plastic morphology of different sponge groups. This was greatly facilitated by the 2002 publication of the two-volume reference work *Systema Porifera* (Hooper & van Soest, 2002a), whose numerous editors compiled a complete systematics of the phylum as understood at that time. The work undertaken in this publication is carried on through the World Porifera Database (van Soest *et al.*, 2016) which aims to provide a readily available continually updated record of sponge systematics in the light of an ever increasing number of revisions based on the addition of molecular data to that of traditional, morphological characters.

Demospongiae Sollas, 1885, currently containing over 7,000 species, is by far the largest of the four extant sponge classes with over 80% of described species,

dwarfing Calcarea Bowerbank, 1862 (~5%); Hexactinellida Schmidt, 1870 (~10%) and Homoscleromorpha Bergquist, 1978 (Maldonado & Riesgo, 2008). The systematics of this class, as previously codified by the *Systema Porifera* (Hooper & van Soest, 2002a) was recently subject to an extensive revision by Morrow and Cárdenas (2015) based on accumulated molecular data (e.g. Cárdenas *et al.*, 2012; Morrow *et al.*, 2012; Morrow *et al.*, 2013; Redmond *et al.*, 2013), and divided into three subclasses containing 22 orders. The main part of the demosponges is found within subclass Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault, 2012, which in turn contains major orders such as Haplosclerida Topsent, 1928; Tetractinellida Marshall, 1876; Tethyida Morrow & Cárdenas, 2015 and Poecilosclerida Topsent, 1928.

1.2 Carnivorous sponges

The aquiferous system forms the central basis for sponge function and nutrient acquisition in all sponges with only one known exception: the carnivorous sponges. Found within the Demospongiae order Poecilosclerida, this group of sponges has evolved the ability to entangle and capture small prey items such as crustaceans rather than relying on suspended particulate matter for nutrition (e.g. Vacelet & Duport, 2004). The aquiferous system is partially or completely reduced within the carnivorous sponges, and most species are composed of a solid spicule skeleton surrounded by looser surface tissue. Carnivorous sponges generally have an erect, branching, pedunculate or pennate morphology, typically with numerous thin filamentous processes facilitating prey capture. Prey items become entangled on the adhesive surface and filaments of the sponge, and the simple, plastic nature of the sponge organization makes it able to slowly draw the prey closer and envelop it using cells that migrate to the area of contact, creating a temporary digestive cavity. The sponges have been shown to not be particularly selective, and can feed on a variety of planktonic organisms upon contact (Vacelet & Duport, 2004). Among the largest and most notable reported prey, small crustaceans such as copepods and amphipods are common, as their numerous appendages allow easier entanglement by the sponge, but

smaller planktonic organisms such as nauplius larvae have also been reported (e.g. Chu & Reiswig, 2014) (Fig. 3).

Figure 3. Examples of diversity within carnivorous sponges. From top left: *Cladorhiza corticocancellata* Lundbeck, 1905; *Lycopodina lycopodium* (Levinsen, 1887); *Abyssocladia dominalba* (Vacelet, 2006); partially digested copepods; *C. gelida* Lundbeck, 1905; *Asbestopluma (Asbestopluma) furcata* Lundbeck, 1905; *Chondrocladia (Chondrocladia) grandis* (Verrill, 1879) (x2).

An early suspicion of carnivory was described by G.O. Sars who in 1872, when describing the first known cladorhizid, *Cladorhiza abyssicola*, wrote: "By means of these innumerable microscopic 'claws' which project everywhere from the surface of the sponge, all the more minute animals and the light floating particles which come into immediate contact with the sponge, become attached to it, and thus probably fulfil an essential condition for its nourishment" (Sars, 1872). Ironically, as general knowledge of the filter-feeding mode of nutrition in other sponges increased with subsequent investigations, later authors discounted the lack of pores or other signs of an aquiferous system (e.g. Lundbeck, 1905; Ridley & Dendy, 1887). Thus, evidence of carnivory in sponges was not firmly established until 1995, when Vacelet and Boury-Esnault published a detailed description of the process of prey capture by

Asbestopluma (Asbestopluma) hypogea (Vacelet & Boury-Esnault, 1996) from a submarine Mediterranean cave (Vacelet & Boury-Esnault, 1995). This initial publication established carnivory as a probable ubiquitous mode of nutrition within other cladorhizid genera (confirmed by e.g. Kübler & Barthel, 1999; Watling, 2007) as well as suspected carnivory within the (at the time) guitarrid genus *Eucheliplum*a (Vacelet, 2007) and certain species of *Esperiopsis* (Ereskovsky & Willenz, 2007).

The unusual feeding strategy of the carnivorous sponges make them comparatively charismatic animals compared to their filter-feeding relatives, and at times various cladorhizids have attracted some attention from the mainstream media including stories in among others the *Sunday Courier* (1996) "Killer sponges on the prowl"[1], *National Geographic* (weird and wild section) (2014) "New killer sponges found in the deep sea"[2], *Wired* (2014) "Absurd creature of the week: World's most beautiful sponge dismantles its victims cell by cell"[3], as well as more muted articles in among others the *Washington Post* (2014) "Biologists discover four new species of carnivorous sponge"[4] and (surprisingly) the *Daily Mail* (2012) "Extraordinary harp-shaped carnivorous sponge discovered living on the Pacific Ocean floor"[5].

The last two decades have also seen a large increase in scientific articles describing new cladorhizids (including, but not limited to Cristobo *et al.*, 2015; Cristobo *et al.*, 2005; Downey & Janussen, 2015; Ereskovsky & Willenz, 2007; Hestetun *et al.*, 2016; Ise & Vacelet, 2010; Kelly & Vacelet, 2011; Lee *et al.*, 2012; Lehnert *et al.*, 2005; Lopes *et al.*, 2011; Lopes & Hajdu, 2014; Lundsten *et al.*, 2014; Reiswig & Lee, 2007; Ríos *et al.*, 2011; Vacelet, 2006; Vacelet, 2008; Vacelet *et al.*, 2009; van

[1]https://news.google.com/newspapers?nid=896&dat=19950126&id=y6RSAAAAIBAJ&sjid=wH0DAAAAIBAJ&pg=6414,3544381&hl=en

[2]http://voices.nationalgeographic.com/2014/04/18/sponges-animals-carnivores-science-weird-new-species/

[3]http://www.wired.com/2014/06/absurd-creature-of-the-week-harp-sponge/

[4]https://www.washingtonpost.com/national/health-science/biologists-discover-four-new-species-of-carnivorous-sponge/2014/04/28/be99c6a2-cbd3-11e3-a75e-463587891b57_story.html

[5]http://www.dailymail.co.uk/sciencetech/article-2228640/Extraordinary-harp-shaped-carnivorous-sponge-discovered-living-Pacific-Ocean-floor.html

Soest & Baker, 2011). While approximately 90 species were known at the end of the 20th century (Vacelet, 2007), over 150 cladorhizids are currently considered valid in the World Porifera Database (van Soest *et al.*, 2016). Much of this increase is due to more marine survey activity in previously little known areas around the world as well as a greater focus on the deep sea and the increased attention given to the group due to its unusual feeding mode. Doubtlessly, the number of new cladorhizid species will continue to grow.

1.2.1 Systematics and history

Carnivorous sponges belong to Poecilosclerida (Porifera; Demospongiae; Heteroscleromorpha), a large order with 20 currently recognized families (van Soest *et al.*, 2016). Originally defined as an order by Topsent (1928), its scope extended to 25 families, 129 genera and 50 subgenera with several thousand species in 2002 when the *Systema Porifera* was published (Hooper & van Soest, 2002b). Consistent with the vital importance of skeletal spicules in sponge taxonomy and systematics, the main diagnostic character and a synapomorphy of Poecilosclerida is the presence of skeletal chela spicules, a type of microsclere (small type of skeletal spicule) derived from the more widespread sigma type spicule (Fig. 4). In *Systema Porifera*, a number of taxa lacking chelae were also assigned to the order based on the presence of other characters shared with members of Poecilosclerida following the interpretation that the lack of chelae represented basal forms or secondary loss (Hooper & van Soest, 2002b). However, molecular data has shown that most of these groups do not form part of the Poecilosclerida (Erpenbeck *et al.*, 2007; Redmond *et al.*, 2013), and the order is now somewhat reduced in scope to a still significant "core" group of chela-bearing taxa that has consistently been recovered as a monophyletic assemblage in molecular analyses (Morrow & Cárdenas, 2015). Megascleres (larger skeletal spicules making up the bulk of the structural skeleton of the sponge) are typically monactinal or diactinal, that is needle-like in shape, with one or both ends either sharpened into a point, rounded, or with a slight swelling. See Table 1 for an overview of spicule terminology used in this thesis[6].

[6] Terminology varies slightly between authors and certain terms represent stages in a morphological continuity. Some notes here. 1) The term sigmancistra implies one or both of two distinct morphological modifications: a clearly flattened inner margin, and contortion; however the precise degree of both can vary between different species, see for instance Fig. 2M vs. 2O. The definition of what constitutes a sigmancistra can be difficult in some cases as borderline morphology is common, leading to some inconsistency in usage of the term. 2) Some chelae, especially in *Abyssocladia* and *Asbestopluma*, have a morphology that is on the border between palmate and arcuate. Here the definitions from Hajdu et al. (1994) are used; however, some chelae have been described as palmate/arcuate based on the ambiguity of their morphology in this thesis. 3) The terms style, mycalostyle, subtylostyle and tylostyle describe different stages in a continuum: the term style is here reserved for clearly non-fusiform monactinal megascleres; mycalostyles for fusiform styles;

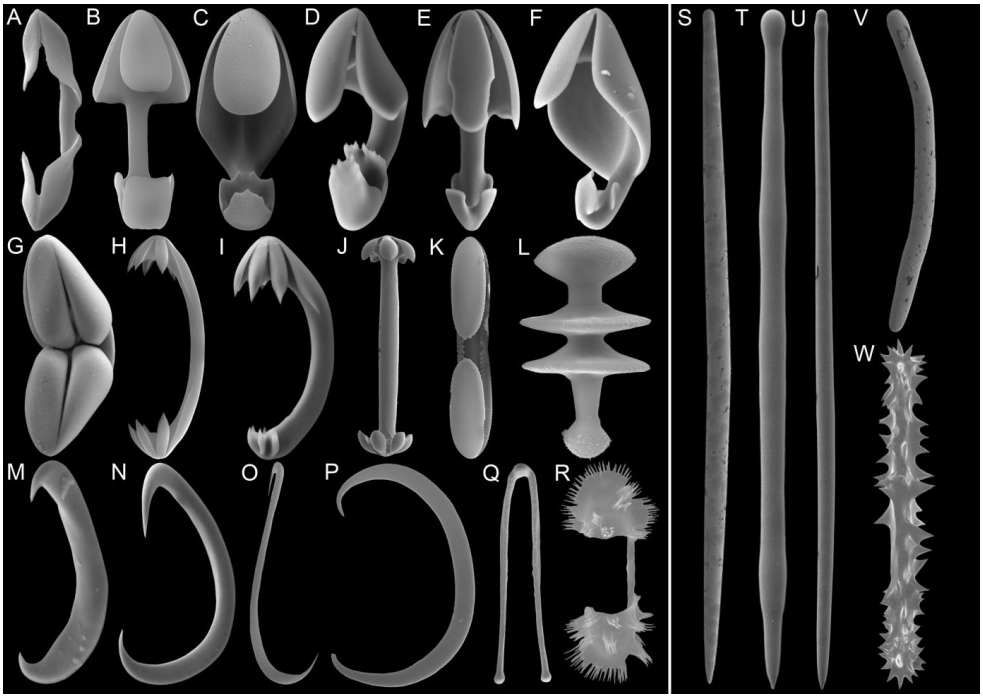

Figure 4. Scanning electron microcopy images showing skeletal spicules of Poecilosclerida with a special emphasis on carnivorous sponges and close relatives. From top left, microscleres: A) *Amphilectus fucorum* (non-carnivorous), palmate chela; B) *Mycale (Mycale) lingua* (non-carnivorous), palmate anisochela; C) *Lycopodina cupressiformis*, palmate anisochela; D) *Lycopodina infundibulum*, arcuate anisochela, E) *Asbestopluma (Asbestopluma) pennatula*, palmate/arcuate anisochela and F) palmate anisochela; G) *Abyssocladia dominalba*, abyssochela; H) *Chondrocladia (Chondrocladia) grandis*, anchorate isochela; I) *Cladorhiza abyssicola*, anchorate anisochela; J) *Cladorhiza mirabilis*,

subtylostyles for styles with faint, slightly offset tyles; and tylostyles for styles with clear, terminal tyles. Obviously there is some ambiguity in the morphology in many cases: A certain category of mycalostyle may feature slight tyles in the thinnest spicules, creating the category mycalostyle/subtylostyle, in other cases the distinction of subtylostyle and tylostyle is difficult to make. 4) Following Lopes et al. (2011) the usage of the term abyssochela is reserved for cleistochelae with a height to width ratio close to one, however, other authors commonly use the term for both cleistochelae and abyssochelae. 5) While the usage of ala/alae is commonly used for all chela appendages, following e.g. Vacelet (2007) the term is used here mostly to refer to lateral appendages partially fused to the shaft, while the term tooth/teeth is used for free/central appendages.

anchorate birotula; K) Eucheliplumа n. sp., placochelae; L) *Ch. (Meliiderma) rodgersi*, trochirhabd; M) *Ab. boletiphora*, sigmancistra; N) *Ab. hemiradiata*, sigma; O) Ch. (C.) grandis, sigmancistra; P) *Ch. (C.) verticillata*, sigma; Q) *L. cupressiformis*, forceps spicule; R) *Guitarra* n. sp., (non-carnivorous) spiny palmate isochela. Megascleres: S) *Ab. hemiradiata*, mycalostyle, T) tylostyle; U) *As. (A.) pennatula*, subtylostyle; V) *Ab. boletiphora*, substrongyle; W) Cl. kenchingtonae, acanthoxea.

Table 1. Glossary of spicule terminology used in this thesis. Descriptions and definitions are taken from the Thesaurus of Sponge Morphology (Boury-Esnault & Rützler, 1997); secondarily from Topsent (1909) (1), Hajdu *et al.* (1994) (2), Vacelet *et al.* (2009) (3), Lopes *et al.* (2011) (4) and Ríos *et al.* (2011) (5).

Megascleres:	
Style	Monaxon spicule with one end pointed, the other (head or base) blunt.
Mycalostyle (2) Fig. 2S	A style with a characteristic fusiform shape where the spicule increases slightly in width from both ends toward the middle, and with a faintly constricted neck. Note: Despite the assertion in Hajdu *et al.* (1994) that the term is a phylogenetic rather than descriptive, it is commonly used for the characteristic style shape in Cladorhizidae and related families.
Subtylostyle Fig. 2U	Tylostyle with one end pointed, the other with a slight swelling or knob; the swelling, more or less distinct, may be displaced along the shaft.
Tylostyle Fig. 2T	A style with a tyle (globular swelling) at the base.
Oxea	Monaxon (diactinal) spicule pointed at both ends. Different types are distinguished by shape and tip morphology.
Strongyle Fig. 2V	An isodiametric, diactinal megasclere with rounded ends.
Acantho- Fig. 2W	Prefix meaning spined, as in acanthostyle or acanthomicrorhabd.
Microscleres	
Chela Fig. 2A-K, R	A microsclere with a curved shaft and recurved alae (wing shapes or derivatives) at each end. Note: The frontal ala(e) or alae only basally connected to the spicule (e.g. arcuate, anchorate chelae) are termed “teeth” by some authors, including here.
Anisochela Fig. 2B-F, I	A chela with unequal ends.
Isochela Fig. 2A, G, J,	A chela with equal ends.

K, R	
Anchorate chela Fig. 2H-I	An isochela with three or more free alae (at each end) in the form of recurved processes shaped like anchor claws (unguiferous) or anchor blades (spatuliferous); with two incipient lateral alae fused with the shaft over their entire length and a gently curved, not abruptly arched shaft. An anchorate chela with three teeth is called a tridentate chela.
Placochela Fig. 2K	A special type of chela with plate-like alae. The alae and the shaft are internally ornamented by radial ridges.
Palmate chela Fig. 2A-C, E-F	An iso- or anisochela in which the lateral alae coalesce with the shaft over their entire length, and the single, median, anterior ala (one at each end) stands free and widens distally.
Arcuate chela Fig. 9-1	An isochela with three free alae and the shaft characteristically curved outward, often bow-shaped.
Cleistochela Fig. 9-3	A chela with the ends (anterior alae) very close to each other.
Abyssochela Fig. 2G, 9-7	Stout cleistochelae where the height x width ration is close to 1 (4).
Cercichela Fig. 17-8	Chela derived microscleres in the form of a laterally flattened, narrow, elongate, oval ring with the two sides slightly unequal in thickness (5).
Birotula Fig. 2J	A type of microsclere with a straight shaft and umbrella-shaped ends.
Sigma Fig. 2M-P	A microsclere of C or S shape.
Sigmancistra Fig. 2M, O	A sigma spicule often contorted about 90 degrees, where the inner margin is flattened, and often with notches close to both ends (1).
Trochirhabd Fig. 2L	Spicule made of a straight, slightly conical rhabd ending in a large, hemispherical bulge at the apex and in a smaller bulge at the thinner end. With one to three thick middle rings (annuli). Hemispherical upper surface of the two bulges covered with short, irregular spines or small buttons (3).
Subtrochirhabd Fig. 11-6	A spear-shaped rudimentary, less developed analogue to a trochirhabd. Originally called meliiderm spicule by Ridley and Dendy (1886) (3).
Forceps spicule Fig. 2Q	A U-shaped microsclere.

Chela morphology, usually varying between palmate, arcuate, anchorate as well as more derived forms, is one of the main diagnostic characters of poecilosclerids both at the family and genus level. However, it has proved difficult to find morphological support for inter-family relationships within the order given the apparent plasticity of chela shape, and a previous chela-based suborder level classification of the Poecilosclerida into Latrunculina, Microcionina, Mycalina and Myxillina (Hajdu *et al.*, 1994; Hooper & van Soest, 2002b; Kelly & Samaai, 2002) has had to be abandoned in the light of molecular evidence (Hajdu *et al.*, 2013; Morrow & Cárdenas, 2015).

Of the 20 recognized poecilosclerid families, almost all carnivorous sponges have long been placed in family Cladorhizidae Dendy, 1922, containing only carnivorous species, with a small number of species assigned to the otherwise non-carnivorous families Guitarridae Dendy, 1924 (all *Eucheliplumа* Topsent, 1909 spp.) and Esperiopsidae Hentschel, 1923 (four *Esperiopsis* Carter, 1882 spp.). In addition to Guitarridae and Esperiopsidae, the non-carnivorous family Mycalidae Lundbeck, 1905 has also been considered a related family based on similarities in spicule morphology (Hajdu & Vacelet, 2002). Within Cladorhizidae species were organized within seven genera (*Abyssocladia* Lévi, 1964; *Asbestopluma* Topsent, 1901; *Cercicladia* Ríos, Kelly & Vacelet, 2011; *Chondrocladia* Thomson, 1873; *Cladorhiza* Sars, 1872; *Lollipocladia* Vacelet, 2008 and *Neocladia* Koltun, 1970) and five subgenera (*Asbestopluma*: *Asbestopluma* Topsent, 1901 and *Helophloeina* Topsent, 1929; *Chondrocladia*: *Chondrocladia* Vacelet, Kelly & Schlacher-Hoenlinger, 2009, *Meliiderma* Ridley & Dendy, 1887 and *Symmetrocladia* Lee et al., 2012).

Cladorhizid genera are generally quite well characterized with diagnostic characters based on chela morphology: The genera *Cladorhiza* (anchorate anisochelae), *Chondrocladia* (anchorate isochelae) and *Asbestopluma* (palmate anisochelae) are all species-rich with a large NE Atlantic representation, and were described in the late 19th and early 20th centuries. *Cladorhiza* and *Chondrocladia* were described as independent genera from their inception (with type species *Cladorhiza abyssicola*

Sars 1872, *Chondrocladia virgata* Thomson, 1873), though some *Chondrocladia* species were described as *Cladorhiza* (Fristedt, 1887; Verrill, 1879). Descriptions of species currently assigned to *Asbestopluma* were originally either assigned to *Cladorhiza* (e.g. Fristedt, 1887; Schmidt, 1875) based on habit similarity, or to *Esperia* Nardo, 1833, later *Esperella* Vosmaer, 1887 (as a replacement for *Esperia*, which was a preoccupied name) (e.g. Carter, 1874; Carter, 1876; Hansen, 1885; Lambe, 1900; Levinsen, 1887), a genus currently synonymized with *Mycale (Aegogropila)*, based on the presence of palmate chelae rather than anchorate unguiferate chelae as found in *Cladorhiza* and *Chondrocladia*. *Asbestopluma* was finally established in its modern form by Topsent (1901). Other genera erected at this time were *Axoniderma* Ridley & Dendy, 1887 (unaccepted, synonymized with *Cladorhiza*), *Crinorhiza* Schmidt, 1880 (unaccepted, synonymized with *Chondrocladia*), *Cometella* Schmidt, 1870 (unaccepted, synonymized with *Asbestopluma*), *Meliiderma* Ridley & Dendy, 1887 (=*Trochoderma*, Ridley & Dendy, 1886) (now considered a valid subgenus of *Chondrocladia*) and *Helophloeina* Topsent, 1929 (now considered a valid subgenus of *Asbestopluma*). *Asbestopluma* was further subdivided into the subgenera *Asbestopluma sensu stricto*, *Cotylina* and *Lycopodina* by Lundbeck (1905). However, following Hentschel (1914), despite *Lycopodina* being raised to genus rank by de Laubenfels (1936), these subgenera have generally not been considered valid as they relied partly on habit rather than spicule characters (Hajdu & Vacelet, 2002; Vacelet & Boury-Esnault, 1996).

These genera were considered part of various groups reflecting the systematic understanding at that time, such as Desmacidonidae, Desmacidinae, Mycalinae and/or Esperellinae (e.g. Fristedt, 1887; Levinsen, 1887; Lundbeck, 1905; Ridley & Dendy, 1887). Cladorhizidae was first erected as subfamily Cladorhizinae by Dendy (1922), and elevated to family rank by de Laubenfels (1936), who also went on to define the genera *Exaxinata* (containing only *C. oxeata* based on the presence of oxeas rather than styles), and *Raoa* (based on the curious *C. tridentata*), both currently unaccepted as they are considered synonyms of *Cladorhiza*.

In the mid-20th century, genera *Abyssocladia* Lévi, 1964 (abyssochelae, arcuate isochelae) and *Neocladia* Koltun, 1970 (birotula-like chelae, anchorate chelae) were established based on newly discovered cladorhizid sponges from the Pacific rather than the Atlantic. These genera were synonymized with *Phelloderma* and *Chondrocladia* respectively in the *Systema Porifera* (Hajdu & Vacelet, 2002), but were later resurrected by Vacelet (2006, 2008). More recently, the genera *Lollipocladia* Vacelet, 2008 (palmate/arcuate isochelae, strongly arched anchorate chelae) and *Cercicladia* Ríos, Kelly & Vacelet, 2011 (cercichelae) were established based on further investigations in the South Pacific. *Meliiderma* and *Helophloeina*, considered as synonyms of *Chondrocladia* and *Asbestopluma* (Hajdu & Vacelet, 2002), were resurrected as subgenera of these respective genera. Lastly, *Symmetrocladia* was described as a monotypic subgenus of *Chondrocladia* (Lee *et al.*, 2012).

In two cases, carnivorous sponges were placed outside Cladorhizidae: genus *Eucheliplum*a and four *Esperiopsis* species (Vacelet, 2007). The close association between *Eucheliplum*a and *Asbestopluma* was recognized by Topsent when he established the genus (Topsent, 1909), with both placed in Mycalinae at that time. Genus *Eucheliplum*a was later placed in Guitarridae based on the presence of placochelae; isochelae with elaborate lamellar ornamentation, that species in the genus shares with the non-carnivorous genus *Guitarra*. The carnivorous species placed within the otherwise non-carnivorous genus *Esperiopsis* were done so based on the simpler shared character of having palmate isochelae.

Different characters, including a carnivorous habit as well as spicule morphology, have been given different levels of consideration in the assignment of carnivorous sponges at the generic and family level. Cladorhizidae lacks a clear spicule-based synapomorphy, being instead a collection of genera with a carnivorous habit, a character given less consideration in the placement of *Eucheliplum*a and *Esperiopsis* spp. in different genera based on spicule characters rather than habit. The question of which morphological characters to prioritize highlights a central uncertainty regarding carnivory in sponges: Whether this feeding mode has evolved several times

in different poecilosclerid lineages, with Cladorhizidae being polyphyletic (as possibly suggested by the great variety of chela types within this family), or whether there has been radiation of spicule morphology in a single carnivorous lineage (Vacelet, 2007) (Fig. 5).

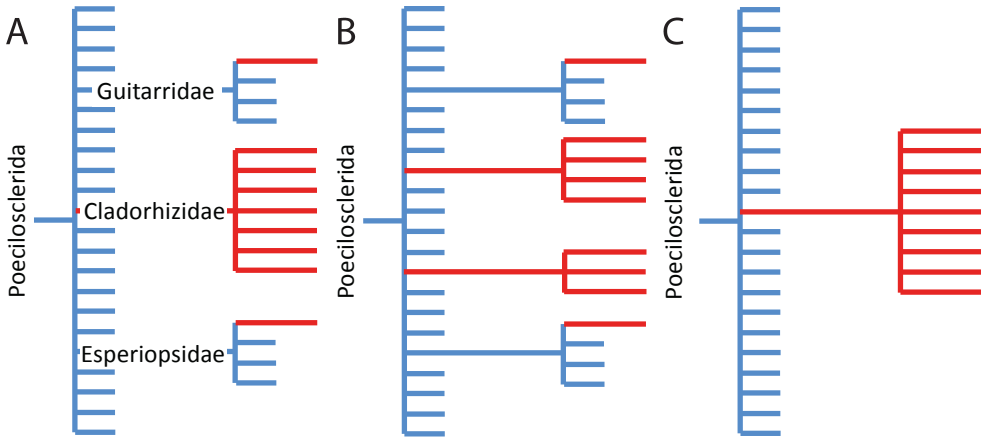

Figure 5. A schematic of systematic relationships within carnivorous sponges. Red branches indicate carnivorous lineages. A) The current systematics previous to the work of this thesis, B) a hypothetical multi-lineage origin of carnivory ("spicule" hypothesis), C) a single-origin hypothesis of carnivory ("habit" hypothesis).

1.2.2 Function

Despite external variation and differences in spicule morphology, all carnivorous sponges share structural and functional characteristics. Carnivorous sponges have different types of erect morphology, including pedunculate, clavate, pennate or branching forms (Fig. 3). Depending on the habitat, they can be connected to the substrate either with a small basal plate (hard bottom), or larger, branching root-like structures (soft bottom). The structural skeleton is composed of needle-like monactinal or diactinal megascleres (Fig. 3, 6): most often mycalostyles or subtylostyles, but sometimes styles, oxeas, or tylostyles.

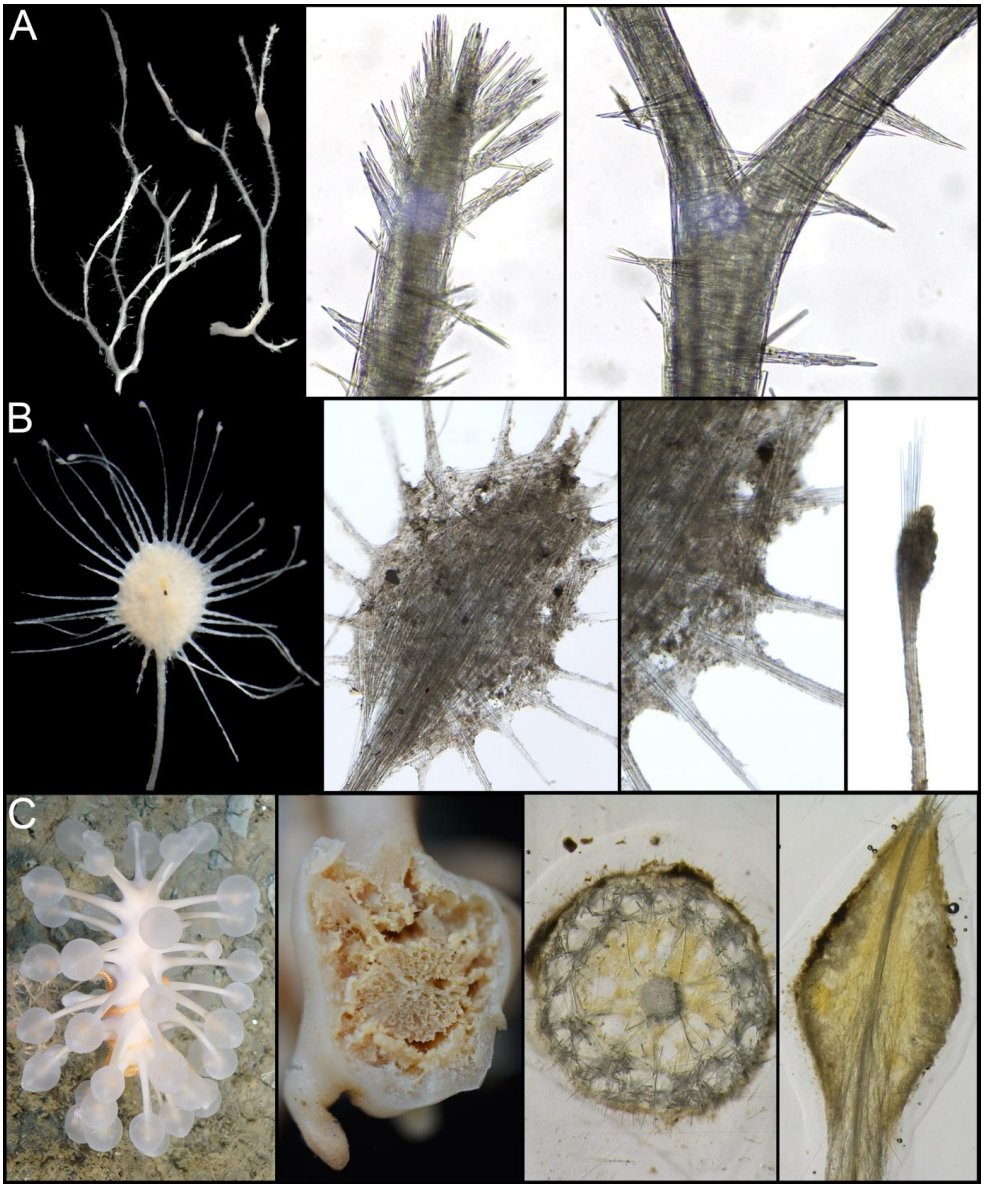

Figure 6. Examples of skeletal organization in carnivorous sponges. A) The branching species *Asbestopluma (A.) ramuscula*, with skeletal detail of a branch end and bifurcating stem; B) a stalked body belonging to *Abyssocladia polycephalus*, with skeletal detail and a partially denuded filament end; C) *Chondrocladia (C.) grandis*, with a cross section of the main stem and side branch, and a longitudinal section through an inflatable swelling.

The stem and any branches are composed of a strong, but flexible central core of tightly packed longitudinally arranged bundles of megascleres points toward the apical part. The central stem is covered by looser tissue with more confusedly arranged megascleres. In *Chondrocladia*, which retains a partial aquiferous system, canals and choanocyte chambers are situated in a middle lacunose layer of the sponge between the central stem and a more solid outer layer (Kübler & Barthel, 1999). The skeleton of the filaments is supported my megascleres in a similar manner as in the stem, and is usually inserted perpendicularly into the main stem skeleton. Pedunculate species have a stem or peduncle supporting the main body of the sponge, which can be spherical or disc-shaped. Here the skeleton is often radial (e.g. Vacelet, 2006). Microscleres, usually a combination of chelae and sigmas or forceps spicules, are found throughout the tissue. In many cases microsclere types are location specific, such as the presence of spiny microtylostyles in the lower stem cover or larger chelae covering the upper stem in many *Asbestopluma* species, or presence of smaller sigmas or sigmancistras in the branch ends of *Cladorhiza* species. At the exterior of the sponge, chelae are usually found with part of their hook-like morphology protruding from the surface, and it is believed that this arrangement facilitates entanglement by providing a "Velcro-like" surface (Lopes *et al.*, 2011; Vacelet & Duport, 2004), which together with a mucous, sticky surface covering (Watling, 2007) are reported mechanisms of prey capture.

A remnant, partial aquiferous system is found within genus *Chondrocladia*, between the central stem and the outer tissue layer (Fig. 6C). While most cladorhizids are fairly small (e.g. a couple of mm to 20 cm tall), many species within *Chondrocladia* are comparatively massive, with a reported length over 50 cm reported in one case (Tendal & Barthel, 1993), and often having thicker stems. The apparent function of this system is not filter-feeding as in non-carnivorous sponges. Rather it is used in order to inflate spherical swellings usually found either terminally or subterminally situated on the branching appendages common in the genus; more solid than the filaments usually found in the other cladorhizid genera. These swellings, which are capable of rapid deflation upon contact, may aid in feeding by trapping prey items (Kübler & Barthel, 1999), but are also important sites of reproduction, as shown for

Chondrocladia (Symmetrocladia) lyra Lee et al., 2012, where careful investigation revealed that terminal swellings were associated with spermatophore production, while mid-branch swellings were sites of oocyte production (Lee *et al.*, 2012).

Excepting *Chondrocladia*, carnivorous sponges completely lack an aquiferous system, and thus they are not able to use this structure for the release of gametes or larvae as in non-carnivorous sponges. While embryos have been recognized in carnivorous sponges by previous authors (Lundbeck, 1905) and are often readily visible through the outer layer of the sponge, only a few studies have investigated reproductive strategies in carnivorous sponges, using *Asbestopluma (A.) occidentalis* (Chu & Reiswig, 2014; Riesgo, 2010; Riesgo *et al.*, 2007) (reassigned to *Lycopodina* following the work in this thesis, see Paper IV). This erect, single-stem species is a viviparous contemporaneous hermaphrodite, and spermatocytes, oocytes and embryos can be present at the same time in a single specimen. In contrast to non-carnivorous sponges, spermatocytes are released in spermatic cysts captured intact by the receiving sponge, allowing synchronous fertilization of multiple oocytes (Riesgo *et al.*, 2007). Surprisingly, release of larvae is, at least in this species, accompanied by disassociation of host tissue, which re-aggregates into asexual propagules, leaving a denuded central stem behind (Chu & Reiswig, 2014). Besides noting the presence of embryos, reproduction in other genera has not been investigated, though arbuscular *Cladorhiza* species often have smaller terminal swellings at their branch ends associated with special sigma spicules suggesting a reproductive function such as gamete production. Reproductive structures seemingly often have special spicules, such as forceps or sigmas (Riesgo *et al.*, 2007), which sometimes can make species identification difficult when these structures are absent.

1.2.3 Biogeography

A prevalent hypothesis regarding the evolution of carnivory is that it is an adaptation to oligotrophic conditions in the deep sea. Carnivorous sponges are generally regarded as deep-sea species, and, together with hexactinellid sponges, increasingly

dominates the sponge fauna at greater depths, where filter feeding is less efficient due to the lower concentration of particulate matter in the water column.

Some cladorhizids have been reported at depths as shallow as >20 m in some habitats (e.g. Chevaldonné *et al.*, 2015; Vacelet, 1996; van Soest & Baker, 2011). Numerous records also exist at depths of 50-300 m, especially in polar areas such as the Barents and Kara Seas or the Southern Ocean (e.g. Fristedt, 1887; Hentschel, 1914; Lambe, 1893; Levinsen, 1887; Lundbeck, 1905). However, cladorhizids become more prevalent at depths >400 m, and are widely distributed on continental shelves worldwide. Furthermore, carnivorous sponges are commonly found in deep sea habitats such as mid-ocean ridges, seamounts and submarine canyons, as well as on abyssal plains and even in hadal trenches. They are frequently found in great numbers in the vicinity of vent and seep systems. Here, chemoautotrophic microorganisms often form the basis of thriving ecosystems, and some carnivorous sponges are able to profit from the increased prey density at these sites (Vacelet, 2007).

While certain species, such as *Cladorhiza gelida* and *Asbestopluma (A.) occidentalis*, have been reported with a eurybathic range (Koltun, 1964; Koltun, 1970a; Lambe, 1893; Lundbeck, 1905), a difference in species composition can generally be discerned for shelf and upper bathyal compared to lower bathyal and abyssal cladorhizid species. As more areas are explored around the world, a comparison of species reported from different areas seems to indicate that shelf faunas appear mostly regionally endemic. Abyssal species, on the other hand, seems to be able to have a much wider distribution (e.g. Paper I). Given the limited dispersal capabilities of sponge larvae, a uniform environment seems to preserve connectivity over greater distances in abyssal carnivorous sponges, though distribution records are often as yet rudimentary.

1.2.4 Carnivorous sponge microbial associations

The importance of the associated microbial community to sponge function and metabolism has been thoroughly established, and sponge microbial diversity is a very active field of research (e.g. Hentschel *et al.*, 2012; Thomas *et al.*, 2016). For

carnivorous sponges specifically, microbial interactions are not well known, but presumably symbiotic microorganism play an important role in the digestion of prey items given the large range of potential metabolic pathways found within prokaryote organisms. Preliminary studies have given some insight into the microbiome of *Asbestopluma (A.) hypogea* Vacelet & Boury-Esnault, 1996 (Dupont *et al.*, 2014; Dupont *et al.*, 2013), though data from other carnivorous sponges are lacking.

Of particular interest is the report of chemoautotrophic symbionts in the carnivorous sponge *Cladorhiza methanophila* Vacelet & Boury-Esnault, 2002 from a mud volcano at the Barbados Accretionary Prism (Vacelet & Boury-Esnault, 2002; Vacelet *et al.*, 1995). Chemoautotrophic symbiosis is well-known in vent and seep fauna such as the giant *Riftia* tube worms, bivalves or crustaceans, but little is known regarding the extent of this kind of metabolism in carnivorous sponges or sponges in general, and *C. methanophila* remains one of only a handful of vent/seep-related chemolithoautotrophic symbioses within the phylum.

1.3 Thesis aims

Since the discovery of carnivory, interest in this group of sponges has been high. Taxonomic articles describing new carnivorous species are published regularly (e.g. Downey & Janussen, 2015; Kelly & Vacelet, 2011; Lopes & Hajdu, 2014; Lundsten *et al.*, 2014), and of the ~150 species known today, some 60 species has been described in the last 20 years. In many cases, spicule characters from new species found in underexplored regions deviate from generic diagnoses, which, originally simple, have as a result grown longer and more qualified. Together with the inconsistent use of a combination of habit and spicule characters in assignment of carnivorous species in Cladorhizidae, Guitarridae and Esperiopsidae, the morphological characters used for carnivorous sponge systematics are not sufficient to resolve the question of whether carnivorous sponges represent one or several lineages: A strict spicule-based approach would imply that Cladorhizidae, containing genera with both anchorate, arcuate and palmate (an)isochela morphology, is polyphyletic; a habit-based approach implies that species assigned to Guitarridae and

Esperiopsidae should be assigned to Cladorhizidae based on the shared carnivorous feeding mode. Thus molecular data, almost non-existent for the cladorhizids at the start of this thesis, is needed to firmly establish the systematic relationships of carnivorous sponges.

The North Atlantic and Arctic are, in terms of deep-sea biology, comparatively well sampled when compared to other marine regions, and a number of species records exist for these areas. However, species descriptions and available data is scattered among many, often old, sources which, while many are of excellent quality, generally lack information considered standard in current publications. No attempt has been made to update species descriptions, bring together available data and provide a comprehensive biogeographical overview of the cladorhizid fauna of these areas. Finally, despite the initial reports of methanotrophic symbiosis within *Cladorhiza methanophila*, the study of microbial interactions within the carnivorous sponges is still in its infancy. Thus, the aims of this PhD project can be summed up in three main objectives:

1. **A taxonomical inventory of cladorhizid sponges in the Atlantic**
   Previous work has provided descriptions of carnivorous sponges from one or a couple of investigations/expeditions. However, there are no larger, regional studies of the cladorhizid fauna from the Atlantic or adjoining Arctic. Information and available distribution information for carnivorous sponges is scattered, and many older species are poorly known as they lack information considered standard in newer species descriptions such as SEM, or even measurements or figures at all. Thus the main taxonomic part of this PhD project has been to provide overviews of the known cladorhizid fauna of the North Atlantic and Arctic, the deep Atlantic, and the Caribbean, and adding to this knowledge using newly collected material and undescribed specimens from existing collections.

   This part of the PhD thesis is based on cladorhizid specimens acquired from a variety of sources such as cruises organized by the Centre for Geobiology at

the University of Bergen, newly collected material from Fisheries and Oceans Canada, the French IFREMER Institute, museum collections at the Copenhagen, London and Bergen natural history museums, the Smithsonian, the Yale Peabody Museum and others. It is addressed in Papers I, II, and III.

2. **The systematics of carnivorous sponges and the origin of carnivory in sponges**

   At the start of this project, almost no molecular data existed with regards to carnivorous sponges, and systematics was based almost solely on morphological characters. Family Cladorhizidae contains genera with a variety of spicule types suggesting the possibility that carnivory had originated several times within the order, and spicule-based systematics by itself is not sufficient to establish the systematics of carnivorous sponges. Using a large, global dataset, a comprehensive phylogeny covering the different genera and morphological diversity of carnivorous sponges is presented in Paper IV, and furthermore connects the results of this phylogeny to morphological characters, revising and updating the previous systematics of this group.

3. **Carnivorous sponge microbial communities**

   Investigations of microbial interactions within the carnivorous sponges are still in their infancy. While some results have been published for microbial interactions within sponges in general, little was known for carnivorous sponges specifically, with the exception of the remarkable finding of methanotrophic, chemoautotrophic symbionts within the seep-associated *Cladorhiza methanophila*. However, recent developments in next generation sequencing (NGS) methods have immensely facilitated investigations of microbial diversity, yielding an incredible increase in genomic data compared to older Sanger sequencing methods.

   In Paper V, we present an overview of the microbiomes of several cladorhizid species using Ion Torrent 16S rRNA amplicon data, in comparison with that of newly collected *C. methanophila* specimens. Together with $\delta^{13}C$ and $\delta^{15}N$

isotope signatures, this data gives further insights into the microbial communities and extent of chemoautotrophic symbiosis within carnivorous sponges.

2 Materials and methods

2.1 List of abbreviations

Geographical names: AMOR, Arctic Mid-Ocean Ridge; GIF Ridge, Greenland-Iceland-Faroe Ridge; GIN Seas, Greenland-Icelandic-Norwegian Seas; MAR, Mid-Atlantic Ridge; NAC, the North Atlantic Current.

Museum collection abbreviations: BMNH, Natural History Museum (UK); CMN, Canadian Museum of Nature; MNHN, Muséum Nationale d'Histoire Naturelle de Paris; MNCN, Museo Nacional de Ciencias Naturales (Spain); NIWA, National Institute of Water and Atmospheric Research (New Zealand); NTNU, NTNU University Museum (Norway); QMG, Queensland Museum (Australia); SMF, Senckenberg Naturmuseum Frankfurt (Germany); USNM, Smithsonian Institution National Museum of Natural History (USA); YPM, Yale Peabody Museum (USA), ZIN RAS, Zoological Institution of Russian Academy of Sciences, Saint-Petersburg (Russia); ZMAPOR, Naturalis Biodiversity Center, Leiden (Netherlands); ZMUC, Natural History Museum of Denmark, Zoological Museum; ZMBN, University Museum of Bergen (Norway).

2.2 Area description

The Atlantic Ocean is the second largest of the world's oceans, with its northern limit bordered by the Arctic and its southern limit bordered by the Southern Ocean, defined by the Polar Front. It contains some 60-70 currently known cladorhizid species. As most of the earliest descriptions of cladorhizids are from the NE Atlantic, cladorhizids from this area were instrumental in creating the diagnoses of the largest of the currently recognized genera within Cladorhizidae (e.g. Carter, 1874; Lundbeck, 1905; Sars, 1872; Thomson, 1873; Topsent, 1901; Topsent, 1909), and the NE Atlantic remains the region where the cladorhizid fauna is best known. For the purposes of delineating distinct cladorhizid faunas, the Atlantic Ocean is roughly divided into five distinct regions here (Fig. 7). These regions should be regarded as

working areas suitable for distinguishing general trends in the currently known cladorhizid fauna rather than more stringently defined systems such as for instance the Marine Ecoregions of the World (MEOWS) (Spalding *et al.*, 2007) or Global Oceans and Deep Seabed (GOODS) (Vierros *et al.*, 2009) classifications:

1. The boreal Atlantic is closely affiliated with adjacent areas in the Arctic. Its southern border is defined here as roughly coinciding with the North Atlantic Current (NAC). This area is comparatively well-explored, and contains most of the first cladorhizid species described by early investigators, though the NW Atlantic including the Eastern USA and Canada has been less explored. It includes the Greenland-Icelandic-Norwegian (GIN) Seas containing the Arctic Mid-Ocean Ridge, the Faroe-Iceland-Greenland (FIG) Ridge, the banks to the west of the British Isles, the North Sea, the Greenland Shelf, New England and Nova Scotian Shelf, as well as adjacent arctic areas such as the Davis Strait, Baffin Bay, and the shallow Barents, Kara, Laptev and Chukchi Seas. This region is defined by large shelf areas, with some differentiation between NE and NW Atlantic and Arctic species.
2. The Southeastern North Atlantic and Mediterranean. This area contains several islands and archipelagoes such as the Azores, Cape Verde, Madeira and the Canary Islands. The species composition in this area has some overlap with the boreal Atlantic fauna, but most species are different.
3. The Caribbean, Gulf of Mexico and adjacent North Atlantic Ocean. A few species of indigenous cladorhizids are known from this area, mainly from the works of Schmidt (Schmidt, 1870; Schmidt, 1880) based partly on the USS Blake surveys in 1877-1880.
4. Southern Atlantic Ocean. Several indigenous species have recently been described on the SE shelf of South America, off Brazil, Argentina as well as Chile, showing the contours of a regional fauna with some affinities to the SW Indian Ocean as well as Pacific species. The cladorhizid fauna of the African shelf is virtually unknown.
5. Atlantic deep-sea basins. At depths of ~2500-3000 m and lower, the cladorhizid species composition typically features different species than in

shallower areas, though this should be regarded more as a rule of thumb than a strict separation. The lower bathyal and abyssal fauna is not well known, and it is the rule rather than the exception that species are known only from one or a small number of specimens. Even so, some species have been reported from several collection localities separated by great distances, a situation similar to that of the abyssal Pacific. The depth distribution also includes features such as the lower slopes of seamounts and the Mid-Atlantic Ridge.

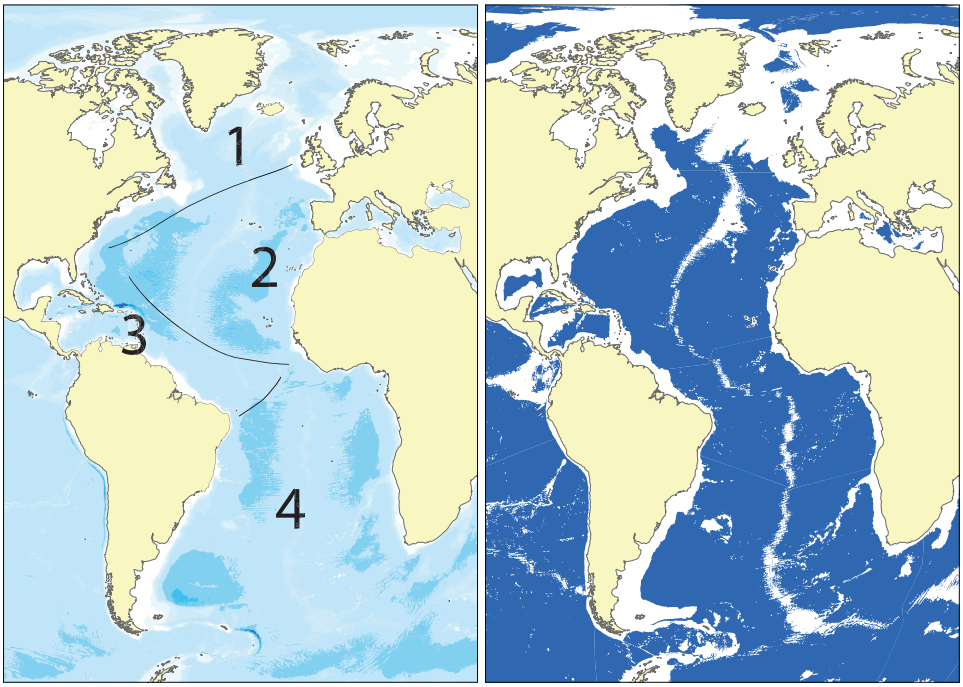

Figure 7. A map showing areas corresponding to the regional cladorhizid faunas of the Atlantic Ocean (left) and lower bathyal and abyssal fauna (>3000 m) (right).

2.3 Specimen collection and overview of samples

The thesis sample material comprises specimens and other data from many different sources. Initially, the main source of cladorhizid specimens was the University of Bergen, Centre for Geobiology cruises to the Arctic Mid-Ocean Ridge (AMOR) from 2006 onwards. As the project progressed, however, this material was complemented

by a wide variety of other sources, which provided essential in providing good coverage of cladorhizid species in the investigations of the cladorhizid fauna in specific parts of the Atlantic Ocean in Papers I, II and III, as well as the phylogenetic analysis in Paper IV (Table 2). Major sample sources for this thesis can be divided into two main sources: Recent research cruises, and samples from museum collections. In both cases, we are greatly indebted to international colleagues, who readily agreed to collaborate on specific projects, and share their specimen material and expertise.

Recent research cruises include the previously mentioned Centre of Geobiology research cruises mapping the Arctic Mid-Ocean Ridge and other areas of interest in the Greenland-Iceland-Norwegian (GIN) Seas such as the Håkon Mosby Mud Volcano. The cladorhizids from these cruises are mainly from the Jan Mayen vent fields and the Schultz Massif Seamount. Additional material from the GIN Seas and Norwegian Shelf was provided by the Norwegian MAREANO cruises, as well as IceAGE 2.

Other major sources of cladorhizid specimens from recent cruises include material collected by the French IFREMER Institute, made available through collaboration with Nicole Boury-Esnault and Jean Vacelet at the Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE) in Marseille; a large number of cladorhizids from the NW Atlantic Ocean and Arctic made available through collaboration with Gabrielle Tompkins-MacDonald and Ellen Kenchington at Fisheries and Oceans Canada (DFO); samples from among others the ATLANTIS and PATAGONIA cruises (Patagonia and Madagascar) made available through collaboration with Javier Cristobo and Pilar Ríos at the Instituto Español de Oceanografía (IEO); newly described and undescribed Pacific cladorhizids for molecular analysis from Michelle Kelly at the New Zealand National Institute of Water and Atmospheric Research (NIWA); and specimens of the methanotrophic cladorhizid *Cladorhiza methanophila* from the 2012 R/V “Atlantis” 21-02 cruise, courtesy of Cindy van Dover at Duke University.

Major contributions from museum collections include the large number of cladorhizids at the Zoological Museum, Natural History Museum of Denmark, from the large number of Danish expeditions mainly to the GIN Seas, from the late 19th to the late 20th centuries, courtesy of Ole Secher Tendal; cladorhizids from the U.S. Smithsonian Institution Museum of Natural History and the Yale Peabody Museum, encompassing both late 19th century material as well as more recently collected cladorhizids from a variety of sources, courtesy of Klaus Rützler and Bill Moser (US National Museum) and Eric Lazo-Wasem and Lourdes Rojas (Yale); the Natural History Museum in London, containing both some of the earliest described cladorhizids from the GIN Seas as well as the species from the HMS "Challenger" expedition, courtesy of Emma Sherlock; more recent samples suitable for molecular analysis from the Naturalis Biodiversity Center in Leiden (Netherlands), courtesy of Rob van Soest and Elly Beglinger; and finally a smaller number of specimens from the Natural history museums in Bergen, Oslo and Trondheim.

Table 2. A list of sources for Atlantic and Arctic cladorhizid specimens examined in the course of this PhD project.

Material	Year	Area	Location	No spec	Paper(s)
NORWAY					
GeoBio cruises	2006-2014	Boreal	Arctic Mid-Ocean Ridge, Norw. shelf	90	Paper II, III, IV, V
Bergen Museum	1880-1980	Boreal, Caribbean, SE North Atlantic	Various	20	Paper III
MAREANO	2013	Boreal	Norwegian EEZ	7	Paper III, IV
IceAGE 2	2013	Boreal	GIF Ridge	7	Paper III, IV
Others	1900-2013	Boreal, Pacific	Various	25	Paper III, IV
Trondheim Museum	1880-1996	Boreal	Norwegian EEZ	10	Paper III, IV
Oslo Museum	1952	Boreal	Norwegian EEZ	4	Paper III

CANADA					
Paamiut	2010-2014	Boreal	Davis Strait, Baffin Bay	54	Paper III
Hudson	2007-2013	Boreal	Scotian Shelf, Flemish Cap	56	Paper III
NEREIDA	2007-2009	Boreal	Flemish Cap	8	Paper III
LAR	2009-2012	Boreal	Davis Strait, Baffin Bay	13	Paper III
NAT. HIST. MUS. COPENHAGEN					
BIOICE	1991-2000	Boreal	GIN Seas/GIF Ridge	35	Paper III
BIOFAR	1987-1990	Boreal	GIN Seas/GIF Ridge	9	Paper III
Ingolf Expedition	1895-1896	Boreal	GIN Seas	43	Paper III
Other	1876-2004	Boreal, SE North Atlantic	Various	77	Paper III
FRANCE					
IFREMER	1981-2003	Abyssal Atlantic	Various	39	Paper I, III
Other	1976-2014	Atlantic, Pacific	Various	6	Paper I, III, IV
NAT. HIST. MUS. LONDON	1873-2011	Boreal, SE North Atlantic, South Atlantic, abyssal Atlantic, Indian Ocean, Pacific	Various	25	Paper III, IV
UNITED STATES					
Smithsonian Institution	1879-1983	Boreal, Caribbean	Scotian Shelf, off Florida, Beata	14	Paper II, III

			Basin		
Yale Peabody Museum	1879-2003	Boreal, Caribbean	Scotian Shelf, Muir Seamount	10	Paper II, III
Harbor Branch Oceanographic Institution	2011	Caribbean	Off Florida	1	Paper II
Duke University	2012	SW North Atlantic	Barbados Accretionary Prism	15	Paper V
SPAIN					
ATLANTIS/ PATAGONIA	2007-2011	SW Atlantic	Patagonian Shelf	20	Paper IV
Other	2011	SW North Atlantic	Gorringe Bank	2	Paper III, IV
NEW ZEALAND					
NIWA	1988-2012	Pacific	Macquarie Ridge, various	14	Paper IV
NETHERLANDS					
Naturalis Biodiversity Center	2000-2007	Boreal	NE Atlantic	10	Paper III, IV
OTHER SOURCES	1997-2013	Atlantic, Southern Ocean, Pacific	Various	37	Paper IV

2.4 Morphological methods

Sponge specimens were photographed submerged in ethanol where possible, with a stand-mounted camera on a black velvet background and detached or ring-mounted flash. Additional pictures of details of interest were obtained with a stereo microscope camera with stacking software. Histology section pictures were obtained with a microscope camera. SEM images were obtained with a Zeiss Supra 55VP Scanning Electron Microscope. Image processing was done using Adobe Photoshop CS5.

Creation of optical microscope and SEM spicule preparations was done through subsampling the specimen in several locations depending on suspected genus and species (as some spicules are typically found at specific locations in the sponge). Subsamples were dissolved using boiling 65% nitric acid, and the spicule suspensions were rinsed several times using ddH_20 and ethanol as described in Boury-Esnault and Rützler (1997). Microscope slides were sealed with Eukitt quick-hardening mounting medium under a suitable cover slide; SEM stubs were prepared by putting a circular glass slide on top of a piece of carbon tape, and then coated after application of spicules.

2.5 Molecular methods

The thesis contains two different types of molecular analysis: Sanger sequencing of cladorhizid gene partitions for phylogenetic analysis, and Ion Torrent prokaryote 16S amplicon sequencing to explore the microbial diversity in specific sponge samples.

In the case of samples used for molecular analysis, DNA extraction was done using either the Qiagen Blood and Tissue kit, or the EZNA Mollusc kit, with an extra step of removing the spicule after tissue lysis, but before adding ethanol (spicules were then often subsequently saved, cleaned and used for spicule preps as detailed above). DNA extracts were kept at 4°C or frozen for longer-term storage. For the NGS 16S amplicon samples, DNA extraction was done in a sterile environment to minimize bacterial contamination issues. For the Sanger analysis, contaminating DNA from microorganisms and prey in the samples remained a consistent issue when sequencing sponge sequence, most severely for the COI gene partition, which was mostly solved by modifying existing primers to more specific variants. Secondary structure issues in the sequenced 28S partition were solved by use of Qiagen "Q-solution" (possibly containing BSA) in PCR samples.

Sequence data was organized and handled mostly using Geneious 6.1.8 (Biomatters). Sanger sequence datasets were analyzed using a combination of Maximum likelihood (with RAxML) and Bayesian (with MrBayes) approaches, and prepared for analysis

using Geneious, gBlocks and Mesquite. For the 16S amplicon dataset, the USEARCH and QIIME pipelines were used to prepare the Ion Torrent fastq output files for further analyses using R (ggplot2, vegan and pheatmap packages, with dependents.

3 Results and discussion

3.1 The cladorhizid fauna of the Atlantic and Arctic Oceans

3.1.1 The boreal Atlantic and Arctic Oceans

The boreal Atlantic and Arctic (Fig. 7-1) covers a large area and there are numerous records of carnivorous sponge species from this region. Large shelf areas in the arctic seas as well as the area bordering the GIN Seas and the coast of Greenland and North America means that there are many records of cladorhizids from shallow to mid-bathyal depths (e.g. 50-2500 m). For this project we were able to examine a number of newly collected cladorhizids from the 2006-2014 Centre of Geobiology cruises, the 2007-2010 "Hudson" cruises, the 2009 NEREIDA cruise, 2009-2015 "Paamiut" cruises, and the 2013 IceAGE 2 and MAREANO cruises. We were also able to examine specimens from several museum collections: The Natural History Museum of Denmark, containing specimens from various early Danish cruises such as the Ryder (1891-1892), Ingolf (1895-1896) and Godthaab (1928) expeditions (including types of specimens from Hansen (1885) and Lundbeck (1905)), as well as substantial material from newer programs such as BIOICE and BIOFAR and other material from various sources; the Natural History Museum in London, containing type material from among other Thomson (1873) and Carter (1874); the Smithsonian Institution National Museum of Natural History and Yale Peabody Museum, containing various cladorhizid material including types and other specimens deposited by Verrill (1879); as well as other sources (Table 2). This allowed us to examine type material for many of the described species from the area. The results from the review investigation into this boreal North Atlantic and Arctic fauna, in all over 400 specimens, are the subject of Paper III.

Genus *Lycopodina* is represented by several common species in the area, including *L. lycopodium* (Levinsen, 1887) and *L. cupressiformis* (Carter, 1874) which share a single-stem erect habit. This genus is challenging from a taxonomic perspective as many species share a very similar spicule complement and general morphology, and

some currently recognized species might actually be several, closely related species (e.g. Koltun, 1970a; van Soest, 2016). Difficulty also arises in the fact that forceps spicules, connected with spermatophores (Riesgo *et al.*, 2007) are sometimes rare or absent. We were able to distinguish a new species, *L. tendali*, from *L. lycopodium*, based on a combination of molecular and morphological results. The status of *L. cupressiformis* is uncertain. While the spicule complement is similar, morphological tendencies suggest that southern specimens from the GIF ridge might be different from more northern specimens from e.g. the AMOR, with *L. robusta* and *L. ruijsi* added as closely related species to *L. cupressiformis* based on habit characters (van Soest, 2016). From the NW Atlantic, we describe *L. novangliae*, which has a morphology intermediate between *L. lycopodium* and *L. tendali*, and *L. cupressiformis*. Other relatives to this group can be found in the North Pacific, with species such as *L. occidentalis* (Lambe, 1893), *L. hadalis* (Lévi, 1964) and *L. gracilis* (Koltun, 1955). An additional species with a small, pedunculate spherical body is *L. hydra* (Lundbeck, 1905) recorded from the GIN Seas. This species is more closely related to species such as the more southern *L. hypogea* (Vacelet & Boury-Esnault, 1996) and the abyssal *L. parvula* (Hestetun *et al.*, 2015) rather than the longer, stem-shaped species such as *L. lycopodium*.

The genus also contains several species with a pedunculate cup-shaped morphology. The most conspicuous of these is *L. infundibulum* (Levinsen, 1887), which is reported mainly from the GIN Seas and Arctic (Levinsen, 1887; Lundbeck, 1905), but also from a few locations in the southern North Atlantic (Boury-Esnault *et al.*, 1994; Hestetun *et al.*, 2015). In addition to *L. infundibulum*, two other closely related species, *L. minuta* (Lambe, 1900) and *L. comata* (Lundbeck, 1905) have also been recorded from the GIN Seas and Davis Strait (Koltun, 1959; Lambe, 1900; Lundbeck, 1905). A fourth species, *L. versatilis* (Topsent, 1890), is known from the area close to the Grand Banks. In this case, as for *L. lycopodium*, molecular evidence helped establish the identity of *L. minuta* as an independent species rather than as a synonym of *L. infundibulum*.

Chondrocladia records from the area are common, and have traditionally been assigned almost exclusively to *C. (C.) gigantea* (Hansen, 1885). In addition to *C. (C.) gigantea*, the species *C. (C.) grandis* (Verrill, 1879) was described based on specimens from the banks of the Nova Scotian Shelf, but as Verrill's descriptions lacked any mention of spicules (Verrill, 1879; Verrill, 1885), no new records have been assigned to that species. Still, previous authors (e.g. Lundbeck, 1905; Topsent, 1930) have noted the similarity between *C. (C.) grandis* and *C. (C.) gigantea*, and speculated whether they could actually represent the same species. By examining most of the original *C. (C.) grandis* material together with *C. (C.) gigantea* type material and additional specimens, together with molecular data, we have been able to show that *C. (C.) gigantea* is a synonym to *C. (C.) grandis* (Paper III). Thus *C. (C.) grandis* should be considered the accepted name of the species. *C. (C.) grandis* is widely distributed in the boreal Atlantic and Arctic, with records from the GIN Seas and shelf areas, the Davis Strait, Baffin Bay, the New England and Nova Scotian shelf and Flemish Cap, with most records between 400-1500 m in depth. It is the only known *Chondrocladia* species in this part of the Atlantic and a close relative of more southern species on each side of the Atlantic including *C. (C.) concrescens* (Schmidt, 1880), *C. (C.) verticillata* Topsent, 1920 and *C. (C.) virgata* Thomson, 1873.

Several *Cladorhiza* species are known from the area. Most species are closely related arbuscular species including *C. abyssicola* Sars, 1872, *C. corticocancellata* Carter, 1876, *C. gelida* Lundbeck, 1905, *C. iniquidentata* Lundbeck, 1905, *C. oxeata* Lundbeck, 1905 and *C. tenuisigma* Lundbeck, 1905. The distribution is a bit different between species with C. abyssicola having a wide geographical distribution also including the Southeast North Atlantic Ocean and Mediterranean, while the others are either known only from the GIN Seas (*C. corticocancellata*, *C. iniquidentata*, *C. tenuisigma*) or have a wider boreal Atlantic and Arctic distribution (*C. gelida*, *C. oxeata*). In addition to these arbuscular species, *C. arctica* Koltun, 1959 has been reported from the slope of the polar basin, and *C. kenchingtonae* from the lower Flemish Cap slope, but these species could more accurately described as lower bathyal and abyssal fauna.

Four *Asbestopluma* species have been described from the boreal Atlantic and Arctic: *A. (A.) bihamatifera* (Carter, 1876), *A. (A.) furcata* Lundbeck, 1905, *A. (A.) pennatula* (Schmidt, 1875) and the newly described *A. (A.) ruetzleri* Hestetun, Tompkins-MacDonald & Rapp, in press. *A. (A.) bihamatifera* was considered a synonym to *A. (A.) pennatula* given its almost identical spicule complement to *A. (A.) pennatula*. However, careful investigation of the large chelae as well as consistent difference in number of spicule rows and differences in molecular sequence (Paper III; Paper IV) shows that it should be resurrected as an independent species. *A. (A.) furcata*, previously known only from the GIN Seas, was shown (Paper III) to also be present in the NW Atlantic. Finally, the new species *A. (A.) ruetzleri*, morphologically similar to *A. (A.) pennatula*, but with clear spicule differences, was discovered upon investigation of specimens from the NW Atlantic and Arctic, where it seems to be the most common *Asbestopluma* species.

The NW Atlantic has been comparatively poorly investigated compared to the NE Atlantic. The cladorhizids examined from this area, from the East coast of the United States to the Davis Strait, yielded two new species, *A. (A.) ruetzleri* and *L. novangliae*, which are close relatives of NE Atlantic species (*A. (A.) pennatula* and *L. lycopodium*). In other cases, examined species from this area turned out to belong to previously described species such as *A. (A.) furcata*, *Ch. (C.) grandis*, *Cl. abyssicola* and *Cl. gelida*, which thus have amphi-boreal Atlantic distributions. The Arctic has, as argued by e.g. Koltun (1970b) a fauna consisting of a subset of adjacent areas. This seems to be true for the cladorhizid fauna: Species reported from the Russian high Arctic Seas (with the exception of *C. arctica*) are species also found in the adjacent Atlantic (e.g. Fristedt, 1887; Gorbunov, 1946; Koltun, 1959; Levinsen, 1887). Thus it would seem that this fauna extends all the way to the Bering Strait, with the Bering Sea and North Pacific home to different species (e.g. Downey & Janussen, 2015; Koltun, 1970a; Lambe, 1893).

Owing to multiple previous investigations in the NE Atlantic and Arctic there is a comparatively good record of cladorhizid species from this region. This is in contrast to the situation in most other areas, where cladorhizid species are usually known from

its collection locality or a couple of records. Including previous collection records in our dataset allowed us to plot individual species giving an outline of their distributions. This showed that distributions of individual species varied significantly, with some species, such as for instance *Cladorhiza oxeata*, having a more northern distribution, while others, such as *C. abyssicola*, are more widespread. However, many species are known almost exclusively from the GIN Seas and adjacent areas, which indicates that this area is still oversampled compared to neighboring areas. While the nature of deep sea research means that this kind of analysis is qualitative and somewhat rudimentary, it is a step up from the almost non-existent distribution information in previous works, where records typically are mentioned in the text only.

In all, 25 cladorhizids are now known from the boreal Atlantic and Arctic, belonging to *Asbestopluma*, *Chondrocladia*, *Cladorhiza* and *Lycopodina*. Of these, we were able to examine specimens from 23 species, and three species were originally described as part of this thesis (Paper III). Our results have also expanded the known distribution of many of the previously known species from the area, and provided an overview of the fauna of the region which is more comprehensive than anything done for cladorhizids in this or other areas.

3.1.2 The southeastern North Atlantic and the Mediterranean

The southeast part of the North Atlantic (Fig. 7-2) has a distinct cladorhizid species composition to that of the boreal Atlantic fauna. Most early records from this area are from Topsent (Topsent, 1904; Topsent, 1909; Topsent, 1929) from the expeditions sponsored by Albert I to the Azores, Tenerife, Cape Verde and other areas, with some exceptions (Arnesen, 1920; Thomson, 1873). Reported cladorhizids indigenous to this area include *Asbestopluma (Helophloeina) stylivarians* Topsent, 1929, *Chondrocladia (C.) virgata* Thomson, 1873, *Cladorhiza flosabyssi* Topsent, 1909, *Cl. grimaldii* Topsent, 1909, *Lycopodina hypogea* (Vacelet, 1996), and the previously non-cladorhizid carnivorous sponge *Eucheliplumа pristina* Topsent, 1909. Some cladorhizids overlap with the boreal fauna reported at a lower depth such as *A. (A.)*

pennatula and *Cl. gelida* (Mid-Atlantic Ridge) (Desbruyères *et al.*, 2001) and *L. infundibulum* (Boury-Esnault *et al.*, 1994). However, most conspicuous is *C. abyssicola*, which has a wide distribution in this area (Boury-Esnault *et al.*, 1994; Topsent, 1909), though recent results show that some records off Mauretania is a new, closely related species (Göcke *et al.*, 2016).

The Mediterranean has a subset of the fauna of the area, with records of *L. hypogea* and *C. abyssicola* only (Aguilar *et al.*, 2011; Babić, 1922; Bakran-Petricioli *et al.*, 2007; Boury-Esnault *et al.*, 1994; Chevaldonné *et al.*, 2015; Vacelet, 1969; Vacelet, 1996).

Chondrocladia (C.) virgata, the type species of *Chondrocladia*, was originally mentioned and pictured by Thomson (1873), and subsequently described by Carter (1874), who misidentified its location as the Faroe Ridge rather than off Gibraltar however. Based on erroneous measurements, Arnesen (1920) later re-described this species as *C. michaelsarsi*. Finally, the species was yet again re-described by Cristobo *et al.* (2015) this time as *C. (C.) robertballardi*. Through a re-examination of the type material from all three species, we were able to establish their identity as the same species using type specimens of *C. (C.) virgata*, *C. (C.) michaelsarsi* and *C. (C.) robertballardi* (Paper III).

3.1.3 The Caribbean Sea and the adjacent Atlantic Ocean

Not many carnivorous sponges are known from the Caribbean Sea and adjacent areas (Fig. 7-3) compared to that of the rest of the North Atlantic, and the currently known fauna consists of five indigenous upper bathyal species (*Asbestopluma (A.) gracilior* (Schmidt, 1870), *Chondrocladia (C.) amphactis* (Schmidt, 1880), *C. (C.) concrescens* (Schmidt, 1880), *C. (C.) verticillata* Topsent, 1920 and *Eucheliplumа congeri* de Laubenfels, 1936), one species also reported from the rest of the North Atlantic, *L. infundibulum* (Paper I), and three species from lower bathyal and abyssal depths (*Abyssocladia polycephalus* Hestetun Pomponi & Rapp, in press, *Asbestopluma (A.) caribica* Hestetun Pomponi & Rapp, in press and *Cladorhiza methanophila* Vacelet & Boury-Esnault, 2002) (Paper II).

The relationship between *C. (C.) concrescens* and *C. (C.) verticillata* is a bit unclear, as these two species were apparently both part of the material described by Schmidt as *C. (C.) concrescens*. However, following the suggestion of (Topsent, 1920), they should in all probability both be considered valid, though very closely related, species. Other close relatives include *C. (C.) grandis* and *C. (C.) virgata*, and an undescribed *Chondrocladia* from Patagonia included in the molecular phylogeny in Paper IV.

Given the poor sampling of this area, the carnivorous sponge fauna of the Caribbean is still quite poorly known, and future investigations will in all probability discover a further number of new cladorhizid species.

3.1.4 The South Atlantic Ocean

The cladorhizid fauna of the South Atlantic is not well known, and the whole sub-equatorial part of the Atlantic is included here as a single region for the purposes of describing the current knowledge of cladorhizids from this area, though in all probability future research will refine this view (Fig. 7-4). A small number of cladorhizids have been previously reported from the abyssal South Atlantic (Cristobo *et al.*, 2005; Ridley & Dendy, 1886; Tendal, 1973; Topsent, 1909). Recent work by Lopes *et al.* (2011) from off the Diego Ramírez Islands (south of Chile), Lopes and Hajdu (2014) and Castello-Branco *et al.* (2016) from off the SW Atlantic off SE Brazil, and Ríos *et al.* (2011) off Patagonia have added in all 16 species to the upper bathyal and shelf of the SW Atlantic Ocean, showing that there is a rich, probably mostly still undiscovered, cladorhizid fauna in the region. Interestingly, this includes the only *Abyssocladia* known from non-abyssal depths in the Atlantic (Lopes & Hajdu, 2014), as well as records of the monotypic genus *Cercicladia* (Ríos *et al.*, 2011). While not yet formally described, several species not belonging to any previously known cladorhizid were collected by the ATLANTIS program off Patagonia and were part of the molecular phylogeny in Paper IV. The eight species from the Diego Ramírez Islands material may strictly speaking be considered SE Pacific or Southern Ocean species, but are included here due to their proximity to the

SW Atlantic as their distribution in all probability includes the Patagonian shelf. The only known shelf cladorhizid from the SE Atlantic Ocean is the new species *Cladorhiza acanthoxea* Hestetun et al., 2015 from off Gabon-Congo (Paper I).

The cladorhizids described in (Lopes *et al.*, 2011) and (Lopes & Hajdu, 2014) show interesting similarities to cladorhizids from the Pacific (Kelly & Vacelet, 2011; Vacelet, 2006) and SW Indian Ocean (Hestetun *et al.*, 2016), with several *Asbestopluma* species having a more anchorate to unguiferate rather than palmate/arcuate morphology, and a larger presence of *Abyssocladia*, showing that there is some affinities in the known southern hemisphere cladorhizid fauna between oceans. Several works on Antarctic cladorhizids have either recently been published or are in prep (Janussen & Tendal, 2007), which will in all probability show more clearly the relationship between the Antarctic fauna to that of the South Atlantic Ocean.

3.1.5 Lower bathyal and abyssal fauna

Most cladorhizid species in the Atlantic have been reported from less than 2500-3000 m depth on shelf areas along the coasts of adjoining continents, but many records also exist of species from lower bathyal and abyssal depths, either on the lower continental slopes, the abyssal plains of the deep-sea Atlantic or on the Mid-Atlantic Ridge (Cristobo *et al.*, 2005; Ridley & Dendy, 1886; Tendal, 1973; Topsent, 1909; Vacelet & Boury-Esnault, 2002). The species reported from these depths are for the most parts different than cladorhizid species from shallower depths. A couple of species, such *as C. (C.) vaceleti* and *L. parvula*, have also been collected from widely different collection localities, suggesting that lower bathyal and abyssal cladorhizids can have a very large distribution, which most likely is related to the uniform conditions of the deep Atlantic basins. In this project, lower bathyal and abyssal species were mainly described in Paper I, with some species also from Paper II and Paper III.

The abyssal *Chondrocladia* fauna is dominated by smaller, stipitate species of the "*Crinorhiza*" form such as *C. (C.) vaceleti*, *C. (C.) guiteli* (Cristobo *et al.*, 2005;

Topsent, 1904), but exceptions exist such as *C. (C.) nicolae*, *C. (C.) albatrossi* and *C. (C.) burtoni* (Cristobo *et al.*, 2005; Tendal, 1973). The methanotrophic species *C. methanophila*, closely related to other, shallower arbuscular *Cladorhiza* species such as *C. abyssicola* has been reported from Barbados Accretionary Prism and the MAR (Vacelet & Boury-Esnault, 2002; Vacelet *et al.*, 1995), and some records of what is probably *C. gelida* are also known from the abyssal North Atlantic (Paper I), with another *Cladorhiza* species, *C. thomsoni*, being reported from the South Atlantic (Paper I) (Topsent, 1909). Other *Cladorhiza* species, *C. flosabyssi*, *C. arctica* and *C. kenchingtonae*, differ from the arbuscular morphology of e.g. *C. abyssicola*, either by having a small, stipitate morphology, or a long, threadlike morphology in the case of *C. kenchingtonae*. Two species of *Asbestopluma* have been reported from the abyssal Atlantic: *A. (A.) caribica*, from the Venezuela basin (Paper II), and *A. (A.) belgicae* (Paper I), otherwise known from shallower depths in the Antarctic (Topsent, 1901).

Several of these species were collected in canyons or at seamounts, such as *Cladorhiza kenchingtonae*. Interestingly, such sites, including the Muir Seamount off Bermuda and the MAR, are also home to three of the four *Abyssocladia*-species recovered from the Atlantic, a genus otherwise almost exclusively found in the Pacific and Indian Oceans (e.g. Hestetun *et al.*, 2016; Vacelet, 2006). As shown by Paper I, the abyssal cladorhizid fauna is severely understudied, and is likely to contain many undescribed species.

3.1.6 Biogeography discussion

The Atlantic Ocean has a species-rich and diverse cladorhizid fauna present. As argued here, the cladorhizid fauna of the Atlantic Ocean can be thought of as a series of related regional faunas at continental shelf and upper bathyal depths, with some overlap in adjacent regions.

In the North Atlantic, the fauna contains a set of arbuscular *Cladorhiza* species related to the type species of the genus, *C. abyssicola*, while the morphology of *Cladorhiza* species in deeper areas, special features such as seamounts and abyssal basins is more diverse, including stipitate and other forms (Paper III). In the case of

Chondrocladia, the four dominating species *C. (C.) grandis*, *C. (C.) virgata*, *C. (C.) verticillata* and *C. (C.) concrescens* are close relatives with an additional undescribed relative in the South Atlantic (Paper IV). These are all large species of the "concrescens" type, and are found at comparatively shallow depths. For *Asbestopluma*, species are relatives of the type species, *A. (A.) pennatula*, with different types of modifications, but looking at the phylogenetic information, *Asbestopluma* species forms a quite diverse group, with species belonging to different clades. Diversity within *Asbestopluma* can often be connected to the morphology of the large type of anisochela, which is often modified into different derived forms (Paper II). For *Lycopodina*, the Atlantic also has a quite diverse fauna, including *L. lycopodium* and *L. cupressiformis* as representatives for long-stalked forms, while *L. infundibulum* and *L. minuta* are two species with a pedunculate morphology and arcuate rather than palmate chelae. *Abyssocladia*, with the exception of *Ab. atlantica* from off SE Brazil, seems to be mainly present at lower bathyal to abyssal depths.

While some of the species at lower bathyal and abyssal depths are morphologically quite similar to shallower species, such as *Cladorhiza methanophila* or *A. (A.) caribica*, others, such as *C. kenchingtonae*, *C. arctica*, C. *flosabyssi*, and stipitate "Crinorhiza" type *Chondrocladia*, have a different morphology, suggesting that they belong to different clades within their respective genera than their shallow water congeners.

As reflected in the length of the discussion here, knowledge of the cladorhizid fauna of the boreal North Atlantic and Arctic is still much higher than for other areas of the Atlantic, though recent studies have begun to rectify this. As knowledge of the southern Atlantic and abyssal basins improve, the number of species deviating from traditional conceptions of the different cladorhizid genera will undoubtedly increase, as already seen in several studies (e.g. Hestetun *et al.*, 2016; Lopes *et al.*, 2011; Lopes & Hajdu, 2014; Vacelet, 2006; Vacelet, 2007). Given the great number of new species continually being described, there is no doubt that the cladorhizid diversity on continental shelves and slopes as well as in the abyssal basins and at features such as

seamounts and mid-ocean ridges is very high, and that a more complete picture will take time to fully emerge.

3.2 Carnivorous sponge systematics

3.2.1 Carnivorous sponge molecular phylogeny

Chela morphology is the most important morphological diagnostic character in classification of poecilosclerid sponges, though other traits such as habit, skeletal organization and the rest of the spicule complement are also used (Hooper & van Soest, 2002b). Most poecilosclerid families are defined by a dominant chela type (together with assumed derivatives), with further refinements at the generic level. However, while Hajdu *et al.* (1994) tried to use, among others, broad patterns of chela morphology to create a subordinal classification of Poecilosclerida, recent molecular studies have shown that microsclere morphology is not always a good systematic indicator, and the suborders have now been abandoned (Hajdu *et al.*, 2013; Morrow & Cárdenas, 2015). Nevertheless, chela morphology is still usually considered valid at the family level.

In contrast, family Cladorhizidae lacks a strong synapomorphy (Hajdu & Vacelet, 2002). Cladorhizidae encompasses genera with several different chela types, including palmate, arcuate and anchorate/unguiferate aniso- and isochelae. Morphological characters associated with a carnivorous feeding strategy such as an erect habit with a completely or partially reduced aquiferous system and filaments/projections have thus, despite the common assertion that habit is unreliable and should be sparingly used for classification, clearly played a defining role in placing the vast majority of carnivorous sponges within Cladorhizidae: A more stringent chela-based classification would likely place the anchorate genera (*Cladorhiza*, *Chondrocladia*) in a different genus than palmate and arcuate groups (*Asbestopluma*, *Abyssocladia*). In two particular examples, chela-based characters have actually been given greater consideration in systematic assignment than habit morphology: *Eucheliplum*a has been placed within family Guitarridae, based on the

presence of placochelae, elaborate, ribbed, modifications of palmate chelae. Four species with arcuate to palmate isochelae have, as this spicule morphology is diagnostic for that family, been placed within *Esperiopsis* (Esperiopsidae), a genus otherwise containing only non-carnivorous sponges.

Recognizing this inconsistency in classification and the lack of a clear synapomorphy, several authors (e.g. Hajdu & Vacelet, 2002; Lopes *et al.*, 2011; Vacelet, 2007) have discussed the possibility that sponge carnivory is a trait that has evolved separately in several poecilosclerid lineages. This is an intriguing possibility in that it implies that characters such as for instance the hook-like chela microscleres make poecilosclerid sponges pre-adapted for a carnivorous feeding strategy to evolve given the right evolutionary pressure (i.e. oligotrophic deep-sea conditions), and make sponge carnivory into something more than a simple one-time oddity.

Thus the question of whether the carnivorous sponges represent a monophyletic assemblage hinges on the degree of plasticity of chela microscleres. In light of recent molecular data, there has been an increasing appreciation of the fact that sponge spicule characters may be more plastic than previously thought (e.g. Cárdenas *et al.*, 2012; Hajdu *et al.*, 2013). Modifications to spicule morphology found in newly described carnivorous sponges support this view. Thus the alternative hypothesis is that carnivory evolved once, and that the diversity of chela morphology represents evolution in spicule characters within one lineage.

Next to no molecular data was available to complement morphological evidence at the start of this PhD project, and one of the main goals of this thesis has been to construct a comprehensive molecular phylogeny of known carnivorous sponges, and use a combination of this molecular data together with known morphological characters to construct an updated and revised systematics of the group. The results of this work are presented in Paper IV, with molecular data for a small number of additional species added in subsequently published Papers II, III and V.

The main aim for the phylogenetic component of this thesis was to test the hypothesis that carnivorous sponges constitute a monophyletic group, which if true has

implications for the ease of evolution of carnivory within sponges. Specifically, this meant providing a comprehensive dataset in terms of genera within Cladorhizidae, as well as a representative each for *Eucheliplura* and a carnivorous *Esperiopsis* species. Secondary aims were to show the position of carnivorous sponges more generally within Poecilosclerida, test the monophyly of cladorhizid genera, and generally elucidate phylogenetic relationships between species. This information was used to update the existing systematics for the group (Paper IV).

Resolving the phylogeny of carnivorous sponges involved two different datasets: The main dataset consisted of 101 sequences from GenBank data and 80 newly sequenced specimens and representing 40 species, belonging to 7 of 9 recognized genera and 4 of 5 recognized subgenera. Only the minor monotypic genera *Lollipocladia* and *Neocladia*, as well as the three species subgenus *Helophloeina* were omitted from the dataset. The main dataset analysis was performed on a concatenated three gene partitioned alignment using maximum likelihood (ML) and Bayesian inference, based on partial 28S rDNA C1-D2 domains (Chombard *et al.*, 1997), partial cytochrome oxidase subunit I (COI) including the Folmer partition and proposed "Erpenbeck" extension, (Folmer *et al.*, 1994; Rot *et al.*, 2006) and the nuclear asparagine-linked glycosylation 11 protein gene (ALG11) (Belinky *et al.*, 2012); in all 2890 base pairs.

A subset consisting of representatives of the majority of carnivorous genera was included in an auxiliary analysis of nearly complete 18S rDNA data. A large number of existing poecilosclerid 18S rDNA sequences was available from GenBank, mainly through the Porifera Tree of Life (PorToL) project (e.g. Redmond *et al.*, 2013). This allowed comparison with a majority of poecilosclerid genera (18 of 20), placing carnivorous sponges in a wider systematic context. In all 148 GenBank sequences and 11 newly sequenced specimens were aligned and fitted to a RNA secondary structure model (Voigt *et al.*, 2008) for use in Bayesian and ML analysis.

Both analyses recovered carnivorous sponges as a monophyletic group, including *Eucheliplura* and carnivorous *Esperiopsis* spp., with high support. Mycalidae and Guitarridae were found to be the closest relatives to the carnivorous sponges, though

the exact relationship between these two families was not established. This strengthens the hypothesis that all known carnivorous sponges represent a single lineage, and suggests that a carnivorous habit should be considered as a main diagnostic character in place of spicule morphology. As a result, Cladorhizidae was retained as a monophyletic family, with *Eucheliplum*a moved to Cladorhizidae as an independent genus, and carnivorous *Esperiopsis* species included as *Abyssocladia* spp.

The genera *Chondrocladia* and *Cladorhiza* were found to be monophyletic. *Asbestopluma* was found to be paraphyletic, with species with a plate-like lower chela morphology and forceps spicules moved to the re-erected genus *Lycopodina*. *Abyssocladia* was found to be monophyletic with the additional inclusion of carnivorous *Esperiopsis* species and *Cercicladia*. The diagnosis of this genus was expanded to allow arcuate isochelae only rather than abyssochelae or cleistochelae. *Eucheliplum*a was recovered basally to the rest of *Abyssocladia*, and was retained as an independent genus. The monotypic genus *Cercicladia* was recovered within *Abyssocladia*, but as only partial COI was recovered from both *Cercicladia* specimens, no changes were made to its status as an independent genus, though future data might show that it belongs within *Abyssocladia*. On the subgeneric level, the *Chondrocladia* subgenera *Chondrocladia*, *Meliiderma* and *Symmetrocladia* were shown to be polyphyletic, implying a split between "*Crinorhiza*" and "*concrescens*" type species as previously used as informal groups, though no formal new subgeneric classification was proposed.

As a particular interesting result, *Chondrocladia* was recovered nested within Cladorhizidae in a derived position related to other genera. This genus retains a partial aquiferous system, which has led to the reasonable assumption that it represents an intermediate between filter-feeding sponges and other cladorhizids, where the aquiferous system has been completely reduced. The phylogenetic results thus imply a parallel complete reduction of the aquiferous system in several independent cladorhizid lineages.

Initially, getting clean sequences from the cladorhizid material proved challenging due to a combination of poorly preserved or old specimens and the high surface to volume ratio and adhesive surface of the sponges. Thus sequences were prone to contamination, especially in the case of the COI Folmer partition, until specific modifications of the Folmer primers suitable for Cladorhizidae were developed. Still, in many cases not all gene partitions were successfully sequenced.

The markers chosen for the phylogenetic analyses worked well in all cases: The two analyses were able to answer the question of monophyly of carnivorous sponges, place the carnivorous sponges within Poecilosclerida, and elucidate relationships at the genus and species levels, with few exceptions. Consistent with the finding that this gene evolves more slowly in basal phyla (Huang *et al.*, 2008) COI, even with the "Erpenbeck" extension, was found to be too conserved to resolve relationships at the species level in some cases, but ALG11 was found to be somewhat less conserved than COI and performed well at both family, genus and species level, making it a good choice for future analysis. The 28S and 18S sequences were adequate for their respective use, but required care in identifying and removing variable loop regions that were unaligneable from the dataset.

Thus the study in Paper IV successfully revealed the major lines in the phylogeny of carnivorous sponges as well as putting to rest the question of whether the carnivorous sponges represented one or several lineages. By applying morphological data to the phylogenetic results, it proved possible to make an updated systematics of the group using an integrative taxonomic approach; a work that will in all probability be further revised and refined in future studies.

3.2.2 Carnivorous sponges and barcoding

COI was employed in a phylogenetic capacity in the analyses employed in Paper IV. This was done in order to more easily accommodate future barcoding of species included in the analysis. However, COI is known to be comparatively conservative in basal phyla including Porifera and Cnidaria (Huang *et al.*, 2008). In some cases, COI by itself was unable to distinguish closely related species in the phylogeny, even with the extension advocated by the sponge barcoding project (http://www.palaeontologie.geo.uni-muenchen.de/SBP/). Initial lab results showed that standard and degenerated Folmer primers picked up significant amount of contamination, limiting their use as barcoding primers for carnivorous sponges in Sanger sequencing applications, an issue that would be less important in NGS approaches, however.

3.3 Microbial community and symbiosis

Sponge microbial ecology studies is a field that has grown exponentially in the last ten years, aided by the increasing ease and decreasing cost provided by the availability of next generation sequencing (NGS) platforms (Hentschel *et al.*, 2012). While early studies typically used pyrosequencing in recent years platforms such as Illumina and Ion Torrent, which are able to provide vast amounts of data at a much lower price, have become more common, allowing the routine use of vast amounts of genomic data in microbial ecology studies as well as other applications.

Using 16S rRNA amplicon data for microbial community sequencing is a standard approach, and has been extensively been applied in microbial ecology since before NGS was available through the use of cloning libraries in Sanger sequencing. The stem regions of the 16S ribosome are extremely conserved, and can thus be used for large-scale phylogeny of prokaryotic organisms. Employing universal prokaryotic primers used together with a NGS platform allows the parallel sequencing of partial 16S rRNA from all prokaryote organisms within the sample (though all primer sets have some inherent taxonomic bias that should be kept in mind when discussing

results). Resulting 16S rRNA amplicon data can be grouped into operational taxonomic units (OTUs) based on percentage similarity, and annotated sequence databases such as SILVA (https://www.arb-silva.de/) or Green genes (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) can then be used to apply taxonomic information to the OTUs, giving an overview of the microbial diversity of the sample. Data pipelines such as QIIME (Caporaso *et al.*, 2010) and/or UPARSE (Edgar, 2013) are used to treat sequence output data, depending on the purpose of the analysis.

Depending on the primers used, results may vary between studies in terms of relative abundance of different prokaryote groups. However, the increasing amount of sponge-centered microbial ecology studies have revealed that sponges have a rich microbial fauna that can be divided into shared and more specific microbial groups depending on the sponge in question (Thomas *et al.*, 2016). For carnivorous sponges in particular, only a preliminary study has been published using the 16S rRNA amplicon approach, however (Dupont *et al.*, 2013).

Paper V is a comparative study of microbiome 16S rRNA amplicon data from several carnivorous sponge species collected near vent and seep sites, including *Cladorhiza methanophila*, which is known to contain methanotrophic symbionts. The results presented as part of this thesis are mostly descriptive, in that they provide an overview of the relative abundance of specific microbial taxa in the different specimens examined. Thus it was possible to show that Proteobacteria and Bacteriodetes are the two largest bacterial phyla present in all samples, divided into certain prevalent bacterial groups such as among others Oceanospirillales, Thiohalorhabdales, Rhodobacterales, Flavobacteriales, and the archaeal *Nitrosopumilus*. Methylococcales was very abundant in *C. methanophila*, confirming the studies describing the symbiosis found in this species. No obvious similar associations were found in any other cladorhizid, however, as also confirmed by diverging $\delta^{13}C$ and $\delta^{15}N$ isotope signatures for this species compared to the rest of the sampled cladorhizids. Thus *C. methanophila* should be considered an isolated case of symbiosis within Cladorhizidae rather than an example of a more widespread phenomenon.

3.4 Diversity of the carnivorous sponges

Many cladorhizid species, especially from the North Atlantic, were described during the late 19th and early to mid-20th centuries, which settled the larger cladorhizid genera such as *Asbestopluma*, *Chondrocladia* and *Cladorhiza* into Cladorhizidae, while the status of the two genera established in the sixties and seventies, *Abyssocladia* and *Neocladia* (now *Koltunicladia*), have not been considered secure until recently (Vacelet, 2006; Vacelet, 2008). The recent discovery of the carnivorous feeding habit led to renewed interest in the group, and in recent years, coinciding with increased research activity in previously unexplored deep-sea areas, the number of articles describing new cladorhizid species has been high: At the turn of the century, 60 species were described, as opposed to ~150 species today (van Soest *et al.*, 2016). These recent studies have shown that the morphological and spicule diversity within Cladorhizidae is greater than what was previously known, though some results (e.g. Koltun, 1970a; Lévi, 1964; Ridley & Dendy, 1887) had hinted at this from earlier Pacific investigations.

Following recent studies describing new carnivorous sponges from the Pacific (Downey & Janussen, 2015; Ise & Vacelet, 2010; Kelly & Vacelet, 2011; Lee *et al.*, 2012; Lehnert *et al.*, 2006; Lehnert *et al.*, 2005; Lopes *et al.*, 2011; Lundsten *et al.*, 2014; Reiswig & Lee, 2007; Vacelet, 2006; Vacelet, 2008; Vacelet & Kelly, 2014), South Atlantic (Castello-Branco *et al.*, 2016; Lopes & Hajdu, 2014; Ríos *et al.*, 2011), Indian Ocean (Hestetun *et al.*, 2016), and Southern Ocean (Dressler-Allame *et al.*, 2016; Goodwin *et al.*, in press; Janussen & Tendal, 2007; van Soest & Baker, 2011), a clearer picture is emerging regarding the cladorhizid fauna worldwide, including the resurrection of *Neocladia* (now *Koltunicladia*), *Meliiderma* and *Helophloeina* (the two latter as subgenera) (Vacelet, 2006; Vacelet, 2008; Vacelet *et al.*, 2009), as well as establishment of the monotypic genera *Cercicladia*, *Lollipocladia*, and subgenus *Symmetrocladia* (Lee *et al.*, 2012; Ríos *et al.*, 2011; Vacelet, 2008). This increase in diversity is not restricted to newly erected groups, and a range of examples of species deviating from the usual spicule morphology have now been described from existing genera, including modifications of chelae between

anchorate and palmate forms, isochelae, anisoplacochelae, monocrepid desmas, or comb-like extensions (Fig. 8).

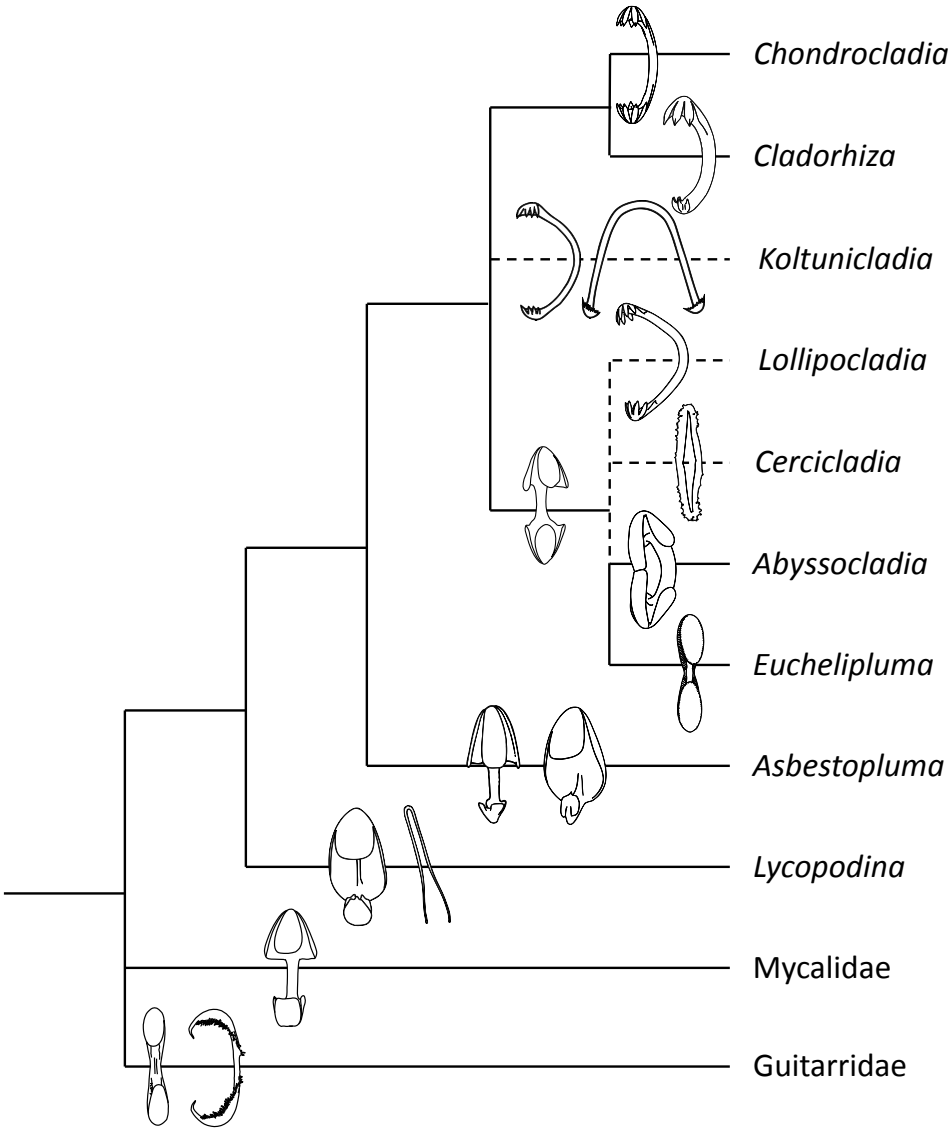

Figure 8. A schematic overview of morphological variation within Cladorhizidae showing the most significant changes in spicule morphology. Solid lines indicate clades that were recovered with at least one species in the phylogeny in Paper IV. Dotted lines indicate

hypothetical relationships based on a qualitative evaluation of morphological characters only, and should be regarded as tentative. Spicules representing *Cercicladia* are drawn from the description by Ríos *et al.* (2011); *Lollipocladia* from Vacelet (2008); *Koltunicladia* from Koltun (1970a); other spicules are drawn from own material.

In most cases, the identity of new species with deviating spicule morphology can be inferred through the rest of the spicule complement. As an example, the larger chela type in *Asbestopluma* is in some cases missing or modified into a non-palmate morphology. However, species belonging to this genus can often still be identified by presence of a combination of mycalostyles, characteristic subtylostyles, smaller chela, together with sigmancistras. In a few cases, several species with the same type of morphological modification have been described (e.g. Hestetun *et al.*, 2016; Kelly & Vacelet, 2011; Lopes & Hajdu, 2014), showing that what was originally thought to be specific aberrations from the standard may instead represent larger clades within the genus.

As shown in this thesis, phylogenetic analysis based on molecular data is an indispensable tool to clarify the relationship between previously known and newly reported variations in spicule morphology, such as the relative intra-generic position of branches with different modification of spicule types. While taxonomic morphological characters have not been combined with molecular data in a quantitative phylogenetic way here, ancestral reconstruction of spicule characters may prove fruitful in the future as the number of species increases even further. In the following text, data from the molecular phylogeny in Paper IV has been used as a baseline for exploring part of the morphological variation within cladorhizid genera. The groups listed below are meant as descriptive hypotheses of possible intra-generic relationships, but are considered working hypotheses based on current (often incomplete) data, and are expected to change in the future as more evidence becomes available.

3.4.1 *Abyssocladia*

Diagnosis. Cladorhizidae most often pedunculate, carrying a disciform or flabelliform body with a radial architecture, in other cases pinnate or branching. Microscleres are a combination of abyssochelae, cleistochelae, arcuate chelae and/or sigmancistras, but not placochelae (from Paper IV).

Abyssocladia was originally described by Lévi based on a specimen collected from the deep Pacific during the 1950-1952 Galathea Expedition, but only a few species (Koltun, 1970a; Lévi, 1964) were described until recently. The original diagnosis of this genus was based on the presence of abyssochelae (called thaumatochelae by Lévi, later renamed in the *Systema Porifera*). Derived from arcuate isochelae, abyssochelae have the upper and lower middle tooth touching or almost touching in the middle of the spicule. The term was later restricted to forms also having a small height to width ratio (Lopes *et al.*, 2011), who used the term cleistochelae to describe forms with teeth touching or nearly touching, but with a general form more closely resembling arcuate isochelae, and arguing for a transformation series from arcuate isochela through cleistochela and finally abyssochela.

The genus was synonymized with *Phelloderma* (Phellodermidae) in the *Systema Porifera* based on a similar chela type in that genus (van Soest & Hajdu, 2002), but was reinstated to Cladorhizidae by Vacelet (2006), later confirmed by molecular COI data (Vargas *et al.*, 2013). The number of known *Abyssocladia* species has increased rapidly in recent years. Several newly described species lack cleistochelae or abyssochelae, having only arcuate isochelae, which means that abyssochelae/cleistochelae no longer can be considered a synapomorphy and necessitating a revision of the diagnosis of the genus. This includes the four *Esperiopsis* spp. added to *Abyssocladia* as a result of the phylogeny included in Paper IV, who have arcuate isochelae only. As *Eucheliplum*a, shown to be a probable sister genus to *Abyssocladia* also has arcuate isochelae in combination with placochelae, *Abyssocladia* at this time lacks a clear synapomorphy (Fig. 9).

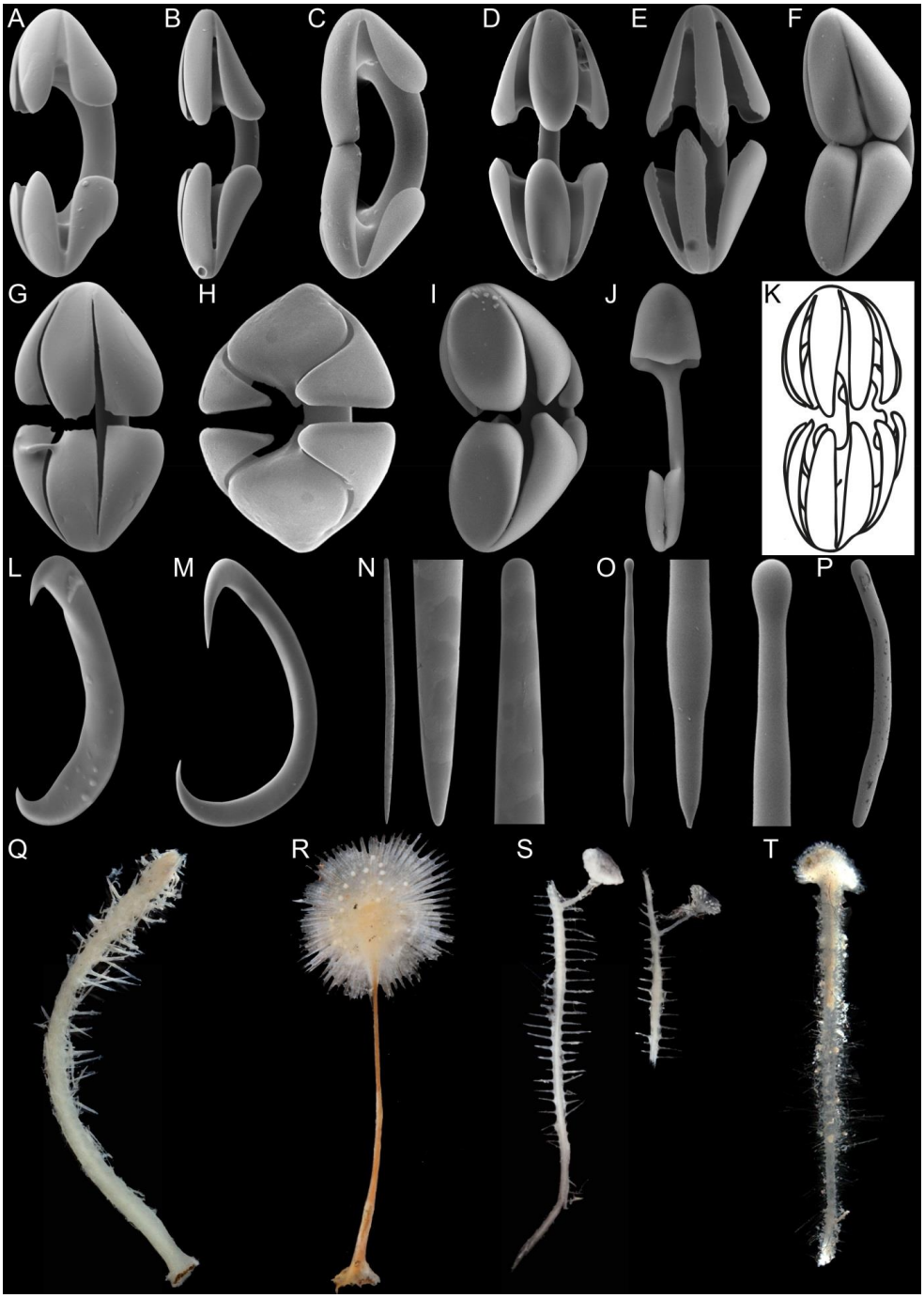

Figure 9. Variation of spicule and habit morphology found within currently described *Abyssocladia*. All images from own data except chela 11, which is reproduced from Kelly and Vacelet (2011). A) Arcuate isochela, *A. boletiphora*; B) arcuate isochela, *A.*

hemiradiata; C) cleistochela, *A. tecta*; D) arcuate isochela, *A. polycephalus*; E) laterally shifted cleistochela, *A. (A.) corniculiphora*; F) closed-off cleistochela, *A. dominalba*; G) abyssochela, *A. boletiphora*; H) abyssochela, *A. tecta*; I) abyssochela, *A. dominalba*; J) contorted palmate isochela, *A. dominalba*; K) multidentate isochela, *A. carcharias*; L) sigmancistra, *A. boletiphora*; M) sigma, *A. hemiradiata*; N) mycalostyle, *A. corniculiphora*; O) polytylote tylostyle, *A. hemiradiata*; P) substrongyle, *A. boletiphora*. Habit of Q) *A. boletiphora*, R) *A. dominalba*, S) *A. corniculiphora* and T) *A. hemiradiata*.

From only three known species previous to 2006, over 20 species have been described to date, mainly from the Pacific. Though four species have now been described including Lopes and Hajdu (2014) and the papers collected in this thesis, the genus is not particularly well represented in the Atlantic, which probably explains its relative obscurity until the last ten years. Most species tend to occur at 2500-5000 m depth meaning that this genus is truly "abyssal" even compared with most other cladorhizid sponges. Species are usually in the range of 2-7 cm, and are often found at places of interest such as seamounts and vent and seep sites.

Many of the initially described *Abyssocladia* species were pedunculate disc-shaped (e.g. Lévi, 1964; Vacelet, 2006), which is still partly reflected in the genus diagnosis, but newly described species show that a range of morphologies are common within the genus, also including flattened single-stem species with filaments in two opposite rows, round single-stem species with filaments in all directions, and species with a branching habit (e.g. Hestetun *et al.*, 2016; Lehnert *et al.*, 2006; Lopes *et al.*, 2011). In some species, curious stalked bodies are found, possibly associated with reproductive processes (Hestetun *et al.*, 2016; Lopes *et al.*, 2011; Vacelet, 2006). Another interesting deviation from the more standard morphology is found in the special branched habit of *A. koltuni* (Ereskovsky & Willenz, 2007), where filaments are as crown-like structures at branch ends only rather than along the stem. Chela morphology, while usually arcuate isochela to abyssochela, can vary between species, with some species, such as *A. carcharias* Kelly & Vacelet, 2011, having specific modifications to morphology, and others having special spicules including orthancistras, desmas or auxiliary palmate anisochelae (Hooper & Lévi, 1989; Vacelet, 2006).

Cercicladia was recovered within *Abyssocladia* in the phylogenetic analysis in Paper IV, though based on partial (592 bp) COI sequence only. While the tentative state of the evidence of inclusion into *Abyssocladia* meant that no formal revision was done in this case, the special cercichelae of that species could be seen as a possible modification of a cleistochela. The phylogeny recovered *Cercicladia australis* within *Abyssocladia*, with *Eucheliplum*a in a basal position. The position of *Eucheliplum*a is not completely settled, but it has clear similarities to *Abyssocladia* in the arcuate chela morphology, and is thus currently considered a sister genus to *Abyssocladia*.

It is at this point difficult to make any hypotheses, even preliminary, regarding any internal divisions regarding the majority of *Abyssocladia* species. Spicule morphology includes the transformation series proposed by Lopes *et al.* (2011): arcuate isochela – cleistochela – abyssochela, however, it is difficult to see any patterns in spicule morphology compared to habit, with the different combinations of these three chelae being found in all listed habit types. Furthermore many *Abyssocladia* specimens from the Paper IV phylogeny remain undescribed, and thus there is a lack of morphological data for drawing further morphological conclusions using the molecular data. *Abyssocladia* is mostly reported from the Pacific, but with a couple of species from the Atlantic and Southern Oceans. Species: *A. atlantica*, *A. boletiphora*, *A. bruuni*, *A. carcharias*, *A. claviformis*, *A. corniculiphora*, *A. desmophora*, *A. diegoramirezensis*, *A. dominalba*, *A. faranauti*, *A. flagrum*, *A. hemiradiata*, *A. huitzilopochtli*, *A. inflata*, *A. koltuni*, *A. lakwollii*, *A. leverhulmei*, *A. myojinensis*, *A. natushimae*, *A. naudur*, *A. oxeata*, *A. polycephalus*, *A. symmetrica*, *A. tecta*, *A. umbellata*.

3.4.2 *Asbestopluma*

Diagnosis. Cladorhizidae with at least one type of palmate, or in one case anchorate unguiferate, anisochela. Usually with a second larger type of palmate to arcuate anisochela that may in some cases be modified to isochela, anisoplacochela or tridentate anchorate chela. Sigmancistras and basal acantho(sub)(tylo)styles are also present with a few exceptions. Never forceps spicules (from Paper IV).

Originally described as a subgenus by Topsent (1901), *Asbestopluma* was elevated to genus rank by Lundbeck (1905). The main diagnostic criterion is the presence of palmate anisochelae in combination with sigmas or sigmancistras. Species in this genus typically have mycalostyles, subtylostyles, two types of palmate anisochelae, sigmancistras, and acanthotylostyles in the basal sheath. More recently described species have shown that the chelae in some species have a different morphology, including isochelae and unguiferate forms (Hestetun *et al.*, 2016; Lopes & Hajdu, 2014), as well as more radical changes such as anisoplacochelae (Kelly & Vacelet, 2011) or diancistras (Paper II). A few *Asbestopluma* species have spear-like microtylostyles instead of acanthotylostyles. These are included in subgenus *Helophloeina*, while other *Asbestopluma* are included in subgenus *Asbestopluma* by default. Monocrepid desmas have been reported for a few species, connected to the basal plate. Species lacking sigmas and with chelae with a special plate-like lower part were, as part of this thesis (Paper IV), taken out of *Asbestopluma* and into the revived genus *Lycopodina* (see below) (Fig. 10).

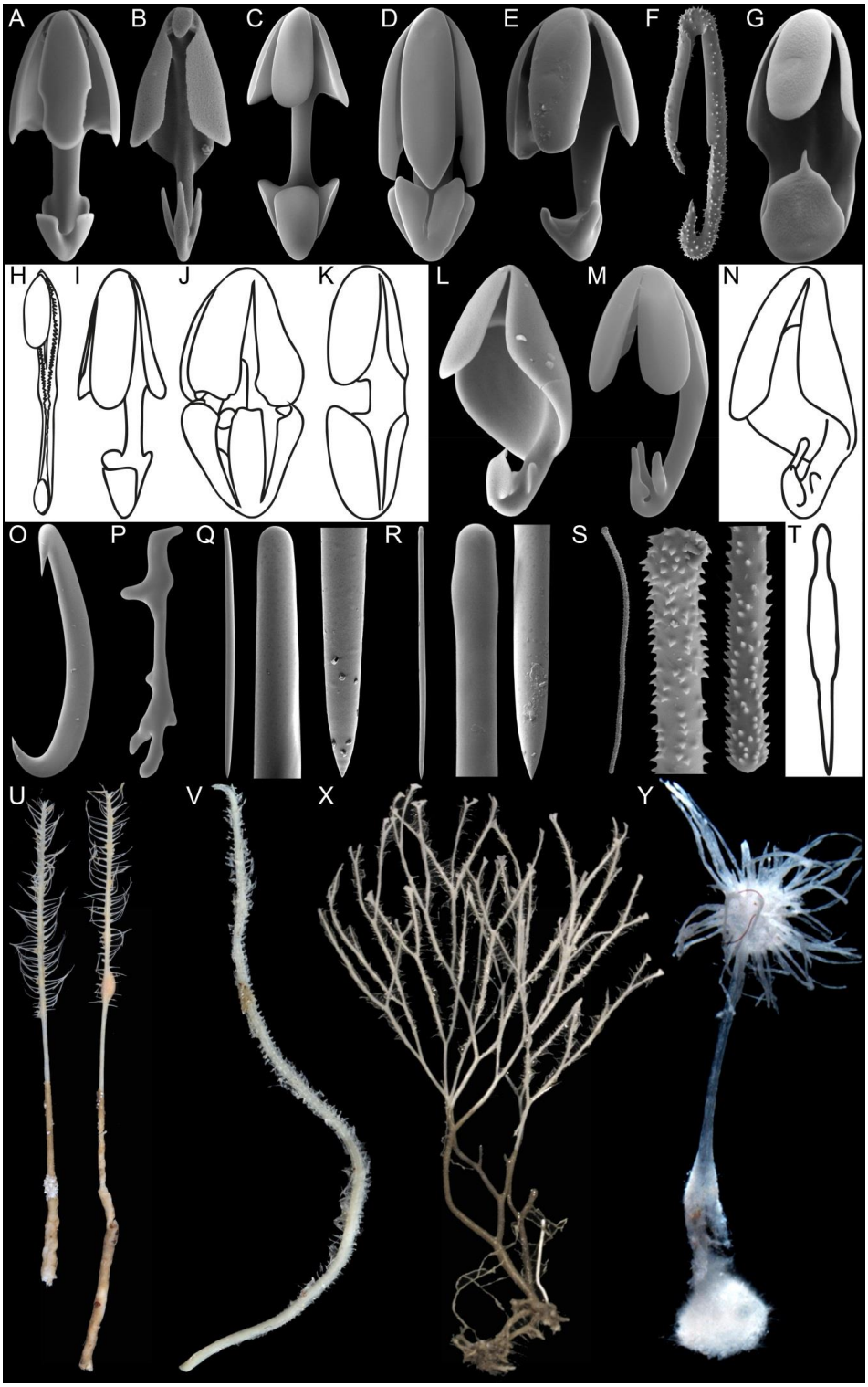
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Figure 10. Variation of main spicule and habit morphology found within currently described *Asbestopluma*. All images from own data except spicules 8-11, 14 and 20, which are reproduced from Kelly and Vacelet (2011), Vacelet (2006), Lopes *et al.* (2011) and Goodwin *et al.* (in press). Large chela types: A) arcuate/palmate anisochela, *A. (A.) pennatula*; B) arcuate anisochela, *A. (A.) unguiferata*; C) arcuate anisochela, *A. (A.) pseudoisochela*; D) cleistoanisochela, *A. (A.) ruetzleri*; E) arcuate anisochela, *A. (A.) jamescooki*; F) anisocercichela, *A. (A.) caribica*; G) laminate palmate anisochela, *A. (A.) laminachela*; H) anisoplacochela, *A. (A.) anisoplacochela*; I) arcuate anisochela, *A. (H.) formosa*; J) abyssochela, *A. (H.) keraia*; K) palmate isochela, *A. (H.) delicata*. Small chela types: L) palmate anisochela, *A. (A.) pennatula*; M) unguiferate anchorate anisochela, *A. (A.) pseudoisochela*; N) palmate anisochela, *A. (H.) formosa*. Other spicules: O) Sigmancistra, *A. (A.) pennatula*; P) desma, *A. (A.) pseudoisochela*; Q) mycalostyle, *A. (A.) pennatula*; R) subtylostyle, *A. (A.) pennatula*; S) acanthotylostyle, *A. (A.) pennatula*; T) spear-like microtylostyle, *A. (H.) formosa*. Habit of U) *A. (A.) pennatula*, V) *A. (A.) pseudoisochela*, X) *A. (A.) furcata* and Y) *A. (A.) laminachela*.

Species belonging to *Asbestopluma* have been reported from shelf areas and upper bathyal features such as seamounts both in the Atlantic, Pacific and Indian Ocean, and it is a common and species-rich genus worldwide, with 24 reported species. A couple of species have also been reported from lower bathyal and abyssal depths.

Asbestopluma species are either single-stem or branching. Filaments are ordered in rows, which gives them either a pennate morphology (two, opposite rows) or a brush-like morphology (4-8 rows). The lower stem is usually covered in a special coating layer, and species have either a basal plate or a system of rhizoids. Based on the currently described species of *Asbestopluma*, a rough division into different subgroups based on spicule morphology overlaid on a simplified version of the results from the molecular phylogeny allows the identification of a couple of morphological tendencies within the genus (Figure 11):

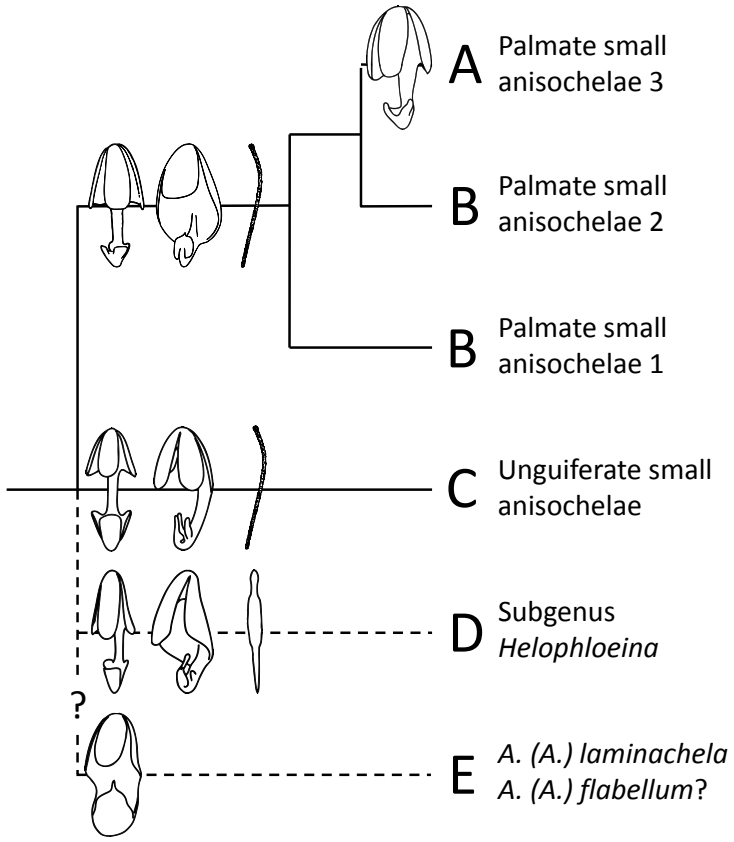

Figure 11. A schematic overview of morphological variation within *Asbestopluma*. Solid lines indicate clades that were recovered with at least one species in the phylogeny in Paper IV. Dotted lines indicate hypothetical relationships based on a qualitative evaluation of morphological characters only, and should be regarded as tentative. Spicules representing group D are drawn from the description by Vacelet (2006) of *A. (H.) formosa*; other spicules are drawn from own material.

Fig. 11A: A subgroup of Pacific and Indian Ocean species where the large anisochela has been somewhat modified into a rounded, arcuate form. Small anisochelae of the common palmate form. Desmas and strongyles present in one species each. Mostly bifurcating, but one single-stem species. Species: *A. (A.) agglutinans, A. (A.) desmophora, A. (A.) jamescooki, A. (A.) ramuscula*.

Fig. 11B: The main group of *Asbestopluma* species with a morphology most resembling that of the type species *A. (A.) pennatula*. The main character is a clearly

palmate form of small anisochela without elongated central lower tooth, and no spear-like microtylostyles. Large anisochelae are palmate to arcuate, but are missing in around half of described species and with specific, derived modifications in the species *A. (A.) anisoplacochela* and *A. (A.) caribica*. Acanthotylostyles are usually present. No record of strongyles or desmas. Around half of species are single-stem, the other half bifurcating. Species: *A. (A.) anisoplacochela*, *A. (A.) belgicae*, *A. (A.) bihamatifera*, *A. (A.) biserialis*, *A. (A.) biserialis* var. *californiana*, *A. (A.) caribica*, *A. (A.) furcata*, *A. (A.) gracilior, A. (A.) magnifica*, *A. (A.) monticola*, *A. (A.) obae*, *A. (A.) pennatula*, *A. (A.) quadriserialis*, *A. (A.) ramosa*, *A. (A.) rickettsi*, *A. (A.) sarsi*, *A. (A.) voyager*, possibly *A. (A.) wolffi sensu* Lévi, 1964.

Fig. 11C: A group of species from the southern hemisphere where the small anisochela has an unguiferate arcuate rather than palmate morphology. Large chelae are mostly arcuate (an)isochelae. Acanthotylostyles are usually present. Both strongyles or desmas common. Both single-stem and bifurcating species. Species: *A. (A.) bitrichela*, *A. (A.) gemmae*, *A. (A.) inexpectata*, *A. (A.) pseudoisochela*, *A. (A.) unguiferata*. Based on the fact that the unguiferate anchorate anisochelae seems identical to those of other species in this group, *Cladorhiza diminuta* Lopes & Hajdu, 2014 may belong here, if it is considered a species that has lost its large anisochelae similar to many species in group B.

Fig. 11D: Subgenus *Helophloeina* are diagnosed based on the presence of spear-shaped microtylostyles, a probable modification of the acanthotylostyles found in other *Asbestopluma* species. Small anisochelae are palmate, but with an elongated central lower tooth. Large chelae morphology is variable: Either lacking, or in the form of arcuate anisochelae, isochelae or even abyssochela-like. Species are bush-like and bifurcating. Species: *A. (H.) formosa*, *A. (H.) delicata*, *A. (H.) keraia*, *A. (H.) stylivarians*.

Fig. 11E: These are pedunculate species with the large type of anisochela missing. The detailed chela morphology of *A. (A.) flabellum* is not apparent from the source. For *A. (A.) laminachela* the lateral alae are fused. Placed within *Asbestopluma* on the

basis of presence of sigmas, however, an alternative hypothesis would be placing them in a clade basal to the rest of *Lycopodina*.

3.4.3 *Chondrocladia*

Diagnosis. Cladorhizidae with anchorate isochelae (from Lee et al., 2012).

The diagnosis of *Chondrocladia* is based on the presence of anchorate isochelae. It contains most of the larger cladorhizid species, and includes species with a remnant aquiferous system. Subgenus *Meliiderma* is diagnosed on the additional presence of trochirhabds or subtrochirhabds, and contains five small, stipitate species with spherical bodies from the Pacific and Indian Oceans. The monotypic subgenus *Symmetrocladia* contains the remarkable *C. (S.) lyra*, a species with a series of vanes or spokes containing numerous vertical branches giving the impression of a harp, and rostriform subtylostyles (Lee *et al.*, 2012). The majority of *Chondrocladia* species are placed in subgenus *Chondrocladia* by default (Fig. 12).

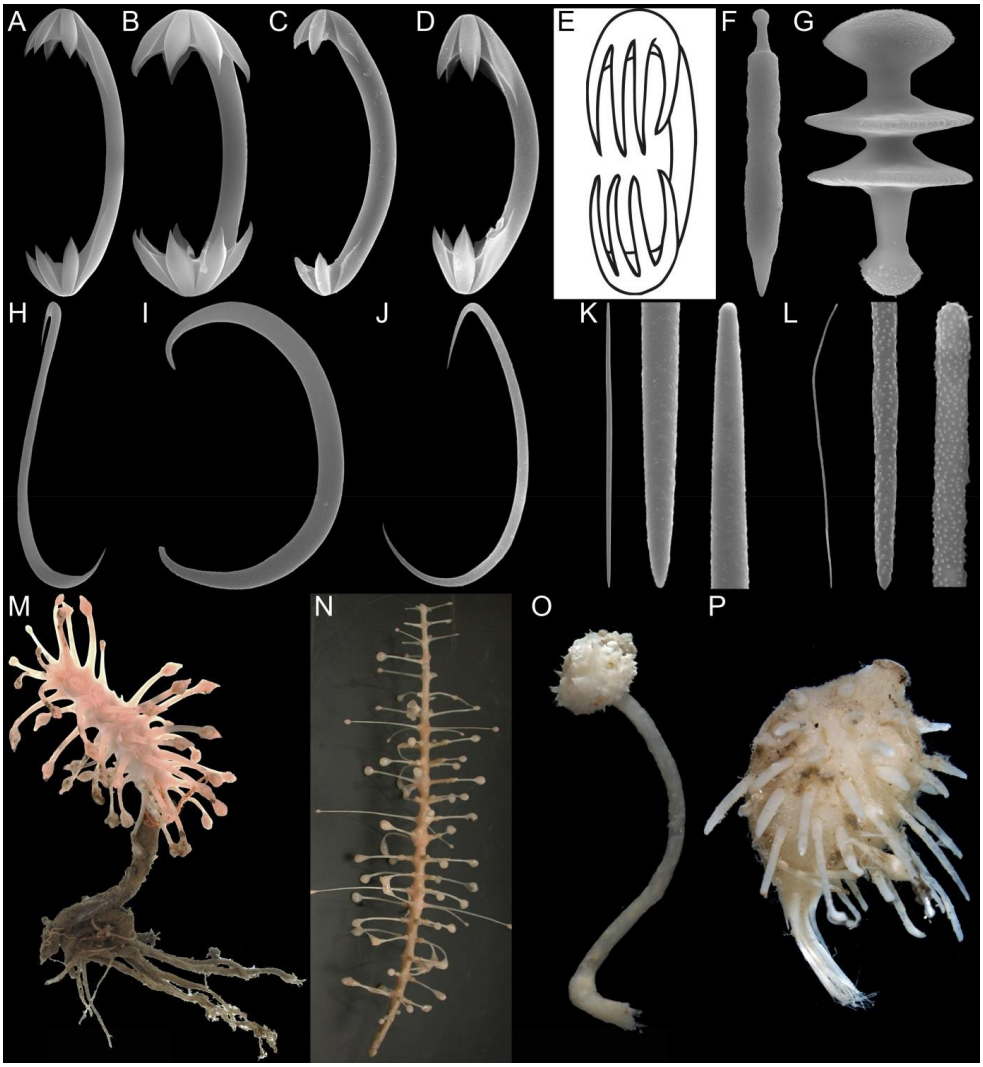

Figure 12. Variation of spicule and habit morphology found within currently described *Chondrocladia*. All images from own data except chela 5, which is reproduced from Schmidt (1880). A) Large anchorate isochela, *C. (C.) grandis*; B) small anchorate isochela, *C. (C.) grandis*; C) large tridentate anchorate isochela, *C. (C.) vaceleti*; D) small tridentate anchorate isochela, *C. (C.) vaceleti*; E) small anchorate isochela, *C. (C.) concrescens*; F) subtrochirhabd, *C. (M.) stipitata*; G) trochirhabd with two annuli, *C. (C.) rodgersi*; H) sigmancistra, *C. (C.) grandis*; I) sigma, *C. (C.) verticillata*; J) sigma, *C. (C.) vaceleti*; K) mycalostyle, *C. (C.) grandis*; L) acanthostyle, *C. (C.) grandis*. Habit of M) *C. (C.) grandis*, N) *C. (C.) verticillata*, O) *C. (M.) rodgersi* and P) *C. (C.) vaceleti*.

Most *Chondrocladia* species have processes that are larger than the filaments common in other cladorhizid genera. In many species, the remnant aquiferous system is used to inflate swellings along or at the end of these branches. Some authors have speculated that rapid deflation of these swellings could have a role in prey capture (Kübler & Barthel, 1999), but they also seem to have a reproductive function (Lee *et al.*, 2012).

Species belonging to subgenus *Chondrocladia* have at times roughly been divided into two groups: The "Crinorhiza" group (named after *Crinorhiza amphactis* Schmidt 1880, currently accepted as *Chondrocladia (C.) amphactis*) contains smaller, stipitate species with branches without obvious swellings issuing from the body and with isochelae with three teeth in each end. The "concrescens" group (named after *Cladorhiza (C.) concrescens* Schmidt, 1880, currently accepted as *Chondrocladia (C.) concrescens*) contains species with an erect, single-stem or branching morphology with numerous side branches at intervals along the stem; isochelae have five teeth in each end. Molecular results from Paper IV confirmed that current subgenus *Chondrocladia* is polyphyletic with respect to *Meliiderma* and *Symmetrocladia* roughly along the lines of "Crinorhiza" and "concrescens" type species, but finding complete and consistent diagnostic characters for these subgenera remains challenging, given exception to characters listed above in some cases.

"Crinorhiza"-like species seem more common at lower bathyal and abyssal depths, while the larger "concrescens" type species are more common at upper bathyal to shallow depths. Interestingly, specimens of *C. (C.) grandis* caught at greater depths seem to have a more "scepter"-like morphology, with a longer stem and more concentrated branching part (Paper III). This was previously reported by Koltun (1970a) who, however, also ended up synonymizing species considered valid today based on perceived habitat-induced variation.

Polynoid polychaetes have been reported from the surface of several *Chondrocladia* species, including *C. (C.) verticillata*, *C. (C.) lampadiglobus, C. (C.) robertballardi* and *C. (C.) virgata*.

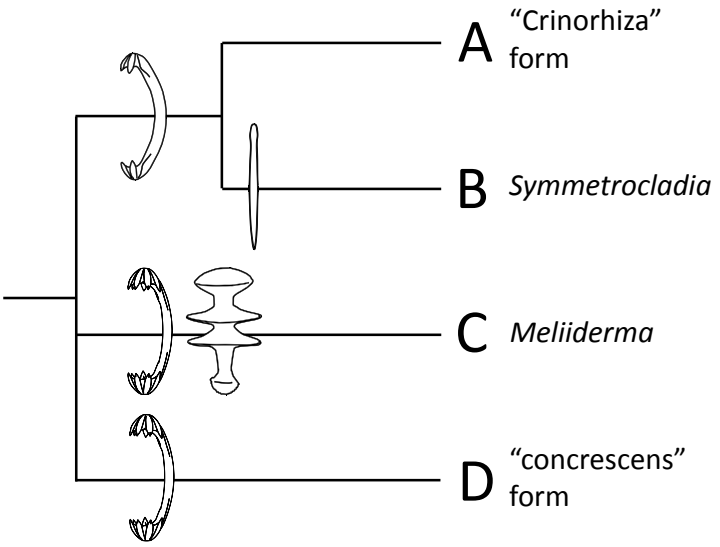

Figure 13. A schematic overview of morphological variation within *Chondrocladia*, based on clades including at least one species in the phylogeny in Paper IV.

Fig. 13A: "Crinorhiza" form species are usually pedunculate with spherical bodies and with tridentate anchorate isochelae. While a few species have been reported from shallower depths, many are lower bathyal and abyssal. Most are reported from the Pacific and Indian Oceans, but with some Atlantic and Southern Ocean species. Similar to pedunculate *Cladorhiza* species, the main peduncle usually penetrates the body of the sponge and protrudes slightly at the apex in many species. Branches/filaments are thinner than in the concrescens group, lack swellings and usually emerge laterally on the body. Most species are comparatively small for the genus (e.g. 20-100 mm). A couple of species have anisochelae with more than three teeth, e.g. *C. (C.) albatrossi*, *C. (C.) guiteli* and *C. (C.) levii*. In the current systematics, this group is polyphyletic with regards to "concrescens" type *Chondrocladia* in subgenus *Chondrocladia*. Species: *C. (C.) albatrossi*, *C. (C.) amphactis*, *C. (C.) antarctica*, *C. (C.) arenifera*, *C. (C.) clavata*, *C. (C.) crinita*, *C.*

(C.) fatimae, *C. (C.) gracilis*, *C. (C.) guiteli*, *C. (C.) levii*, *C. (C.) nani*, *C. (C.) pulvinata*, *C. (C.) scolionema*, *C. (C.) vaceleti*.

Fig. 13B: The monotypic subgenus *Symmetrocladia* contains the spectacular Eastern Pacific species *C. (S.) lyra*. This large species is composed of a central root system connecting a radial system of horizontal vanes set with long, vertical branches giving the impression of a harp or lyre. Inflatable swellings are found both terminally and subterminally on the long, vertical branches, which also feature secondary filaments; uniquely in the genus.

Fig. 13C: Subgenus *Meliiderma* contains a few comparatively small, pedunculate species defined by the presence of trochirhabds or subtrochirhabds. These spicules are usually found in an outer stem layer, and may be derived from the finely spiny styles that fulfil a similar function in "concrescens" type *Chondrocladia* species. Isochelae have five teeth. No obvious remnant aquiferous system. Species have been reported mostly from the Southern hemisphere in the Indian and Pacific Oceans at medium depths (1000-3000 m). Species: *C. (M.) latrunculioides*, *C. (M.) occulta*, *C. (M.) rogersi*, *C. (M.) stipitata*, *C. (M.) tasmaniensis*, *C. (M.) turbiformis*.

Fig. 13D: Species with the "concrescens" form represent the largest known cladorhizids. The habit is composed of a single or branching stem, the upper part of which is set with branches projecting in all directions. Branches have inflatable swellings, usually terminally, but subterminally in the case of at least one species (*C. (C.) virgata*). The upper part may be elongated and comprising most of the stem, or be reduced in length to the top part, in what Koltun (1970) evocatively referred to as a "scepter"-form. Isochelae most often have 6-9 teeth with a couple of exceptions. A rough covering layer usually containing finely spiny styles and a system of rhizoids is usually reported for specimens where the basal part is recovered. About an equal number of species have been reported from the Atlantic and Pacific, and species are known both from upper bathyal, lower bathyal and abyssal depths. This group is polyphyletic with regards to "Crinorhiza" forms in the current systematics. Species: *C. (C.) asigmata*, *C. (C.) burtoni*, *C. (C.) concrescens*, *C. (C.) dichotoma*, *C. (C.)*

grandis, *C. (C.) koltuni*, *C. (C.) lampadiglobus*, *C. (C.) multichela*, *C. (C.) nicolae*, *C (C.) robertballardi*, *C. (C.) saffroni*, *C. (C.) schlatteri*, *C. (C.) verticillata*, *C. (C.) virgata*, *C. (C.) yatsui*. The species *C. (C.) magna* is also placed here tentatively, though the description of this species (Tanita, 1965) is somewhat ambiguous; lacking size measurements and with no swellings on branches.

3.4.4 *Cladorhiza*

Diagnosis. Cladorhizidae with only anchorate/unguiferate anisochelae (from Lopes and Hajdu, 2014).

Cladorhiza contains cladorhizid species with anchorate anisochelae, including the first cladorhizid species described, *Cladorhiza abyssicola* Sars, 1872. The genus has an uncomplicated taxonomic history, and was recovered as a monophyletic group in the phylogenetic analyses of Paper IV as sister group to *Chondrocladia*.

Most *Cladorhiza* species may either have an arbuscular, single-stem, or stipitate morphology (Reiswig & Lee, 2007). In the latter case, the body, suspended on a peduncle, may be cup-shaped or umbrella-shaped, with filaments emerging from the rim, and the main stem protruding through the body and creating an apical extension. Interestingly, stipitate *Cladorhiza* species have anchorate anisochelae with three teeth, while arbuscular forms have five teeth. Additionally, stipitate forms are generally smaller, and are typically found at greater depths than larger, branching forms, a situation that mirrors that of *Chondrocladia*. Indeed, “Crinorhiza” form was actually used by early authors (e.g. Ridley & Dendy, 1886) to also refer to stipitate species of *Cladorhiza* in addition to *Chondrocladia* (Fig. 14).

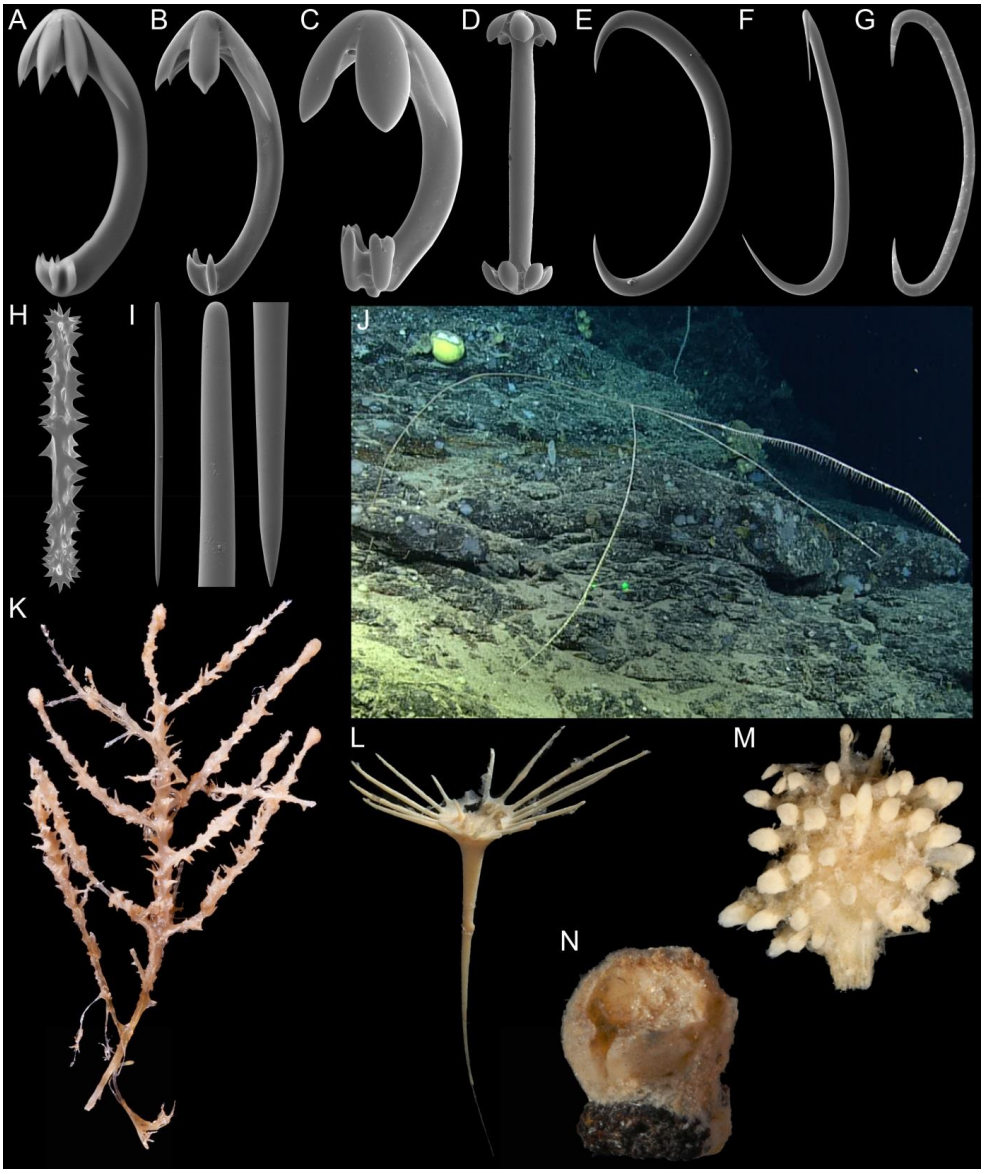

Figure 14. Variation of spicule and habit morphology found within currently described *Cladorhiza*. All images from own data. A) Five-toothed anisochela, *C. abyssicola*; B) tridentate anisochela, *C. moruliformis*; C) tridentate anisochela with lower teeth bifurcated, *C. tridentata*; D) birotula-like "pseudoamphiaster", *C. mirabilis*; E) sigma, *C. abyssicola*; F) sigmancistra, *C. abyssicola*; G) sigma, *C. moruliformis*; H) acanthoxea, *C. kenchingtonae*; I) mycalostyle, *C. abyssicola*. Habit of J) *C. kenchingtonae*, K) *C. abyssicola*, L) *C. inversa*, M) *C. tridentata* and N) *C. moruliformis*.

The parallel division in both *Cladorhiza* and *Chondrocladia* into stipitate tridentate and arbuscular five-toothed chela forms is puzzling, and raises a possible alternative hypothesis: That stipitate *Cladorhiza* species might be more closely related to stipitate *Chondrocladia* species rather than the rest of *Cladorhiza*, making *Cladorhiza* polyphyletic. On the other hand, evolutionary pressure adapting to lower bathyal and abyssal depths could have induced parallel evolution in both closely related genera, with similar morphological results, though it does not adequately explain the reduction in number of teeth in both clades. Regrettably, current molecular data is heavily biased towards the arbuscular North Atlantic species such as *C. abyssicola* and its relatives, and no sequences are currently available for stipitate *Cladorhiza*, and this hypothesis would need additional evidence to test its viability (Fig. 15).

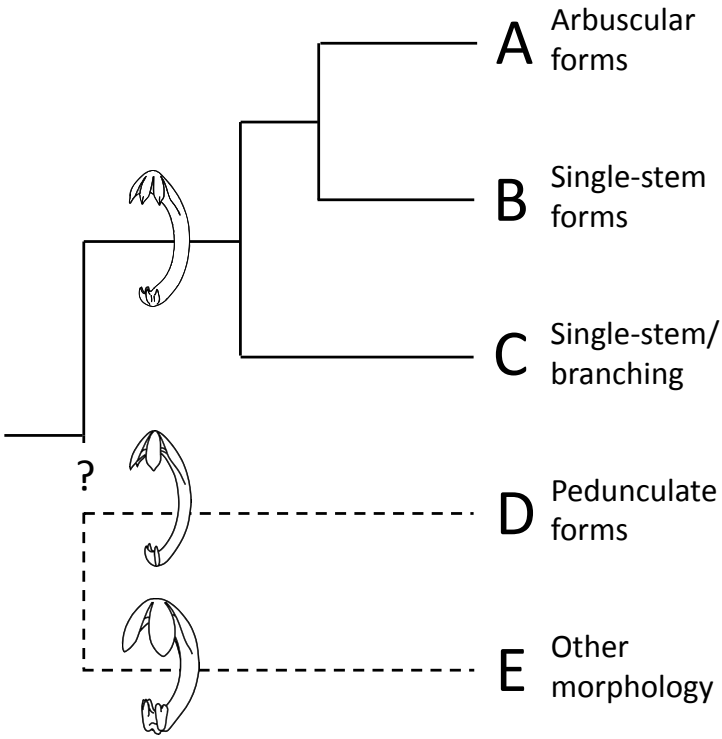

Figure 15. A schematic overview of morphological variation within *Cladorhiza*. Solid lines indicate clades that were recovered with at least one species in the phylogeny in Paper IV. Dotted lines indicate hypothetical relationships based on a qualitative evaluation of morphological characters only, and should be regarded as tentative.

Fig. 15A: *Cladorhiza* species with a branching, bush-like morphology such as the type species of the genus, *C. abyssicola*. Branches are set with filaments in all directions and often end in slight swellings that are associated with reproductive structures. Spicules usually include one type of mycalostyle, five-toothed anchorate anisochelae, sigmas and contorted sigmancistras. Sigmancistras may be rare or absent, and are usually associated with branch end swellings. Known species are almost exclusively Atlantic, and often found in comparatively shallow waters (e.g. 200-2000 m), though some species are deeper. Species: *C. abyssicola*, *C. corticocancellata*, *C. gelida*, *C. iniquidentata*, *C. methanophila*, *C. oxeata*, *C. scanloni*, *C. tenuisigma*, *C. thomsoni*.

Fig. 15B: *Cladorhiza* species with a single-stem morphology set with filaments in all directions ("bottle-brush" shape). Spicule complement similar to arbuscular forms, with mycalostyles, five-toothed (sometimes seven) anchorate anisochelae, sigmas, and often contorted sigmancistras. Some species are described from fragments and are placed here simply based on lack of evidence of branching. A couple of Atlantic representatives, but mostly Pacific, typically depths of 2000 m and below. Species: *C. acanthoxea*, *C. caillieti*, *C. evae*, *C. grimaldii*, *C. linearis*, *C. penniformis*, *C. rectangularis*, *C. segonzaci*, *C. septemdentalis*. Also possibly *C. microchela* (tridentate).

Fig. 15C: *Cladorhiza kenchingtonae*: A long filiform, basal stem, a middle branching point, and three long filiform branches ~2 m long, with two ventral, oblique filament rows. No known relative (though possibly one or more species known only from fragments could have a similar morphology). Two types of mycalostyle, five-toothed anchorate anisochelae, acanthoxeas and sigmas. Placement here based on molecular evidence from Paper III.

Fig. 15D: Pedunculate *Cladorhiza* species with tridentate anisochelae. Morphology variation includes conical umbrella and upturned umbrella-like species, spherical, pyriform, and derived morphology (e.g. *C. pteron*). Typically the peduncle grows though the sponge body and emerges apically, either as a short swelling or a longer,

filiform structure; a region that seems related to reproduction. Filaments are usually arranged laterally, either as an upwards or downwards-facing crown, or more loosely arranged outwards, similar to that of "Crinorhiza"-type *Chondrocladia* species. Atlantic, Pacific and Indian Ocean species. Many abyssal, but some also shallower. Typically smaller than 10 cm, with two known exceptions, the >30 cm species *C. pteron* and *C. corona*. Species: *C. arctica*, *C. bathycrinoides*, *C. corona*, *C. flosabyssi*, *C. inversa*, *C. longipinna*, *C. mani*, *C. mirabilis*, *C. moruliformis*, *C. nematophora*, *C. nicoleae*, *C. pentacrinus*, *C. pteron*, *C. similis*.

Fig. 15E: A few species with an uncommon or unclear morphology: The solid, cup-shaped *C. tridentata*; and the species *C. ephyrula* and *C. schistochela*, that though different in morphology, cannot readily be assigned to any of the other groups. All three species have tridentate anisochelae with unusually large upper teeth and articulated, bifurcated lower teeth.

3.4.5 *Lycopodina*

Diagnosis. Cladorhizidae pedunculate with body either in the form of an erect stem or sphere with filaments in all directions, or cup-shaped. Megascleres are mycalostyles and commonly shorter (tylo)styles. Microscleres are one type of arcuate or palmate anisochela where the smaller end is in the shape of a central plate and two rudimentary, flat, lateral teeth, all with serrated edges towards the middle. To this forceps spicules are often added, but may be rare or absent in particular species or specimens of a single species. Never sigmas or sigmancistras (from Paper IV).

Lycopodina contains species with a single type of palmate to arcuate anisochelae with a characteristic plate-like lower part, and in many cases with forceps spicules. Originally erected as a subgenus of *Asbestopluma* by Lundbeck (1905), *Lycopodina* was elevated to genus rank by de Laubenfels (1936), but was not considered valid until molecular data (Paper IV) showed that genus *Asbestopluma* was paraphyletic with respect to the species currently assigned to *Lycopodina*. Subgenus *Cotylina* Lundbeck, 1905, erected to account for stipitate *Lycopodina* species, is synonymized with *Lycopodina* based on molecular evidence. The genus is among the major

cladorhizid genera, with approximately 25 described species. Species are usually either single stem, in most cases brush-like with filaments in all directions; or they are small, pedunculate, with a spherical or cup-shaped body (Fig. 16).

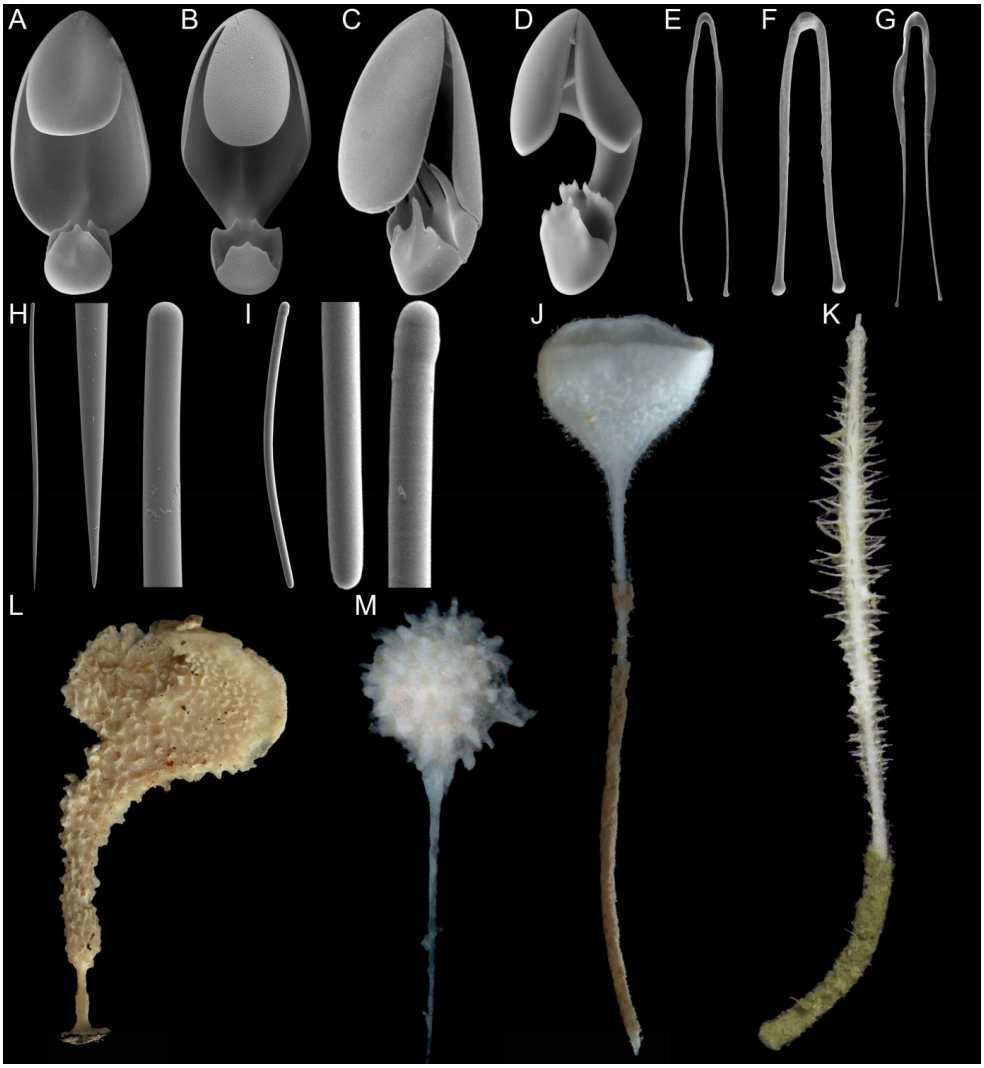

Figure 16. Variation of spicule and habit morphology found within currently described *Lycopodina*. All images from own data. A) Palmate anisochela, *L. lycopodium*; B) palmate anisochela, *L. cupressiformis*; C) palmate anisochela, *L. rastrichela*; D) arcuate anisochela, *L. infundibulum*; E) forceps, *L. lycopodium*; F) forceps, *L. cupressiformis*; G) forceps, *L. tendali*; H) style, *L. lycopodium*; I) microsubtylostrongyle, *L. cupressiformis*. Habit of J) *L. cupressiformis*, K) *L. parvula*, L) *L. infundibulum* and M) *L. lycopodium*.

The major difference in spicule morphology within the genus (excepting certain specific modifications such as the long comb-like extensions of the lower part of the anisochelae of *L. rastrichela*) is between the clearly palmate chelae of e.g. *L. lycopodium*, and the arcuate type of e.g. *L. infundibulum*. Based on currently known species and available molecular data, it would seem that clearly palmate anisochelae are more widespread and present in most of the genus, while the arcuate type is confined to a single, smaller clade within the genus associated with stipitate cup-shaped species.

Central to the current genus diagnosis is the presence of forceps spicules (uniquely among the Cladorhizidae) and lack of any sigmas or sigmancistras. There are some signs that this might be a simplification of actual phylogenetic relationships: The species *A. (A.) laminachela* Hestetun, Rapp and Xavier, 2016 from the Indian Ocean, while assigned to *Asbestopluma* based on the presence of sigmas, has several characters suggesting a placement within *Lycopodina* such as palmate chela morphology reminiscent of *Lycopodina* and a pedunculate morphology. Sigmas or sigmancistras are found in all other genera within Cladorhizidae, as well as in Mycalidae and Guitarridae, suggesting their secondary loss within *Lycopodina*, alternatively that forceps spicules are derived from sigmas. A probable explanation for the presence of sigmas within some *Lycopodina* species would be that these represent a basal branch within the genus, before loss/modification of sigmas in the rest of the genus.

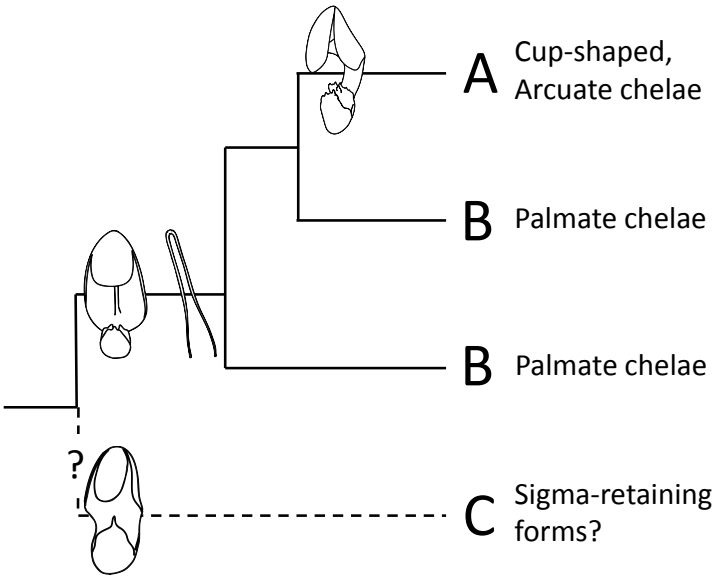

Figure 17. A schematic overview of morphological variation within *Lycopodina*. Solid lines indicate clades that were recovered with at least one species in the phylogeny in Paper IV. Dotted lines indicate hypothetical relationships based on a qualitative evaluation of morphological characters only, and should be regarded as tentative.

Fig. 17A: A particular form of arcuate morphology retaining the plate-like lower part of the spicule diagnostic for *Lycopodina* is associated with a cup-shaped morphology within the genus. The molecular results in Paper IV established that this group is a derived clade within the rest of the *Lycopodina*, and the arcuate form should thus be considered a modification of the palmate form in group B. Species are usually smaller than 50 mm, with a cup-shaped body that may be closed off into a flattened pyriform shape, however. Sometimes the rim is lined with tiny filaments. Species are known from the Atlantic, Southern and Pacific Oceans. Species: *L. calyx*, *L. comata*, *L. infundibulum*, *L. infundibulum orientalis*, *L. minuta*, *L. pediculifera*, *L. rhaphidiophorus*, *L. versatilis*. Also placed here is *L. bilamellata*, a curious species made up of an elongated stem with two blade-like structures and that might be a modification of the cup-shaped habit, though the specie s description is not sufficient to establish precise morphology (Lévi, 1993).

Fig. 17B: This group contains species with the most common anisochela morphology in the genus: A clearly palmate anisochela with a plate-like lower end. Species are either elongate single stem or pedunculate spherical. In either case filaments emerge in all directions, and the stem morphology could thus be seen as an elongated version of the spherical or subspherical morphology. Common in the Atlantic, Pacific and Southern Oceans. Some species very shallow, e.g. *L. hypogea*, *L. vaceleti*; some very deep, e.g. *L. hadalis*, *L. parvula*, *L. wolffi* (*sensu* Koltun). Some confusion exists regarding the exact identification of *L. occidentalis-like* species from the North Pacific (e.g. *L. lebedi*, *L. hadalis*, *L. occidentalis*, *L. gracilis*). The species *L. globularis* has a "globular", deviating morphology from the rest. The species *L. rastrichela* has anisochelae where the lower plate tips have been extended into long protrusions. Species: *L. callithrix*, *L. communis*, *L. cupressiformis*, *L. drakensis*, *L. ecoprof*, *L. globularis*, *L. gracilis*, *L. hydra*, *L. hypogea*, *L. lebedi*, *L. lycopodium*, *L. hadalis*, *L. microstrongyla, L. novangliae*, *L. occidentalis*, *L. parvula*, *L. rastrichela*, *L. rhabdostylophora*, *L. robusta*, *L. ruijsi*, *L. tendali*, *L. vaceleti*. Possibly also including *L. wolffi sensu* Koltun, 1970.

Fig. 17C: If the argument is accepted that forceps spicules represent a derived form of the sigma spicules present in all related genera, there is a possibility that there exists a basal clade within *Lycopodina* with sigma-bearing forms. Group C represents a possible alternative hypothesis regarding the placement of the two species *A. (A.) flabellum* and *A. (A.) laminachela* that are currently placed in *Asbestopluma* on the basis of presence of sigmas, but otherwise have several characters lacking for that genus: Habit is small, pedunculate with filaments in all directions; a morphology otherwise not present in the genus; megascleres are polytylote and different from the common subtylostyle shape in *Asbestopluma*; finally the chelae of *A. (A.) laminachela*, while unique, are more similar to those of *Lycopodina*; the chelae of *A. (A.) flabellum* are not visible in detail from the species description.

3.4.6 Other cladorhizid genera

Other genera within *Cladorhizidae* include *Cercicladia*, *Eucheliplum*a, *Koltunicladia* (formerly *Neocladia*, see Paper IV) and *Lollipocladia*. Compared with the larger cladorhizid genera, containing around 140 species, these genera collectively contain only 11 species, and except *Eucheliplum*a, are monotypic. Molecular data shows that *Eucheliplum*a and *Cercicladia* are associated with *Abyssocladia*, though their exact positions are uncertain. Sequence data is lacking for *Koltunicladia* and *Lollipocladia*, though a combination of spicule and habit characters display some similarity to *Abyssocladia*, and possibly *Chondrocladia* and *Cladorhiza*. An overview of diagnostic spicules is given in Fig. 18.

*Eucheliplum*a contains five species and is defined by the presence of placochelae. This spicule type has an elaborate, ribbed morphology, and is also found within Guitarridae, where the genus was placed previous to the molecular evidence presented in Paper IV. *Eucheliplum*a species also have arcuate isochelae, a shared character with *Abyssocladia*, and the phylogenetic analyses recovered *Eucheliplum*a (with variable support, however) as a sister group to the rest of *Abyssocladia*. *Eucheliplum*a species have a single stem pennate or branching morphology, and have been reported from Japan, the Aleutian Islands, Cape Verde, the Caribbean and the Patagonian shelf.

The main diagnostic criterion of the monotypic genus *Cercicladia* (containing *C. australis* Ríos, Kelly & Vacelet, 2011) is the presence of ring-shaped modified chelae named cercichelae. For the molecular analyses, only the COI extension was successfully sequenced (592 bp, see paper IV), which placed it within *Abyssocladia*. Given the relative lack of molecular data, no systematic revision synonymizing *Cercicladia* with *Abyssocladia* was done in Paper IV. However, a similar type of reduction of a palmate anisochela in the *Asbestopluma* species *A. (A.) caribica* (Paper II), lends additional support to the hypothesis that cercichela morphology represents a reduction of an arcuate chela or cleistochela.

Koltunicladia and *Lollipocladia* are both monotypic genera containing *K. flabelliformis* (Koltun, 1970a) and *L. tiburoni* Vacelet, 2008 respectively. They are defined of the basis of strongly curved birotula-like microscleres and sigmancistras in the case of *Koltunicladia*, and large strongly curved anchorate isochelae in combination with palmate isochelae and sigmancistras in the case of *Lollipocladia*. Given the relative plasticity of spicule morphology and their stipitate disc-shaped habit, they could possibly be part of the *Abyssocladia* clade systematically, but their positions have not been tested with molecular evidence.

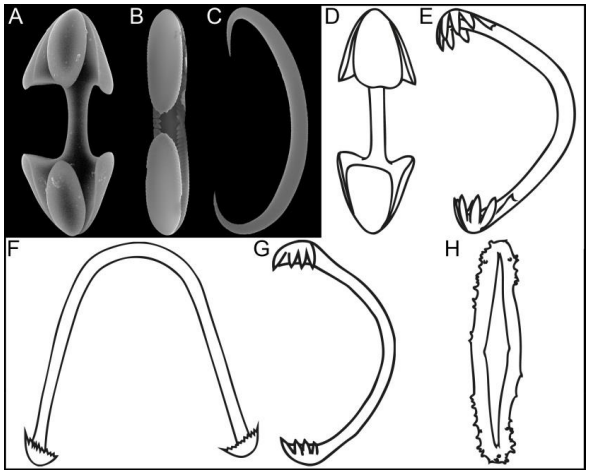

Figure 18. Variation of spicule and habit morphology found within other cladorhizid genera. First three images from own data, otherwise reproduced from Vacelet (2008), Koltun (1970a) and Ríos *et al.* (2011). A) palmate/arcuate isochela, *E. pristina*; B) placochela, *E.* n. sp.; C) sigmancistra, *E.* n. sp.; D) palmate/arcuate isochela, *L. tiburoni*; E) arched anchorate isochela, *L. tiburoni*; F) arched, birotula-like anchorate isochela, *K. flabelliformis*; G) arched anchorate isochela, *K. flabelliformis*; H) cercichela, *C. australis*.

4 Closing remarks

Twenty years after the discovery of carnivory in sponges, progress has been rapid in furthering knowledge of this exceptional group of sponges: Numerous studies have been published both greatly increasing the number of known species as well as elucidating aspects of physiology and reproduction within the group, and over 150 species have currently been described. Carnivory is an exceptional example of evolution using an existing body plan to develop a completely different life strategy in the face of selective evolutionary pressure, and is clearly a very successful development helping sponges colonize the deep sea.

The Atlantic is home to a species-rich, highly diverse cladorhizid fauna, and in a first for carnivorous sponges, sufficient information allowing for a complete updated set of species descriptions and biogeographic records have been compiled in a single publication in the case of the boreal North-Atlantic and Arctic. Records for other parts of the Atlantic are still at a more rudimentary stage, but progress has been made in several of these regions here and by other authors, to be added to in the future. Increasing the amount of available data allow increasingly accurate comparative estimates of biogeographic and depth distributions between cladorhizid species.

The work contained in this thesis has helped establish that carnivory arose only once within currently known sponges rather than in several separate lineages, a finding that has major implications for assessing the difficulty in transitioning from a filter-feeding to a carnivorous nutrient acquisition strategy. Furthermore, the fact that the only clade retaining a remnant aquiferous system is nested within other cladorhizid taxa shows that evolutionary pressure for reduction of the aquiferous system once carnivorous is high. Systematic reassignments here have simplified carnivorous sponge classification and reorganized it to correspond to phylogenetic relationships within the group, facilitating the addition of new species to Cladorhizidae and resolving long-standing questions regarding inter- and intrageneric relationships of the morphological variation found within carnivorous sponges.

The great amount of newly reported data is increasingly showing a more complete picture of total diversity within the carnivorous sponges, moving focus away from the relatively narrow previous understanding of the group based mostly on North Atlantic species, and showing that morphological variation within carnivorous sponges is greater than previously thought. The most striking example of this is *Abyssocladia*, a genus re-erected only ten years ago, now shown to contain over 20 species with a huge amount of spicule variation, and found in all of the world's major oceans, but a similar re-appreciation of intrageneric diversity has also happened in, for instance, *Asbestopluma* and *Lycopodina*.

Finally, microbiome studies, which aided by NGS techniques have started to become more prevalent in sponges in general, are still in their infancy for carnivorous sponges. However, results from this thesis represent the first multi-species investigation into carnivorous sponge microbial communities, showing that carnivorous sponges have a distinct microbial composition that is partly overlapping from species to species. They also show that Cladorhiza methanophila represents an exception in its use of chemoautotrophic symbionts to obtain a significant part of its nutrition among currently known carnivorous sponges. Given the affinity of carnivorous sponges to vent and seep areas around the world, it is possible, however, that new examples of similar symbioses will be reported in the future.

5 Future perspectives

Cladorhizid species continue to be described at a high rate. This is highlighting the diversity within the carnivorous sponges as new species have new spicule morphologies, but is also adding species with similar spicule modifications to previous species with morphologies deviating from the standard of the genus, giving a fuller picture of the diversity and relative size of different clades of carnivorous sponges, and bridging gaps between known species.

This diversity also highlights the evolution and plasticity of spicule morphology, showing that spicules by themselves are not always sufficient to establish systematic relationships in the Cladorhizidae, and increasing the strain on spicule-based diagnoses, which have tended to become longer in recent times, with repeated amendments.

Future species will undoubtedly shed more light on the relationships between groups of carnivorous sponges, especially when morphological characters are augmented by molecular data. While the classification presented in Paper IV has provided a much needed update to existing systematics of the group and answered basic questions regarding the monophyly of family Cladorhizidae, new data will undoubtedly lead to further revisions and amendments in the future.

As the monophyly of carnivorous sponges has been more securely established, questions remain about the exact relationship between Cladorhizidae and the two closest related families Guitarridae and Mycalidae, with the goal of identifying preadaptations that could explain the evolutionary event leading to carnivory in Cladorhizidae. In addition to morphological studies, NGS sequencing methods would make it feasible to look further into differences in gene expression and microbiome components on either side of the carnivorous habit. Special interest could be given to genus *Chondrocladia*: Some species (such as *Meliiderma* spp.) seem to lack a remnant aquiferous system present in the rest of the genus. The role of the aquiferous

system and associated gene expression would prove an interesting study, given the apparent strong evolutionary pressure to reduce this system entirely.

The microbiome work in this thesis has increased knowledge of the diversity of microbial organisms within Cladorhizidae. Prokaryotes have an important contribution to the metabolism and working of the host sponges. Future work could more thoroughly look into the pathways and enzymatic activity utilized by prokaryote symbionts in the metabolism of the host. In terms of autotrophy, only *C. methanophila* is as yet known, with few other observations of such high biomass right at sites of emission, rather more usual slightly higher biomass feeding on vent animals. This still remains the most obvious use of autotrophic symbionts in a vent and seep setting among sponges in general, and no autotrophic activity was found even in closely related species. The possibility thus exists that further exploration of vent and seep habitats will yield additional autotrophic symbioses between autotrophic symbionts and carnivorous sponges.

While stemming from a single evolutionary event, carnivory in sponges has proved to be a successful strategy, and from being treated as exotics, increasingly, it is shown that carnivorous sponges are present in a variety of habitats worldwide, also in shallower habitats. Carnivory represents a radical departure from the usual habit of sponges, and thus can give valuable insights into early animal evolution.

6 References

Adamska, M., Degnan, B., Green, K. and Zwafink, C. (2011). What sponges can tell us about the evolution of developmental processes. *Zoology*, **114**. doi:10.1016/j.zool.2010.10.003

Aguilar, R., Correa, M.L., Calcinai, B., Pastor, X., De la Torriente, A. and Garcia, S. (2011). First records of *Asbestopluma hypogea* Vacelet and Boury-Esnault, 1996 (Porifera, Demospongiae Cladorhizidae) on seamounts and in bathyal settings of the Mediterranean Sea. *Zootaxa*, (2925), 33–40.

Antcliffe, J.B., Callow, R.H. and Brasier, M.D. (2014). Giving the early fossil record of sponges a squeeze. *Biological Reviews*, **89**(4), 972-1004. doi:10.1111/brv.12090

Arnesen, E. (1920). "Spongia". *Report on the Scientific Results of the "Michael Sars" North Atlantic Deep-Sea Expedition, 1910.* vol. III, part 2, Bergen: John Grieg, pp 1–29.

Babić, K. (1922). Monactinellida und Tetractinellida des Adriatischen Meeres. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere*, **46**(2), 217–302.

Bakran-Petricioli, T., Vacelet, J., Zibrowius, H., Petricioli, D. and Chevaldonné, P. (2007). New data on the distribution of the 'deep-sea'sponges *Asbestopluma hypogea* and *Oopsacas minuta* in the Mediterranean Sea. *Mar. Ecol.*, **28**(s1), 10-23. doi:10.1111/j.1439-0485.2007.00179.x

Belinky, F., Szitenberg, A., Goldfarb, I., Feldstein, T., Wörheide, G., Ilan, M.*, et al.* (2012). ALG11–A new variable DNA marker for sponge phylogeny: Comparison of phylogenetic performances with the 18S rDNA and the COI gene. *Mol. Phylogen. Evol.*, **63**(3), 702-713. doi:10.1016/j.ympev.2012.02.008

Bergquist, P.R. (1978). *Sponges*. Berkeley and Los Angeles: University of California Press.

Borchiellini, C., Manuel, M., Alivon, E., Boury-Esnault, N., Vacelet, J. and Le Parco, Y. (2001). Sponge paraphyly and the origin of Metazoa. *J. Evol. Biol.*, **14**(1), 171-179. doi:10.1046/j.1420-9101.2001.00244.x

Boury-Esnault, N., Pansini, M. and Uriz, M.J. (1994). Spongiaires bathyaux de la mer d'Alboran et du golfe ibéro-marocain. *Mémoires du Muséum national d'Histoire naturelle*, (160), 1-174.

Boury-Esnault, N. and Rützler, K. (1997). *Thesaurus of Sponge Morphology*. Washington, D.C.: Smithsonian Institution Press.

Bowerbank, J. (1862). On the anatomy and physiology of the Spongiadae. Part II. *Philosophical transactions of the Royal Society of London*, **152**, 747-836.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, **7**(5), 335-336. doi:10.1038/nmeth.f.303

Cárdenas, P., Pérez, T. and Boury-Esnault, N. (2012). Advances in Sponge Science: Phylogeny, Systematics, Ecology. Chapter 2: Sponge Systematics Facing New Challenges. *Adv. Mar. Biol.*, **61**, 79-209. doi:10.1016/b978-0-12-387787-1.00010-6

Carter, H.J. (1874). Descriptions and Figures of Deep-sea Sponges and their Spicules from the Atlantic Ocean, dredged up on board H.M.S.'Porcupine', chiefly in 1869; with Figures and Descriptions of some remarkable Spicules from the Agulhas Shoal and Colon, Panama. *Annals and Magazine of Natural History (4)*, **14**(79), 207-221, 245-257, pls.XIII-XV.

Carter, H.J. (1876). Descriptions and Figures of Deep-Sea Sponges and their Spicules, from the Atlantic Ocean, dredged up on board H.M.S.'Porcupine', chiefly in 1869 (concluded). *Annals and Magazine of Natural History (4)*, **18**(105-108), 226-240, 307-324, 388-410, 458-479, pls XII-XVI.

Carter, H.J. (1882). Some sponges from the West Indies and Acapulco in the Liverpool Free Museum described, with general and classificatory remarks. *Annals and Magazine of Natural History (5)*, **9**(52), 266-301, 346-368, pls XI-XII.

Castello-Branco, C., Hestetun, J.T., Rapp, H.T. and Hajdu, E. (2016). Taxonomy of *Cladorhiza* in the deep SW Atlantic: *C. nicoleae* sp. nov. and redescription of *C. inversa* (Cladorhizidae, Poecilosclerida, Demospongiae). *J. Mar. Biol. Assoc. U.K.*, **96**(02), 297-303. doi:10.1017/S0025315415000211

Chevaldonné, P., Pérez, T., Crouzet, J.M., Bay-Nouailhat, W., Bay-Nouailhat, A., Fourt, M., *et al.* (2015). Unexpected records of 'deep-sea'carnivorous sponges *Asbestopluma hypogea* in the shallow NE Atlantic shed light on new conservation issues. *Mar. Ecol.*, **36**(3), 475-484. doi:10.1111/maec.12155

Chombard, C., Boury-Esnault, N., Tillier, A. and Vacelet, J. (1997). Polyphyly of "sclerosponges" (Porifera, Demospongiae) supported by 28S ribosomal sequences. *The Biological Bulletin*, **193**(3), 359-367. doi:10.2307/1542938

Chu, J.W. and Reiswig, H.M. (2014). Mechanisms of propagule release in the carnivorous sponge *Asbestopluma occidentalis*. *Invertebr. Biol.*, **133**(2), 109-120. doi:10.1111/ivb.12045

Cristobo, F.J., Ríos, P., Pomponi, S.A. and Xavier, J.R. (2015). A new carnivorous sponge, *Chondrocladia robertballardi* sp. nov. (Porifera: Cladorhizidae) from two north-east Atlantic seamounts. *J. Mar. Biol. Assoc. U.K.*, **95**(7), 1345-1352. doi:10.1017/S0025315414001325

Cristobo, F.J., Urgorri, V. and Ríos, P. (2005). Three new species of carnivorous deep-sea sponges from the DIVA-1 expedition in the Angola Basin (South Atlantic). *Org Divers Evol*, **5**, 203-213. doi:10.1016/j.ode.2004.11.004

de Laubenfels, M.W. (1936). *A Discussion of the Sponge Fauna of the Dry Tortugas in Particular and the West Indies in General, with Material for a Revision of the Families and Orders of the Porifera*. Carnegie Institute of Washington Publication 467.

Dendy, A. (1922). The Percy Sladen Trust Expedition to the Indian Ocean in 1905, under the Leadership of Mr. J. Stanley Gardiner, M.A. Volume VII. No. I.—Report on the Sigmatotetraxonida collected by H.M.S. "Sealark" in the Indian Ocean. *Transactions of the Linnean Society of London. 2nd Series: Zoology*, **18**(1), 1-164, pls 161-118.

Dendy, A. (1924). "Porifera. Part I. Non-Antarctic sponges". *Natural History Report. British Antarctic (Terra Nova) Expedition, 1910.* vol. Zoology 6(3), pp 269-392, pls I-XV.

Desbruyères, D., Biscoito, M., Caprais, J.-C., Colaço, A., Comtet, T., Crassous, P.*, et al.* (2001). Variations in deep-sea hydrothermal vent communities on the Mid-Atlantic Ridge near the Azores plateau. *Deep Sea Research Part I: Oceanographic Research Papers*, **48**(5), 1325-1346. doi:10.1016/S0967-0637(00)00083-2

dos Reis, M., Thawornwattana, Y., Angelis, K., Telford, Maximilian J., Donoghue, Philip C.J. and Yang, Z. (2015). Uncertainty in the timing of origin of animals and the limits of precision in molecular timescales. *Curr. Biol.*, **25**(22), 2939-2950. doi:10.1016/j.cub.2015.09.066

Downey, R.V. and Janussen, D. (2015). New insights into the abyssal sponge fauna of the Kurile–Kamchatka plain and trench region (Northwest Pacific). *Deep Sea Research Part II: Topical Studies in Oceanography*, **111**, 34-43. doi:10.1016/j.dsr2.2014.08.010

Dressler-Allame, M., Göcke, C., Kersken, D., Plotkin, A. and Janussen, D. (2016). Carnivorous sponges (Cladorhizidae) of the deep Weddell Sea, with descriptions of two new species. *Deep Sea Research Part II: Topical Studies in Oceanography*. doi:10.1016/j.dsr2.2016.08.006

Dunn, C.W., Hejnol, A., Matus, D.Q., Pang, K., Browne, W.E., Smith, S.A.*, et al.* (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*, **452**(7188), 745-749. doi:10.1038/nature06614

Dunn, C.W., Leys, S.P. and Haddock, S.H.D. (2015). The hidden biology of sponges and ctenophores. *Trends Ecol. Evol.*, **30**(5), 282-291. doi:10.1016/j.tree.2015.03.003

Dupont, S., Carre-Mlouka, A., Domart-Coulon, I., Vacelet, J. and Bourguet-Kondracki, M.-L. (2014). Exploring cultivable Bacteria from the prokaryotic community associated with the carnivorous sponge *Asbestopluma hypogea*. *FEMS Microbiol. Ecol.*, **88**(1), 160-174. doi:10.1111/1574-6941.12279

Dupont, S., Corre, E., Li, Y., Vacelet, J. and Bourguet-Kondracki, M.-L. (2013). First insights into the microbiome of a carnivorous sponge. *FEMS Microbiol. Ecol.*, **86**(3), 520-531. doi:10.1111/1574-6941.12178

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*, **10**(10), 996-998. doi:10.1038/nmeth.2604

Ereskovsky, A.V. and Willenz, P. (2007). *Esperiopsis koltuni* sp. nov. (Demospongiae: Poecilosclerida: Esperiopsidae), a carnivorous sponge from deep water of the Sea of Okhotsk (North Pacific). *J. Mar. Biol. Assoc. U.K.*, **87**(6), 1379-1386. doi:10.1017/S0025315407058109

Erpenbeck, D., List-Armitage, S., Alvarez, B., Degnan, B.M., Wörheide, G. and Hooper, J.N.A. (2007). The systematics of Raspailiidae (Demospongiae: Poecilosclerida: Microcionina) re-analysed with a ribosomal marker. *J. Mar. Biol. Assoc. U.K.*, **87**(6), 1571-1576. doi:10.1017/S0025315407058201

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, **3**(5), 294.

Fortunato, S., Adamski, M., Bergum, B., Guder, C., Jordal, S., Leininger, S.*, et al.* (2012). Genome-wide analysis of the sox family in the calcareous sponge Sycon ciliatum: multiple genes with unique expression patterns. *EvoDevo*, **3**(1), 1-11. doi:10.1186/2041-9139-3-14

Fristedt, K. (1887). "Sponges from the Atlantic and Arctic Oceans and the Behring Sea". In A.E. Nordenskiöld (ed) *Vega-Expeditionens Vetenskapliga Iakttagelser.* vol. IV, Stockholm: F&G Beijers Förlag, pp 403-471.

Gazave, E., Lapébie, P., Ereskovsky, A.V., Vacelet, J., Renard, E., Cárdenas, P.*, et al.* (2012). No longer Demospongiae: Homoscleromorpha formal nomination as a fourth class of Porifera. *Hydrobiologia*, **687**(1), 3-10. doi:10.1007/s10750-011-0842-x

Goodwin, C., Berman, J., Downey, R. and Hendry, K. (in press). Carnivorous sponges (Porifera, Demospongiae, Poecilosclerida, Cladorhizidae) from the Drake Passage (Southern Ocean) with a description of eight new species and a review of the family Cladorhizidae in the Southern Ocean. *Invertebr. Syst.*

Gorbunov, G. (1946). Bottom life of the Novosiberian shoalwaters and the central part of the Arctic Ocean [In Russian]. *Transactions of the Drifting Expedition of the Main Administration of the Northern Sea Route on the Icebreaker" G. Sedov" 1937-1940*, **3**, 30-138.

Göcke, C., Hestetun, J.T., Uhlir, C., Freiwald, A., Beuck, L. and Janussen, D. (2016). *Cladorhiza corallophila* sp. nov., a new carnivorous sponge (Cladorhizidae, Demospongiae) living in close association with *Lophelia pertusa* and *Madrepora oculata* (Scleractinia). *Zootaxa*, **4168**(3), 512-524.

Hajdu, E., de Paula, T.S., Redmond, N.E., Cosme, B., Collins, A.G. and Lôbo-Hajdu, G. (2013). Mycalina: another crack in the Poecilosclerida framework. *Integr. Comp. Biol.*, **53**(3), 462-472. doi:10.1093/icb/ict074

Hajdu, E. and Vacelet, J. (2002). "Family Cladorhizidae Dendy, 1922". In J.N. Hooper and R.W. van Soest (eds) *Systema Porifera. A guide to the classification of sponges.* vol. 1, New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/ Plenum, pp 636-641.

Hajdu, E., van Soest, R.W.M. and Hooper, J.N.A. (1994). "Proposal for a phylogenetic subordinal classification of poecilosclerid sponges". In R.W. van Soest, T.M. van Kempen and J.-C. Braekman (eds) *Sponges in Time and Space.* Rotterdam: Balkema, pp 123-139.

Halanych, K.M. (2015). The ctenophore lineage is older than sponges? That cannot be right! Or can it? *J. Exp. Biol.*, **218**(4), 592-597. doi:10.1242/jeb.111872

Hansen, G.A. (1885). "Spongiadæ". *The Norwegian North-Atlantic Expedition 1876-1878.* vol. 13 (Zoology), pp 1-26, pls I-VII.

Hejnol, A., Obst, M., Stamatakis, A., Ott, M., Rouse, G.W., Edgecombe, G.D*., et al.* (2009). Assessing the root of bilaterian animals with scalable phylogenomic methods. *Proceedings of the Royal Society of London B: Biological Sciences*, **276**(1677), 4261-4270. doi:10.1098/rspb.2009.0896

Hentschel, E. (1914). "Monaxone Kieselschwämme und Hornschwämme der Deutschen Südpolar-Expedition 1901-1903". *Deutsche Südpolar-Expedition, 1901-03.* vol. XV-1, pp 35-141, pls IV-VIII.

Hentschel, E. (1923). "Erste Unterabteilung der Metazoa: Parazoa, Porifera-Schwämme". In W. Kükenthal and T. Krumbach (eds) *Handbuch der Zoologie. Eine Naturgeschichteder Stämme des Tierreiches. Vol. 1, Protozoa, Porifera, Coelenterata, Mesozoa.* Berlin and Leipzig: Walter de Gruyter und Co.

Hentschel, U., Hopke, J., Horn, M., Friedrich, A.B., Wagner, M., Hacker, J*., et al.* (2002). Molecular evidence for a uniform microbial community in sponges from different oceans. *Appl. Environ. Microbiol.*, **68**(9), 4431-4440. doi:10.1128/AEM.68.9.4431-4440.2002

Hentschel, U., Piel, J., Degnan, S.M. and Taylor, M.W. (2012). Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology*, **10**(9), 641-654. doi:10.1038/nrmicro2839

Hestetun, J., Fourt, M., Vacelet, J., Boury-Esnault, N. and Rapp, H. (2015). Cladorhizidae (Porifera, Demospongiae, Poecilosclerida) of the deep Atlantic collected during Ifremer cruises, with a biogeographic overview of the Atlantic species. *J. Mar. Biol. Assoc. U.K.*, **95**(7), 1311-1342. doi:10.1017/S0025315413001100

Hestetun, J.T., Rapp, H.T. and Xavier, J. (2016). Carnivorous sponges (Porifera, Cladorhizidae) from the Southwest Indian Ocean Ridge seamounts. *Deep Sea Research Part II: Topical Studies in Oceanography*, 1-24. doi:10.1016/j.dsr2.2016.03.004

Hoffmann, F., Radax, R., Woebken, D., Holtappels, M., Lavik, G., Rapp, H.T., *et al.* (2009). Complex nitrogen cycling in the sponge *Geodia barretti*. *Environ. Microbiol.*, **11**(9), 2228-2243. doi:10.1111/j.1462-2920.2009.01944.x

Hooper, J.N. and van Soest, R.W. (2002a). "Systema Porifera. A guide to the classification of sponges". *Systema Porifera.* Springer, pp 1-7.

Hooper, J.N.A. and Lévi, C. (1989). *Esperiopsis desmophora* n. sp. (Porifera: Demospongiae): a desma-bearing Poecilosclerida. *Mem. Queensl. Mus.*, **27**(2), 437-441.

Hooper, J.N.A. and van Soest, R.W.M. (2002b). "Order Poecilosclerida Topsent, 1928". In J.N. Hooper and R.W. van Soest (eds) *Systema Porifera. A guide to the classification of sponges*. vol. 1, New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/ Plenum, pp 403-408.

Huang, D., Meier, R., Todd, P.A. and Chou, L.M. (2008). Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. *J. Mol. Evol.*, **66**(2), 167-174. doi:10.1007/s00239-008-9069-5

Ise, Y. and Vacelet, J. (2010). New carnivorous sponges of the genus *Abyssocladia* (Demospongiae, Poecilosclerida, Cladorhizidae) from Myojin Knoll, Izu-Ogasawara Arc, southern Japan. *Zool. Sci.*, **27**(11), 888-894. doi:10.2108/zsj.27.888

Janussen, D. and Tendal, O.S. (2007). Diversity and distribution of Porifera in the bathyal and abyssal Weddell Sea and adjacent areas. *Deep Sea Research Part II: Topical Studies in Oceanography*, **54**(16), 1864-1875. doi:10.1016/j.dsr2.2007.07.012

Kelly, M. and Samaai, T. (2002). "Suborder Latrunculina subord. nov. incertae sedis.". In J.N. Hooper and R.W. van Soest (eds) *Systema Porifera. A guide to the classification of sponges*. vol. 1, New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/ Plenum, pp 707.

Kelly, M. and Vacelet, J. (2011). Three new remarkable carnivorous sponges (Porifera, Cladorhizidae) from deep New Zealand and Australian (Macquarie Island) waters. *Zootaxa*, (2976), 55-68.

Kennedy, J., Flemer, B., Jackson, S.A., Morrissey, J.P., O'Gara, F. and Dobson, A.D. (2014). Evidence of a putative deep sea specific microbiome in marine sponges. *PLoS ONE*, **9**(3), e91092. doi:10.1371/journal.pone.0091092

Koltun, V.M. (1955). Sponges. Atlas of the Invertebrate Fauna of the Far-Eastern Seas of the USSR. [In Russian]. *Akademiya Nauk SSSR*, 45-50, pls IV-VI.

Koltun, V.M. (1959). "Siliceous horny sponges of the northern and fareastern seas of the U.S.S.R. [In Russian]". *Opredeliteli po faune SSR, izdavaemye Zoologicheskim muzeem Akademii nauk. 67.* pp 1-236.

Koltun, V.M. (1964). Sponges (Porifera) collected in the Greenland seas and from the region to the north of the Spitzbergen and Franz Josef Land, from expeditions of the 'F. Litke' 1955, 'Obb' 1956 and 'Lena' 1957 and 1958. Scientific results of the higher latitudes. Oceanographic Expeditions to the northern part of the Greenland Sea and the adjacent arctic basin (In Russian). *Publications Arctic Antarctic Scientific Institute*, (259), 143-166.

Koltun, V.M. (1970a). "Sponge fauna of the northwestern Pacific from the shallows to the hadal depths". In V. Bogorov (ed) *Fauna of the Kurile-Kamchatka Trench and its environment. Institute of Oceanology of the Academy of Sciences of the U.S.S.R. [English translation, 1972]*. vol. 86, Jerusalem: Israel Program for Scientific Translations Ltd., pp 1-372, pls I-VIII.

Koltun, V.M. (1970b). Sponges of the Arctic and Antarctic: a faunistic review. *Symp Zool Soc Lond*, **25**, 285-297.

Kübler, B. and Barthel, D. (1999). A carnivorous sponge, *Chondrocladia gigantea* (Porifera: Demospongiae), the giant deepsea clubsponge from the Norwegian Trench. *Mem. Queensl. Mus.*, **44**, 289-298.

Lambe, L.M. (1893). Sponges from the Pacific coast of Canada. *Proceedings and Transactions of the Royal Society of Canada*, **11**(4), 25-43, pls II-IV.

Lambe, L.M. (1900). Sponges from the coasts of northeastern Canada and Greenland. *Trans R Soc Can*, (6), 19-49.

Lanna, E. (2015). Evo-devo of non-bilaterian animals. *Genet. Mol. Biol.*, **38**, 284-300. doi:10.1590/S1415-475738320150005

Lee, W.L., Reiswig, H.M., Austin, W.C. and Lundsten, L. (2012). An extraordinary new carnivorous sponge, *Chondrocladia lyra*, in the new subgenus *Symmetrocladia* (Demospongiae, Cladorhizidae), from off of northern California, USA. *Invertebr. Biol.*, **131**(4), 259-284. doi:10.1111/Ivb.12001

Lehnert, H., Stone, R. and Heimler, W. (2006). New species of deep-sea demosponges (Porifera) from the Aleutian Islands (Alaska, USA). *Zootaxa*, (1250), 1-35.

Lehnert, H., Watling, L. and Stone, R. (2005). *Cladorhiza corona* sp. nov. (Porifera : Demospongiae : Cladorhizidae) from the Aleutian islands (Alaska). *J. Mar. Biol. Assoc. U.K.*, **85**(6), 1359-1366. doi:10.1017/S0025315405012531

Lévi, C. (1964). "Spongiaires des zones bathyale, abyssale et hadale". *Galathea Report. Scientific Results of The Danish Deep-Sea Expedition Round the World, 1950-52.* vol. 7, pp 63-112, pls II-XI.

Levinsen, G.M.R. (1887). "Kara-Havets Svampe (Porifera)". *Dijmphna-Togtets zoologisk-botaniske Udbytte.* vol. 1, pp 339-372, pls XXIX-XXXI.

Lopes, D.A., Bravo, A. and Hajdu, E. (2011). New carnivorous sponges (Cladorhizidae: Poecilosclerida: Demospongiae) from off Diego Ramírez Archipelago (south Chile), with comments on taxonomy and biogeography of the family. *Invertebr. Syst.*, **25**(5), 407-443. doi:10.1071/is11015

Lopes, D.A. and Hajdu, E. (2014). Carnivorous sponges from deep-sea coral mounds in the Campos Basin (SW Atlantic), with the description of six new species (Cladorhizidae, Poecilosclerida, Demospongiae). *Mar. Biol. Res.*, **10**(4), 329-356. doi:10.1080/17451000.2013.797587

Lundbeck, W. (1905). "Porifera. (Part II.) Desmacidonidae (pars.)". *The Danish Ingolf-Expedition 6(2).* Copenhagen: BiancoLuno, pp 1-219, pls I-XX.

Lundsten, L., Reiswig, H.M. and Austin, W.C. (2014). Four new species of Cladorhizidae (Porifera, Demospongiae, Poecilosclerida) from the Northeast Pacific. *Zootaxa*, **3786**(2), 101-123. doi:10.11646/zootaxa.3786.2.1

Maldonado, M., Aguilar, R., Bannister, R.J., Bell, J., Conway, K.W., Dayton, P.K.*, et al.* (2016). "Sponge grounds as key marine habitats: a synthetic review of types, structure, functional roles, and conservation concerns". In S. Rossi, L. Bramanti, A. Gori and C. Orejas (eds) *Marine Animal Forests – The Ecology of Benthic Biodiversity Hotspots.* Switzerland: MeteorSpringer.

Maldonado, M. and Riesgo, A. (2008). Reproduction in Porifera: a synoptic overview. *Treballs de la Societat Catalana de Biologia*, (59), 24-49. doi:10.2436/20.1501.02.56

Marshall, W. (1876). Ideen über die Verwandtschaftsverhältnisse der Hexactinelliden. *Zeitschrift für wissenschaftliche Zoologie*, **27**(1), 113-136.

Morrow, C.C. and Cárdenas, P. (2015). Proposal for a revised classification of the Demospongiae (Porifera). *Front Zool*, **12**(7), 1-27. doi:10.1186/s12983-015-0099-8

Morrow, C.C., Picton, B.E., Erpenbeck, D., Boury-Esnault, N., Maggs, C.A. and Allcock, A.L. (2012). Congruence between nuclear and mitochondrial genes in Demospongiae: a new hypothesis for relationships within the G4 clade (Porifera: Demospongiae). *Mol. Phylogen. Evol.*, **62**(1), 174-190. doi:10.1016/j.ympev.2011.09.016

Morrow, C.C., Redmond, N.E., Picton, B.E., Thacker, R.W., Collins, A.G., Maggs, C.A.*, et al.* (2013). Molecular phylogenies support homoplasy of multiple morphological characters used in the taxonomy of Heteroscleromorpha (Porifera: Demospongiae). *Integr. Comp. Biol.*, **53**(3), 428-446. doi:10.1093/icb/ict065

Nardo, G. (1833). Auszug aus einem neuen System der Spongiarien, wonach bereits die Aufstellung in der Universitäts-Sammlung zu Padua gemacht ist. *Isis, oder Encyclopädische Zeitung Coll. Oken, Jena*, 519-523.

Nosenko, T., Schreiber, F., Adamska, M., Adamski, M., Eitel, M., Hammel, J.*, et al.* (2013). Deep metazoan phylogeny: When different genes tell different stories. *Mol. Phylogen. Evol.*, **67**(1), 223-233. doi:10.1016/j.ympev.2013.01.010

Philippe, H., Derelle, R., Lopez, P., Pick, K., Borchiellini, C., Boury-Esnault, N.*, et al.* (2009). Phylogenomics revives traditional views on deep animal relationships. *Curr. Biol.*, **19**(8), 706-712. doi:10.1016/j.cub.2009.02.052

Pisani, D., Pett, W., Dohrmann, M., Feuda, R., Rota-Stabelli, O., Philippe, H.*, et al.* (2015). Genomic data do not support comb jellies as the sister group to all other animals. *Proceedings of the National Academy of Sciences*, **112**(50), 15402-15407. doi:10.1073/pnas.1518127112

Redmond, N.E., Morrow, C.C., Thacker, R.W., Diaz, M.C., Boury-Esnault, N., Cárdenas, P.*, et al.* (2013). Phylogeny and systematics of Demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. *Integr. Comp. Biol.*, **53**(3), 388-415. doi:10.1093/icb/ict078

Reiswig, H.M. and Lee, W.L. (2007). A new species of *Cladorhiza* (Porifera: Cladorhizidae) from S California (USA). *Porifera research: biodiversity, innovation, sustainability. Série Livros*, **28**, 517-523.

Ridley, S.O. and Dendy, A. (1886). Preliminary Report on the Monaxonida collected by HMS 'Challenger'. *Annals and Magazine of Natural History*, **5**(18), 325–351, 470–493. doi:10.1080/00222938609459998

Ridley, S.O. and Dendy, A. (1887). "Report on the Monaxonida collected by H.M.S. 'Challenger' during the years 1873-1876". *Report on the Scientific Results of the Voyage of H.M.S. 'Challenger', 1873-1876. Zoology 20(59): i-lxviii.* Edinburgh: Neill and Company, pp 1-275, pls I-LI.

Riesgo, A. (2010). Phagocytosis of sperm by follicle cells of the carnivorous sponge *Asbestopluma occidentalis* (Porifera, Demospongiae). *Tissue and Cell*, **42**(3), 198-201. doi:10.1016/j.tice.2010.03.001

Riesgo, A., Taylor, C. and Leys, S.P. (2007). Reproduction in a carnivorous sponge: the significance of the absence of an aquiferous system to the sponge body plan. *Evol Dev*, **9**(6), 618-631. doi:10.1111/j.1525-142X.2007.00200.x

Ríos, P., Kelly, M. and Vacelet, J. (2011). *Cercicladia australis*, a new carnivorous sponge with novel chelae from the Tasman Basin and the Argentine Patagonian Margin (Porifera, Cladorhizidae). *Zootaxa*, (3131), 52-62. doi:10.11646/zootaxa.3131.1.3

Rot, C., Goldfarb, I., Ilan, M. and Huchon, D. (2006). Putative cross-kingdom horizontal gene transfer in sponge (Porifera) mitochondria. *BMC Evol. Biol.*, **6**(1), 71. doi:10.1186/1471-2148-6-71

Sars, G.O. (1872). *On Some Remarkable Forms of Animal Life from the Great Deeps Off the Norwegian Coast. Part 1, partly from posthumous manuscripts of the late professor Dr. Michael Sars. University Program for the 1st half-year 1869*. Christiania: Brøgger & Christie.

Schmidt, O. (1870). *Grundzüge einer Spongien-Fauna des atlantischen Gebietes*. Leipzig: Wilhelm Engelmann.

Schmidt, O. (1875). "Spongien". *Die Expedition zur physikalisch-chemischen und biologischen Untersuchung der Nordsee im Sommer 1872. Jahresbericht der Commission zur Wissenschaftlichen Untersuchung der Deutschen Meere in Kiel.* vol. 2-3, pp 115-120, pl I.

Schmidt, O. (1880). "Die Spongien des Meerbusen von Mexico (Und des caraibischen Meeres). Heft II. Abtheilung II. Hexactinelliden. Abtheilung III. Tetractinelliden. Monactinelliden und Anhang. Nachträge zu Abtheilung I (Lithistiden)". *Reports on the dredging under the supervision of Alexander Agassiz, in the Gulf of Mexico, by the USCSS 'Blake'.* Jena: Gustav Fischer, pp 33-90, pls V-X.

Sollas, W. On the physical characters of calcareous and siliceous sponge-spicules and other structures. *1885.* Royal Dublin Society.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M.*, et al.* (2007). Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience*, **57**(7), 573-583. doi:10.1641/B570707

Sperling, E.A., Pisani, D. and Peterson, K.J. (2007). Poriferan paraphyly and its implications for Precambrian palaeobiology. *Geological Society, London, Special Publications*, **286**(1), 355-368. doi:10.1144/sp286.25

Tanita, S. (1965). "Porifera". In Y. Okada, S. Uchida and T. Uchida (eds) *New Illustrated Encyclopedia of the Fauna of Japan [In Japanese]*. vol. 1, Tokyo: Hokuryukan, pp 138–166.

Taylor, M.W., Thacker, R.W. and Hentschel, U. (2007). Evolutionary insights from sponges. *Science*, **316**(5833), 1854-1855. doi:10.1126/science.1144387

Tendal, O.S. (1973). Sponges collected by the Swedish deep sea expedition. *Zool. Scr.*, **2**(1), 33-38.

Tendal, O.S. and Barthel, D. (1993). *Chondrocladia gigantea* (Demospongiae) - The giant clubsponge of the Northeast Atlantic. *Deep-Sea Newsl*, (20), 12-15.

Thomas, T., Moitinho-Silva, L., Lurgi, M., Bjork, J.R., Easson, C., Astudillo-Garcia, C.*, et al.* (2016). Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun*, **7**. doi:10.1038/ncomms11870

Thomson, W.C. (1873). *The Depths of the Sea*. London: Macmillan and Co.

Topsent, E. (1890). Notice préliminaire sur les spongiaires recueillis durant les campagnes de l'Hirondelle. *Bull. Soc. Zool. Fr.*, **15**, 26-32, 65-71.

Topsent, E. (1901). "Spongiaires". *Résultats du voyage du S.Y. 'Belgica'en 1897-99 sous le commandement de A. de Gerlache de Gomery. Expédition antarctique belge.* vol. Zoologie 4, pp 1-54, pls I-VI.

Topsent, E. (1904). *Spongiaires des Açores. Résultats des campagnes scientifiques accomplies sur son yacht par le Prince Albert I. Monaco*. Monaco: Govt. of Monaco.

Topsent, E. (1909). Etude sur quelques *Cladorhiza* et sur *Eucheliplum pristina* n. g. et n. sp. *Bulletin de l'Institut océanographique de Monaco*, (151), 1-23.

Topsent, E. (1920). Spongiaires du Musée Zoologique de Strasbourg. Monaxonides. *Bulletin de l'Institut océanographique, Monaco*, (381), 1-36.

Topsent, E. (1928). *Spongiaires de l'Atlantique et de la Méditerranée, provenant des croisières du Prince Albert 1er de Monaco*. Imprimerie de Monaco.

Topsent, E. (1929). Notes sur *Helophloeina stylivarians*, n. g., n. sp., Mycaline à desmes des Canaries. *Bulletin de l'Institut océanographique de Monaco*, (533), 1-8.

Topsent, E. (1930). *Chondrocladia yatsui* n. sp., de la Baie de Sagami. *Annotationes zoologicae japonenses*, (12), 421-432.

Vacelet, J. (1969). Eponges de la Roche du Large et de l'étage bathyal de Méditerranée (Récoltes de la soucoupe plongeante Cousteau et dragages). *Mémoires du Muséum national d'Histoire naturelle (A, Zoologie)*, **59**(2), 145-219.

Vacelet, J. (1996). "Deep-sea sponges in a Mediterranean cave". In F. Uiblein, J. Ott and M. Stachowitsch (eds) *Deep-sea and extreme shallow-water habitats: affinities and adaptations. Biosystematics and Ecology Series 11.* Österreichische Akademie der Wissenschaften, pp 299-312.

Vacelet, J. (2006). New carnivorous sponges (Porifera, Poecilosclerida) collected from manned submersibles in the deep Pacific. *Zool. J. Linn. Soc.*, **148**(4), 553-584. doi:10.1111/j.1096-3642.2006.00234.x

Vacelet, J. (2007). Diversity and evolution of deep-sea carnivorous sponges. *Porifera research: biodiversity, innovation and sustainability. Série Livros*, **28**, 107-115.

Vacelet, J. (2008). A new genus of carnivorous sponges (Porifera: Poecilosclerida, Cladorhizidae) from the deep N-E Pacific, and remarks on the genus *Neocladia*. *Zootaxa*, (1752), 57-65.

Vacelet, J. and Boury-Esnault, N. (1995). Carnivorous sponges. *Nature*, **373**(6512), 333-335.

Vacelet, J. and Boury-Esnault, N. (1996). A new species of carnivorous sponge (Demospongiae: Cladorhizidae) from a Mediterranean cave. *Bulletin van het Koninklijk Belgisch Instituut voor Natuurwetenschappen. Biologie/Bulletin de l'Institut Royal des Sciences Naturelles de Belgique. Biologie*, **66**(Suppl.).

Vacelet, J. and Boury-Esnault, N. (2002). A new species of carnivorous deep-sea sponge (Demospongiae : Cladorhizidae) associated with methanotrophic bacteria. *Cah. Biol. Mar.*, **43**(2), 141-148.

Vacelet, J., Boury-Esnault, N., Fiala-Medioni, A. and Fisher, C.R. (1995). A methanotrophic carnivorous sponge. *Nature*, **377**(6547), 296-296. doi:10.1038/377296a0

Vacelet, J. and Duport, E. (2004). Prey capture and digestion in the carnivorous sponge *Asbestopluma hypogea* (Porifera: Demospongiae). *Zoomorphology*, **123**(4), 179-190. doi:10.1007/s00435-004-0100-0

Vacelet, J. and Kelly, M. (2014). A new species of *Abyssocladia* (Porifera, Demospongiae, Poecilosclerida, Cladorhizidae) and other carnivorous sponges from the far eastern Solomon Islands. *Zootaxa*, (3815), 386-396. doi:10.11646/zootaxa.3815.3.4

Vacelet, J., Kelly, M. and Schlacher-Hoenlinger, M. (2009). Two new species of *Chondrocladia* (Demospongiae: Cladorhizidae) with a new spicule type from the deep south Pacific, and a discussion of the genus *Meliiderma*. *Zootaxa*, (2073), 57-68.

van Soest, R.W., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N.J.*, et al.* (2012). Global diversity of sponges (Porifera). *PLoS ONE*, **7**(4), e35105. doi:10.1371/journal.pone.0035105

van Soest, R.W.M. (2016). Sponge-collecting from a drifting ice floe: the Porifera obtained in the Kara Sea by the Dutch Polar Expedition 1882-83. *Contrib Zool*, **85**(3), 311-336.

van Soest, R.W.M. and Baker, B.J. (2011). A new carnivorous shallow-water sponge from McMurdo Sound, Antarctica (Porifera, Poecilosclerida). *Marine Biodiversity*, **41**(4), 495-501. doi:10.1007/s12526-010-0076-6

van Soest, R.W.M., Boury-Esnault, N., Hooper, J.N.A., Rützler, K., de Voogd, N.J., Alvarez, B.d.G.*, et al.* (2016). World Porifera Database.

van Soest, R.W.M. and Hajdu, E. (2002). "Family Phellodermidae fam. nov.". In J.N. Hooper and R.W. van Soest (eds) *Systema Porifera. A guide to the classification of sponges.* vol. 1, New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/Plenum, pp 621-624.

Vargas, S., Erpenbeck, D., Göcke, C., Hall, K.A., Hooper, J.N., Janussen, D.*, et al.* (2013). Molecular phylogeny of *Abyssocladia* (Cladorhizidae: Poecilosclerida) and *Phelloderma* (Phellodermidae: Poecilosclerida) suggests a diversification of chelae microscleres in cladorhizid sponges. *Zool. Scr.*, **42**(1), 106-116. doi:10.1111/j.1463-6409.2012.00560.x

Verrill, A.E. (1879). Notice of recent additions to the marine invertebrates of the northeastern coast of America, with descriptions of new genera and species, and critical remarks. *Proceedings of the United States National Museum*, **2**, 165-205.

Verrill, A.E. (1885). "Results of the explorations made by the Steamer Albatross off the northern coast of the United States, in 1883". *Annual Report of the United States Commission of Fish and Fisheries, Washington, 1883.* Washington: Govt. Printing Office, pp 503-699. no. 11].

Vierros, M., Cresswell, I., Rice, J. and Ardron, J. (2009). Global open oceans and deep seabed (Good): Bio-geographic classification.

Voigt, O., Erpenbeck, D. and Wörheide, G. (2008). Molecular evolution of rDNA in early diverging Metazoa: First comparative analysis and phylogenetic application of complete SSU rRNA secondary structures in Porifera. *BMC Evol. Biol.*, **8**(1), 69. doi:10.1186/1471-2148-8-69

Vosmaer, G. (1887). "Klassen und Ordnungen der Spongien (Porifera)". In H. Bronn (ed) *Die Klassen und Ordnungen des Thierreichs.* vol. 2, Leipzig & Heidelberg, pp 1-499.

Watling, L. (2007). Predation on copepods by an Alaskan cladorhizid sponge. *J. Mar. Biol. Assoc. U.K.*, **87**(06), 1721-1726. doi:10.1017/S0025315407058560

Whelan, N.V., Kocot, K.M., Moroz, L.L. and Halanych, K.M. (2015). Error, signal, and the placement of Ctenophora sister to all other animals. *Proceedings of the National Academy of Sciences*, **112**(18), 5773-5778. doi:10.1073/pnas.1503453112

Wörheide, G., Dohrmann, M., Erpenbeck, D., Larroux, C., Maldonado, M., Voigt, O.*, et al.* (2012). Deep Phylogeny and Evolution of Sponges (Phylum Porifera). *Adv. Mar. Biol.*, **61**, 1-62. doi:10.1016/B978-0-12-387787-1.00007-6

Yin, Z., Zhu, M., Davidson, E.H., Bottjer, D.J., Zhao, F. and Tafforeau, P. (2015). Sponge grade body fossil with cellular resolution dating 60 Myr before the Cambrian. *Proceedings of the National Academy of Sciences*, **112**(12), E1453-E1460. doi:10.1073/pnas.1414577112

Paper IV

The systematics of carnivorous sponges

IV

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The systematics of carnivorous sponges ☆

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ABSTRACT

Carnivorous sponges are characterized by their unique method of capturing mesoplanktonic prey coupled with the complete or partial reduction of the aquiferous system characteristic of the phylum Porifera. Current systematics place the vast majority of carnivorous sponges within Cladorhizidae, with certain species assigned to Guitarridae and Esperiopsidae. Morphological characters have not been able to show whether this classification is evolutionary accurate, and whether carnivory has evolved once or in several lineages.

In the present paper we present the first comprehensive molecular phylogeny of the carnivorous sponges, interpret these results in conjunction with morphological characters, and propose a revised classification of the group. Molecular phylogenies were inferred using 18S rDNA and a combined dataset of partial 28S rDNA, COI and ALG11 sequences. The results recovered carnivorous sponges as a clade closely related to the families Mycalidae and Guitarridae, showing family Cladorhizidae to be monophyletic and also including carnivorous species currently placed in other families. The genus *Lycopodina* is resurrected for species currently placed in the paraphyletic subgenus *Asbestopluma* (*Asbestopluma*) featuring forceps spicules and lacking sigmas or sigmancistras. The genera *Chondrocladia* and *Cladorhiza* are found to be monophyletic. However, results indicate that the subgenus *Chondrocladia* is polyphyletic with respect to the subgenera *Meliiderma* and *Symmetrocladia*. *Eucheliplumа*, formerly Guitarridae, is retained, but transferred to Cladorhizidae. The four known carnivorous species currently in *Esperiopsis* are transferred to *Abyssocladia*. *Neocladia* is a junior homonym and is here renamed *Koltunicladia*.

Our results provide strong evidence in support of the hypothesis that carnivory in sponges has evolved only once. While spicule characters mostly reflect monophyletic groups at the generic level, differences between genera represent evolution within family Cladorhizidae rather than evolution of carnivory in separate lineages. Conflicting spicule characters can be reinterpreted to support the inclusion of all carnivorous sponges within Cladorhizidae, and a carnivorous habit should thus be considered the main diagnostic character in systematic classification.

1. Introduction

An aquiferous system used to filter water for particulate matter is generally considered a defining feature of sponges (e.g. Bergquist, 1978). The only known exceptions are the carnivorous sponges (Demospongiae: Poecilosclerida) which have developed the ability to trap, envelop, and digest prey items, representing a unique evolutionary innovation within the phylum Porifera. Prey capture is dependent on the plastic nature of the sponge and happens through initial entanglement of the prey followed by migration and complete envelopment into the sponge by amoebocytes, which are able to digest the prey over a period of several days (Vacelet and Duport, 2004). Morphological adaptations to carnivory include an erect body morphology, a complete or partial reduction of the aquiferous system and the presence of filaments or inflatable spheres with an adhesive surface to catch and digest suitable prey. Typical prey items are small crustaceans, but the

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sponges are not very selective, and prey suitability seems to be governed mainly by the prey having appendages that can become entangled in contact with the sponge (Vacelet, 2007; Vacelet and Duport, 2004).

This carnivorous feeding strategy is generally considered to be an evolutionary adaptation to the oligotrophic conditions of the deep sea, where filter feeding is less viable for obtaining nutrients (e.g. Vacelet, 2007). Carnivorous sponges are thus mainly considered deep-sea sponges, and they constitute a large part of the sponge fauna at bathyal, abyssal and even hadal depths, with a depth record of 8840 m (Koltun, 1970). They are, however, also very much present in shallower habitats on the upper slope and shelf and are not uncommon up to a depth of a couple of hundred meters. Certain species have been reported even shallower (<100 m), and a couple of species are known as shallow as 20 m, mainly, but not exclusively, in cave habitats (Aguilar et al., 2011; Bakran-Petricioli et al., 2007; Chevaldonné et al., 2014; Vacelet, 1996; Vacelet and Boury-Esnault, 1996; van Soest and Baker, 2011). Carnivorous sponges are frequently found in the general enrichment zones around hydrothermal vents and seeps, benefiting from the increased prey availability at these sites (Vacelet, 2006b). Chemoautotrophic symbiotic bacteria have been reported from two species of carnivorous sponges, but the extent of symbiotic relationships is not known (Riesgo et al., 2007; Vacelet and Boury-Esnault, 2002; Vacelet et al., 1995, 1996). However, the symbiotic microbiome could be involved in the digestion process (Dupont et al., 2014, 2013; Vacelet and Duport, 2004).

Approximately 130 species with a morphology suggesting carnivory have been described to date. Due to the poor sampling of most of the world's oceans this number probably represents only a portion of the total amount of carnivorous sponges, and new species are continually being described (Kelly and Vacelet, 2011). The vast majority of carnivorous sponges are presently placed within Cladorhizidae Dendy, 1922 (Porifera: Demospongiae: Poecilosclerida). This family currently contains seven genera and five subgenera accepted by the World Porifera Database (van Soest et al., 2015): *Abyssocladia* Lévi, 1964; *Asbestopluma* (*Asbestopluma*) Topsent, 1901; *Asbestopluma* (*Helophloeina*) Topsent, 1929; *Cercicladia* Ríos, Kelly & Vacelet, 2011; *Chondrocladia* (*Chondrocladia*) Thomson, 1873; *Chondrocladia* (*Meliiderma*) Ridley and Dendy, 1887; *Chondrocladia* (*Symmetrocladia*) Lee et al., 2012; *Cladorhiza* Sars, 1872; *Lollipocladia* Vacelet, 2008 and *Neocladia*, Koltun, 1970. Morphological adaptations suggesting a carnivorous feeding strategy (erect morphology, filaments, lack of aquiferous system, observations of partially digested prey) are also present in certain other taxa such as *Euchelipluma* spp. Topsent, 1909 (Guitarridae Dendy, 1924) and some species currently placed within *Esperiopsis* Carter, 1882 (Esperiopsidae Hentschel, 1923).

Carnivorous sponges belong to order Poecilosclerida, which forms part of the clade Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault, 2012 in molecular analyses (Borchiellini et al., 2004; Cárdenas et al., 2012; Erpenbeck and Wörheide, 2007; Lavrov et al., 2008; Morrow et al., 2012; Redmond et al., 2013). Chela microscleres are unique to the order Poecilosclerida, and a clade containing chela-bearing poecilosclerids is usually recovered in molecular analyses close to several polyphyletic assemblages of mostly hadromerid sponges (Erpenbeck et al., 2007a; Erpenbeck and Wörheide, 2007; Lavrov et al., 2008; Morrow et al., 2012; Redmond et al., 2013; Thacker et al., 2013). Within the order, chela morphology was previously one of the major characters forming the basis of the subordinal classification, comprising Microcionina, Latrunculina, Myxillina and Mycalina (Hajdu et al., 1994; Hooper and van Soest, 2002; Kelly and Samaai, 2002). Molecular evidence shows that this classification does not describe the true evolutionary relationships of the order (Erpenbeck and Wörheide, 2007; Hajdu et al., 2013) and it is no longer considered valid in a newly proposed classification of the Demospongiae (Morrow and Cárdenas, 2015).

The systematics of the carnivorous sponges is currently based primarily on spicule characters, with a special emphasis on chela type at the generic level (Hajdu and Vacelet, 2002). While the current cladorhizid genera are quite well characterized, they collectively contain a large range of chela morphologies, including both palmate and anchorate forms of both iso- and anisochelae as well as more particular forms such as abyssochelae, cleistochelae and cercichelae. This has caused several authors to question the monophyly of family Cladorhizidae (Lopes et al., 2011; Vacelet, 2006a). Thus morphological characters alone have not been able to answer the question of whether carnivory has evolved multiple times within the Poecilosclerida, or whether the carnivorous sponges represent a monophyletic group with a wide range of spicule assemblages (see Kelly and Vacelet, 2011). Only a few molecular sequences are available for carnivorous sponges (Borchiellini et al., 2004; Chevaldonné et al., 2014; Riesgo et al., 2014; Vargas et al., 2013) and there has been no comprehensive attempt to establish the phylogenetic relationships of the group and their relationship to other poecilosclerids.

Accordingly, we investigated (1) whether carnivory in sponges has evolved once or several times, i.e. whether the carnivorous sponges constitute a monophyletic group, (2) the systematic position of carnivorous sponges in relation to other poecilosclerids, (3) the accuracy of the current intra-family systematics of family Cladorhizidae as well as (4) the systematics of carnivorous species currently assigned to other families. In this study, our overall aim has been to construct the first comprehensive phylogeny of the carnivorous sponges using molecular data, and relate the molecular findings to morphological characters. Based on our findings, we propose a revision of the current systematics of the carnivorous sponges and provide an overview of and key to identification of carnivorous genera and subgenera.

2. Materials and methods

2.1. Collection, preservation and identification

Specimens from the North Atlantic and Arctic were collected on board the Norwegian Institute of Marine Research and University of Bergen research vessels RV "G.O. Sars" and RV "Hans Brattström", and the German GEOMAR research vessel RV "Poseidon". Specimens from the New Zealand EEZ were collected on board the National Institute of Water & Atmospheric Research (NIWA) research vessel RV "Tangaroa". SW Atlantic specimens were collected on board the RV "Miguel Oliver" as part of the ATLANTIS project mapping the continental margin off Argentina. SW Indian Ocean specimens were collected on board the RV "Vizconde de Eza" in April 2009 during the MAINBAZA cruise to study benthic biodiversity of the continental margin off Mozambique, and the RV "James Cook" cruise no. 66 to the Southwest Indian Ocean Ridge (SWIOR) in 2011. Additional specimens were obtained from the collections at the Naturalis Biodiversity Center (Leiden) as well as single specimens from several sources (Table 1).

Most of the material was preserved in 96% ethanol. Some material originally preserved in 70% ethanol was also successfully sequenced, though in many cases only partially. All samples used for the phylogenetic analyses, as well as additional specimens used for morphological comparison, were examined and identified to species level. Species yet to be formally described have been assigned alphabetical characters to distinguish separate species. Taxonomic descriptions of these species will be presented in forthcoming papers.

Table 1
List of specimens used in this study with taxonomic identification of voucher given, accession numbers and collection localities. Sequences from GenBank in bold. Abbreviations used for voucher identification: BMNH, Natural History Museum (London, UK); CASIZ, California Academy of Sciences (USA); MNHN, Muséum National d'Histoire Naturelle (Paris, France); NIWA, National Institute of Water and Atmospheric Research (Wellington, New Zealand); NTNU, Museum of Natural History and Archaeology (Trondheim, Norway); QM, Queensland Museum (Brisbane, Australia); WAM, Western Australian Museum; ZMAPOR, Porifera collections of Naturalis Biodiversity Center (Leiden, Netherlands); ZMBN, The Natural History Collections, University Museum of Bergen (Norway). AT and PAT abbreviations refer to 2008–2009 "Atlantis" and "Patagonia" cruise material deposited at Centro Oceanográfico de Gijón.

Species	Voucher	28S rDNA	COI	ALG11	Collection locality
Carnivorous sponges					
Abyssocladia					
A. dominalba	ZMBN 103443	LN870577		LN870543	Lau Basin
A. dominalba	ZMBN 103444	LN870578	LN870440	LN870544	Lau Basin
A. dominalba	ZMBN 103445	LN870579	LN870441	LN870545	Lau Basin
A. dominalba	ZMBN 103446	LN870580	LN870442	LN870546	Lau Basin
A. lakwollii	NIWA 81378	LN870581	LN870443		Solomon Islands
A. n. sp. A	NIWA 52674	LN870582	LN870444		Macquarie Ridge
A. n. sp. B	NIWA 41033	LN870583	LN870445		Macquarie Ridge
A. n. sp. C	NIWA 40540	LN870584	LN870446		Macquarie Ridge
A. sp.	**SMF 11750**		**HE611581**		
Asbestopluma					
A. (A.) cf. *belgicae*	193-8 AGT 12	LN870588	LN870450	LN870518	Antarctica
A. (A.) bihamatifera	ZMBN 103447	LN870587	LN870449	LN870517	Btw. Iceland/Faroe
A. (A.) cupressiformis	ZMBN 103448	LN870589	LN870451	LN870519	Jan Mayen
A. (A.) cupressiformis	ZMBN 103449	LN870590	LN870452	LN870520	Jan Mayen
A. (A.) cupressiformis	ZMBN 103450	LN870591	LN870453	LN870521	Jan Mayen
A. (A.) cupressiformis	ZMBN 103451	LN870592	LN870454	LN870522	Jan Mayen
A. (A.) cupressiformis	ZMBN 103452	LN870593	LN870455	LN870523	Jan Mayen
A. (A.) desmophora	QM G331844[a]	LN870594	LN870456		Macquarie Ridge
A. (A.) furcata	ZMBN 103453	LN870595	LN870457	LN870524	Mid-Arctic Ridge
A. (A.) furcata	ZMBN 103454	LN870596	LN870458	LN870525	Mid-Arctic Ridge
A. (A.) furcata	ZMBN 103455	LN870597	LN870459	LN870526	Jan Mayen
A. (A.) furcata	ZMBN 103456	LN870598	LN870460	LN870527	Jan Mayen
A. (A.) furcata	ZMBN 103457	LN870599	LN870461	LN870528	Jan Mayen
A. (A.) furcata	ZMBN 103458	LN870600	LN870462	LN870529	Jan Mayen
A. (A.) furcata	ZMBN 103459	LN870601	LN870463	LN870530	Jan Mayen
A. (A.) hypogea			**HE611582**		W. Mediterranean
A. (A.) infundibulum	ZMBN 103460	LN870602	LN870464		Nyegga (Norw. Shelf)
A. (A.) infundibulum	ZMBN 103461	LN870603	LN870465	LN870531	Nyegga (Norw. Shelf)
A. (A.) lycopodium	ZMBN 103462	LN870604	LN870466		Nyegga (Norw. Shelf)
A. (A.) lycopodium	ZMBN 103463	LN870605	LN870470	LN870532	Jan Mayen
A. (A.) lycopodium	ZMBN 103464	LN870606	LN870467	LN870533	Skjold (Norw. Shelf)
A. (A.) lycopodium	ZMBN 103465	LN870607	LN870468	LN870534	Nyegga (Norw. Shelf)
A. (A.) lycopodium	ZMBN 103466	LN870608	LN870469	LN870535	Skjold (Norw. Shelf)
A. (A.) obae	**NIWA 28893**		**HE611583**		**Ross Sea**
A. (A.) occidentalis	**MCZ:DNA 105732**		**JX999062**		
A. (A.) pennatula	ZMBN 103467	LN870613	LN870475	LN870540	Barents Sea
A. (A.) pennatula	ZMBN 103468	LN870614	LN870476	LN870541	Barents Sea
A. (A.) pennatula	ZMBN 103469	LN870615	LN870477	LN870542	Barents Sea
A. (A.) n. sp. A	BMNH 2015.6.4.1	LN870609	LN870471	LN870536	SW Indian Ocean
A. (A.) n. sp. A	BMNH 2015.6.4.2	LN870610	LN870472	LN870537	SW Indian Ocean
A. (A.) n. sp. A	BMNH 2015.6.4.3	LN870611	LN870473	LN870538	SW Indian Ocean
A. (A.) n. sp. B	AT-0309 6LO15	LN870612	LN870474	LN870539	Patagonia
Cercicladia					
C. australis	NIWA 39599		LN870478		Macquarie Ridge
C. australis	PAT-0108 77DR5		LN870479		Patagonia
Chondrocladia					
C. (C.) antarctica	**SMF 11752**		**HE611586**		**E. Weddell Sea**
C. (C.) fatimae	BPCP 3729	LN870616	LN870480	LN870557	Papua New Guinea
C. (C.) fatimae	BPCP 3736	LN870617	LN870481	LN870558	Papua New Guinea
C. (C.) gigantea	NTNU 15204a	LN870618	LN870482		Ross Isl. (Svalbard)
C. (C.) gigantea	NTNU 15204b	LN870619	LN870483	LN870559	Ross Isl. (Svalbard)
C. (C.) nani	MNHN.D.NBE.1088		LN870492		NW of Kerguelen
C. (C.) robertballardi	MNHN.D.CL.4110	LN870627	LN870493	LN870567	Gorringe Bank
C. (C.) vaceleti	AT-0308 9LO93	LN870628	LN870494	LN870568	Patagonia
C. (C.) vaceleti	PAT-0108 51DR16		LN870495		Patagonia
C. (C.) vaceleti	PAT-0108 76DR15		LN870496		Patagonia
C. (C.) n. sp. A	AT-0308 8LO115	LN870622	LN870486	LN870562	Patagonia
C. (C.) n. sp. A	AT-0308 2DR3	LN870621		LN870561	Patagonia
C. (C.) n. sp. A	PAT-0108 52DR16	LN870623	LN870487	LN870563	Patagonia
C. (C.) n. sp. A	PAT-1208 46DR5	LN870624	LN870488	LN870564	Patagonia
C. (C.) n. sp. A	PAT-1208 58DR5	LN870625	LN870489	LN870565	Patagonia
C. (C.) n. sp. B	NIWA 25838		LN870491		Ross Sea
C. (C.) n. sp. C	NIWA 25826	LN870626	LN870490	LN870566	Otago (NZ)
C. (M.) n. sp. A	BMNH 2015.6.4.4		LN870485	LN870560	SW Indian Ocean
C. (S.) lyra	CASIZ 18877	LN870620	LN870484		South Escanaba Ridge

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher	28S rDNA	COI	ALG11	Collection locality
Cladorhiza					
C. abyssicola	ZMBN 103470	LN870631	LN870499	LN870550	Skagerrak
C. abyssicola	ZMA POR 19500	LN870629	LN870497	LN870548	SE Rockall Bank
C. abyssicola	ZMA POR 19732	LN870630	LN870498	LN870549	SE Rockall Bank
C. corticocancellata	ZMBN 103471	LN870632	LN870500	LN870551	Nyegga (Norw. Shelf)
C. cf. gelida	**SMF 11753**		**HE611584**		**Greenland Sea**
C. gelida	ZMBN 103472	LN870633	LN870501	LN870552	Mid-Arctic Ridge
C. gelida	ZMBN 103473	LN870634	LN870502	LN870553	Jan Mayen
C. gelida	ZMBN 103474	LN870635	LN870503	LN870554	Jan Mayen
C. penniformis	**SMF 11751**		**HE611585**		**E. Weddell Sea**
C. tenuisigma	ZMBN 103475	LN870636	LN870504	LN870555	Bear Isl. Shelf
C. tenuisigma	ZMBN 103476	LN870637	LN870505	LN870556	Faroe-Shetland Channel
Eucheliplumа					
E. n. sp. A	PAT-0108 75DR15	LN870640	LN870508	LN870571	Patagonia
E. n. sp. A	AT-0309 14LO103	LN870639	LN870507	LN870570	Patagonia
E. n. sp. A	PAT-1208 96DR5	LN870641	LN870509	LN870572	Patagonia
Esperiopsis					
E. koltuni	ZIN RAS 10774	LN870638	LN870506	LN870569	Sea of Okhotsk
Poecilosclerida					
Coelosphaeridae					
Lissodendoryx (A.) fibrosa	**NCI 401**	**KC869529**			**Malaysia**
Lissodendoryx (E.) arenaria	**NCI 321**	**KC869561**			**South Africa**
Lissodendoryx (L.) complicata	ZMBN 103478	LN870644	LN870513	LN870574	Mid-Arctic Ridge
Crambeidae					
Crambe crambe	**UCM PWC 933**	**AY561883**			
Crambe crambe			**AF526297**		**Mediterranean**
Monanchora arbuscula	**SI06x202**	**KC869447**			**Panama**
Desmacididae					
Desmapsamma anchorata	**UCM PWC 1660**		**HE591461**	**HE591451**	**Panama**
Esperiopsidae					
Amphilectus fucorum	ZMA POR 19817	LN870585	LN870447		Helgoland
Amphilectus fucorum	ZMA POR 22561	LN870586	LN870448	LN870547	North Sea
Amphilectus fucorum	**BELUM:Mc5093**	**HQ379226**			**Wales**
Ulosa stuposa	ZMA POR 22527	LN870648	LN870516	LN870576	Roscoff, Eng. Channel
Guitarridae					
Guitarra antarctica	ECOQUIM 786e	LN870642	LN870510	LN870573	Weddell Sea
Guitarra fimbriata	**NCI 405**	**KC869537**			**South Africa**
Guitarra n. sp. A	WAM Z31763		LN870511		Off. Ningaloo Coast
Mycalidae					
Mycale (A.) laxissima	**SBP S44**		**EF519651**		**Belize**
Mycale (A.) mirabilis	**QM BG306269**		**HE611590**		**Queensland**
Mycale (A.) mirabilis	**QM BG307148**		**HE611591**		**Queensland**
Mycale (A.) mirabilis	**QM BG305553**		**HE611592**		**Queensland**
Mycale (A.) mirabilis	**QM BG300561**		**HE611589**		**Shark Bay, W. Aus.**
Mycale (M.) lingua	ZMBN 103479	LN870646	LN870514		Langenuen, W. Norw.
Mycale (M.) lingua	ZMA POR 20445	LN870645			Skagerrak
Mycale (R.) marshallhalli	ZMA POR 20471	LN870647	LN870515	LN870575	Skagerrak
Podospongiidae					
Negombata magnifica	**TAU:25198**		**NC_010171**	**FR819668**	**Red Sea**
Merliida (Hamacanthidae)					
Hamacantha (V.) falcula	ZMBN 103477	LN870643	LN870512		Korsfjord, W. Norw.

[a] Originally NIWA 41013.

2.2. Datasets

We created one main multi-gene dataset to infer the phylogeny of the carnivorous sponges, and one 18S rDNA dataset with larger outgroup sampling to infer the general position of the carnivorous sponges within Poecilosclerida. For the multi-gene dataset, including all available carnivorous sponges as well as outgroup species, we chose three independent molecular markers with suitable resolution for species, genus and family level phylogeny: the 28S rDNA C1–D2 partition (Chombard et al., 1997), the overlapping "Folmer" and "Erpenbeck" fragments of COI (Folmer et al., 1994; Rot et al., 2006), and part of the protein-coding nuclear gene ALG11 (Belinky et al., 2012). Due to a combination of variable preservation quality, large surface to mass ratio and adhesive surface, the carnivorous sponges represented a challenging material. Thus we used modifications of standard barcoding primers for COI to exclude contamination.

For the 28S rDNA, COI and ALG11 dataset we sequenced specimens from the carnivorous family Cladorhizidae Dendy, 1922 and the families Guitarridae Dendy, 1924, Mycalidae Lundbeck, 1905 and Esperiopsidae Hentschel, 1923. These families are regarded as close relatives to Cladorhizidae, and Guitarridae and Esperiopsidae

Table 2
List of primers used in this study.

Partition	Sequence	Source
18S rDNA		
1F18S	5′-AAC CTG GTT GAT CCT GCC AGT-3′	Redmond et al. (2007)
600R18S	5′-CGA GCT TTT TAA CTG CAA PCR-3′	Redmond et al. (2007)
400F18S	5′-CCT GAG AAA CGG CTA CCA CA-3′	Redmond et al. (2007)
1350R18S	5′-CGG GAC TAG TTA GCA GGT TAA-3′	Redmond et al. (2007)
1200F18S	5′-TAA TTT GAC TCA ACA CGG G-3′	Redmond et al. (2007)
1800R18S	5′-GTT CAC CTA CYG AAA CCT TGT T PCR-3′	Redmond et al. (2007)
28S rDNA		
Ep1b′	5′-GTG GCC GGG AGA GGC AGC-3′	Chombard et al. (1997)
D2	5′-TCC GTG TTT CAA GAC GGG-3′	Chombard et al. (1997)
ALG11		
ALG11-D1	5′-TTY CAY CCN TAY TGY AAY GCN GGN GG-3′	Belinky et al. (2012)
ALG11-R1	5′-ATN CCR AAR TGY TCR TTC CAC AT-3′	Belinky et al. (2012)
ALG11-D2	5′-TGY AAY GCN GGN GGN GGN GA-3′	Belinky et al. (2012)
ALG11-R2	5′-CCR AAR TGY TCR TTC CAC ATN GTR TG-3′	Belinky et al. (2012)
COI		
LCO1490-Neg	5′-TTT CAA CAA ATC ATA AGG ATA TAG G-3′	This study (original primer Folmer et al., 1994)
HCO2198-Cla	5′-TAA ACC TCC GGG TGG CCA AAA AAC CA-3′	This study (original primer Folmer et al., 1994)
COX1-D2-Cla	5′-AAC ACA GCT TTT TTT GAT CCT GCG GG-3′	This study (original primer Rot et al., 2006)
COX1-R1	5′-TGT TGR GGG AAA AAR GTT AAA TT-3′	Rot et al. (2006)

are considered to contain carnivorous species. Additionally, we sequenced *Lissodendoryx (Lissodendoryx) complicata* (Hansen, 1885) and added poecilosclerid sequences from GenBank (Table 1). As it is considered a sister group to Poecilosclerida, *Hamacantha* (*Vomerula*) *falcula* (Bowerbank, 1874) (Merliida) was chosen as outgroup for the analyses. Eighty specimens representing 40 species were successfully partially or completely sequenced for this dataset, giving a total of 101 taxa including GenBank sequences.

For the 18S rDNA dataset, we sequenced a subset of 11 specimens (7 cladorhizids, *Eucheliplum*a n. sp. A, *Guitarra antarctica* Hentschel, 1914, *Ulosa stuposa* (Esper, 1794) and *Hamacantha (V.) falcula*). A large number of nearly complete demosponge 18S rDNA sequences have been made available through the Porifera Tree of Life (PorTOL) project (e.g. Redmond et al., 2013). As 18S rDNA is more conserved than the markers chosen for the combined 28S rDNA, COI and ALG11 analysis, adding carnivorous species to existing 18S rDNA sequence data from order Poecilosclerida presented an opportunity to put the carnivorous sponges into a wider systematic context.

Molecular analyses have shown that several taxa previously placed within Poecilosclerida (chiefly Raspailiidae but also others) are polyphyletic to other poecilosclerids (e.g. Erpenbeck et al., 2007a; Redmond et al., 2013), and in the newly proposed classification of Morrow and Cárdenas (2015) the scope of the order has been reduced to encompass only those families who have been consistently recovered as a monophyletic assemblage. We added all available 18S rDNA sequences from GenBank belonging to Poecilosclerida *sensu* Morrow and Cárdenas (2015) >1250 bp in length (with a large majority >1700 bp in length), as well as sister taxa including *Merlia normani* Kirkpatrick, 1908 (Merliida), *Desmacella* spp. (Desmacellida) and *Xenospongia patelliformis* Gray, 1858 (Tethyida) for a total of 159 specimens (accession numbers given in the supplementary information phylogenetic trees). The dataset comprises 18 of the 20 currently recognized families of Poecilosclerida, including Mycalidae, Guitarridae and Esperiopsidae, and provides comprehensive coverage of the order.

2.3. Extraction, amplification and sequencing

DNA extraction was performed using the Qiagen Blood and Tissue kit (QIAGEN) according to the manufacturer's instructions, with the additional step of removing spicules by pipetting after lysis before adding ethanol to the mixture. The mitochondrial and ALG11 markers were amplified in 25 μl reactions using TaKaRa Ex Taq HS DNA Polymerase (TaKaRa Bio) following the recommended quantities of the manufacturer. The ribosomal sequences were amplified using TaKaRa Ex Taq HS taq with Qiagen buffer adding 5 μl Q-solution to each reaction (QIAGEN). PCR products were purified using ExoSAP-IT (USB Europe, Germany).

For 18S rDNA the primer pairs SP18aF-600R18S, 400F18S-1350R18S and 1200F18S-18SgR were used (Redmond et al., 2007) (Table 2) (1 cycle [5 min/94 °C; 2 min/48–54 °C, 2 min/72 °C]; 35 cycles [1 min/94 °C, 30–50 s/48–54 °C, 1 min/72 °C]; 7 min/72 °C). For the C1–D2 28S partition the Ep1b′ and D2 primer pair was used (Chombard et al., 1997) (Table 2) (1 cycle [5 min/94 °C, 2 min/62 °C, 2 min/72 °C]; 35 cycles [1 min/94 °C, 45 s/62 °C, 1 min/72 °C]; 7 min/72 °C). For the ALG11 fragment a nested approach using the D1 and R1, then the D2 and R2 primer pairs was used using the same PCR cycling profile both times (Belinky et al., 2012) (Table 2) (1 cycle [5 min/94 °C; 2 min/54 °C, 2 min/72 °C]; 35 cycles [1 min/94 °C, 30 s/54 °C, 1 min/72 °C]; 7 min/72 °C). For the overlapping Folmer and Erpenbeck fragments the modified LCO1490-Neg and HCO2198-Cla, and COX1-D2-Cla and COX1-R1 primers were used (Folmer et al., 1994; Rot et al., 2006) (Table 2) (5 min/94 °C; 5 cycles [45 s/94 °C, 30 s/45 °C, 1 min/72°]; 30 cycles [45 s/94 °C, 30 s/50 °C, 1 min/72 °C]; 7 min/72 °C). The LCO1490-Neg primer modification is based on the full mitochondrial genome of *Negombata magnifica* (NC_010171) from GenBank, while the internal primers HCO2198-Cla and COX-D2-Cla are modifications to the original primers based on initial results from this study.

2.4. Alignment

Sequence contigs were assembled, quality checked, and trimmed using Geneious 6.1.7 by Biomatters (www.geneious.com), and sequences were checked for contamination using BLAST searches (http://blast.ncbi.nlm.nih.gov).

Alignment of the protein-coding COI and ALG11 partitions was done using MUSCLE 3.8.425 (Edgar, 2004), and alignments were examined and corrected as necessary using Geneious 6.1.7. The COI alignment contains 1216 characters of which 587 are variable

and 447 parsimony informative while the ALG11 alignment contains 940 characters of which 644 are variable and 578 parsimony informative. As an additional quality check and in order to identify codon positions, sequence alignments were translated using BioEdit 7.2.3 (Hall, 1999). Codon saturation plots were examined for all three positions in each gene using DAMBE 5.3.108 (Xia, 2013) with no saturation detected.

Several studies have shown the utility of using secondary structure in analysis of ribosomal sequences (e.g. Erpenbeck et al., 2007b; Voigt et al., 2008). Accordingly, initial alignment of 18S rDNA was done using ClustalW 2.1 (Larkin et al., 2007), and this alignment was manually fitted to a modified version of the consensus 18S rRNA secondary structure published by Voigt et al. (2008) using SeaView 4 (Gouy et al., 2010), creating a 1784 bp sequence alignment embedded in a 1864 bp secondary structure model alignment to preserve stem and loop structure. Two ambiguously aligned loop regions 10 and 32 bp long were identified using a combination of manual examination and gBlocks 0.91b (Castresana, 2000; Talavera and Castresana, 2007) with default parameters except all gap positions were set to allowed. These regions were excluded from further analysis. The resulting alignment contains 1742 characters of which 350 are variable and 259 are parsimony informative.

Alignment of 28S rDNA was done in MAFFT7 (Katoh and Standley, 2013) using the Q-INS-i algorithm, and the alignment was trimmed using gBlocks 0.91b with default parameters except all gap positions were set to allowed, reducing alignment size from 957 to 734 characters, of which 351 are variable and 447 parsimony informative. As we only had a partial 28S rDNA sequence, which we incorporated into a concatenated phylogenetic analysis (see Section 2.5) we did not employ a secondary structure model for this partition.

2.5. Phylogenetic analysis

The use of multispecies coalescent models has been shown to outperform analyses of concatenated (supermatrix) datasets in recent studies (e.g. Heled and Drummond, 2010; Lambert et al., 2015). However, the majority of species in our main dataset are represented by single specimens only precluding the use of these models, and thus we used a concatenated dataset of 28S rDNA, COI and ALG11 (2890 bp) for multi-locus analyses. In this dataset, 28S (AY561883) and COI (AF526297) sequences from two different GenBank *Crambe crambe* specimens were combined into a chimeric sequence.

All phylogenetic analyses were performed both by Bayesian inference using MrBayes 3.2.2 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003) and maximum likelihood (ML) using RAxML 8.0.20 (Stamatakis, 2014b). JModelTest 2.1.5 (Darriba et al., 2012; Guindon and Gascuel, 2003) was used to find the optimal site model for each gene and codon position (where applicable). In each case the GTR + G or GTR + I + G model was considered the most appropriate according to the Akaike Information Criterion. As argued by among others Stamatakis (2014a), invariable sites represents an additional approach to incorporate rate heterogeneity already covered by the gamma distribution parameter, and thus we choose to not employ invariable sites in our analyses.

For 18S rDNA the dataset was partitioned into stem and loop regions according to the secondary structure alignment, with special substitution models used for the paired sites of the stem regions. The Perl script 2analysis, available from the Porifera SSU rRNA secondary structures Database (http://www.palaeontologie.geo.lmu.de/molpal/RRNA/) was used to build the MrBayes model from the 18S alignment. In MrBayes the GTR + G model was used for loop regions while the supported RNA16B substitution model was used for stem regions. The Markov chain Monte Carlo (MCMC) analysis was performed with two four-chain runs for $12 * 10^6$ generations with $3 * 10^6$ generations burn-in and sampling every 1000 generations, with final deviation of split frequencies at 0.004 and log likelihood ESS at 5030. In RAxML we used the GTRGAMMA model with the RNA7D substitution model applied for stem regions with 2000 rapid bootstraps, the default hill climbing algorithm and a randomized stepwise addition starting MP tree.

For 28S rDNA, COI and ALG11, MrBayes and RAxML analyses were run both on the concatenated dataset and on each gene separately. For both the MrBayes and the RAxML analyses the concatenated dataset was partitioned into seven unlinked partitions comprising 28S rDNA and the three codon positions of COI and ALG11 respectively. Codon partitioning was also employed for single-gene COI and ALG11 analyses. The MCMC analysis comprised two four-chain runs for $16.56 * 12^6$ generations with $4.12 * 10^6$ generations burn-in for the concatenated dataset and $12 * 10^6$ generations with $3 * 10^6$ generations burn-in for single-gene analyses, with sampling every 1000 generations. Chain convergence was monitored by observation of log likelihood values and standard deviation of split frequencies (in the range of 0.002–0.007 for all analyses). Posterior log likelihood ESS scores were examined using Tracer 1.6 (Drummond et al., 2012) (in the range of 2500–7000 for all analyses). For the ML analysis in RAxML the GTRGAMMA model was used with 2000 rapid bootstraps and the default rapid hill climbing algorithm with a randomized stepwise addition starting MP tree.

3. Results

With the exception of the monotypic genera *Lollipocladia* and *Neocladia* (the latter here renamed *Koltunicladia*, see Section 4.9) and the *Asbestopluma* subgenus *Helophloeina* (containing three species), we were able to obtain sequence data from all recognized taxa with known carnivorous sponges at the subgenus level or higher, providing a comprehensive dataset representing the known diversity of carnivorous sponges. The RAxML best trees from the 18S rDNA and concatenated three gene dataset are illustrated (Figs. 1 and 2) with bootstrap values (BS) over 50 from the ML analyses and posterior probabilities (PP) over 0.5 from the Bayesian analyses shown. Genetrees for each analysis, including individual 28S rDNA, COI and ALG11 analyses, are given in supplementary Figures A–J.

3.1. 18S rDNA tree

The general results from the 18S rDNA genetree are comparable to those of Redmond et al. (2013) (Fig. 1). Two clades of *Merlia* and *Hamacantha (V.) falcula* together with *Desmacella* Schmidt, 1870 were recovered as sister groups to the rest of Poecilosclerida. *Amphilectus fucorum* (Esper, 1794) (Esperiopsidae, non-carnivorous) was recovered in a separate clade close to the families Isodictyidae Dendy, 1924 and Podospongiidae de Laubenfels, 1936 and is not closely related to the carnivorous sponges nor to Mycalidae or Guitarridae. While earlier studies has found *Ulosa stuposa* (BELUM:Mc4523; KC901912) outside order Poecilosclerida (Morrow et al., 2012; Redmond et al., 2013), our specimen identified as *U. stuposa* was recovered within the Esperiopsidae clade (BS = 82; PP = 0.88). All included carnivorous sponges were recovered in a well-supported clade with *Guitarra* Carter, 1874 (BS = 77; P = 0.95) and Mycalidae (BS = 96; P = 0.99) as sister groups, providing support to the hypothesis that carnivorous sponges represent a monophyletic assemblage, though the dataset is too conserved to confidently resolve relationships within the carnivorous sponges.

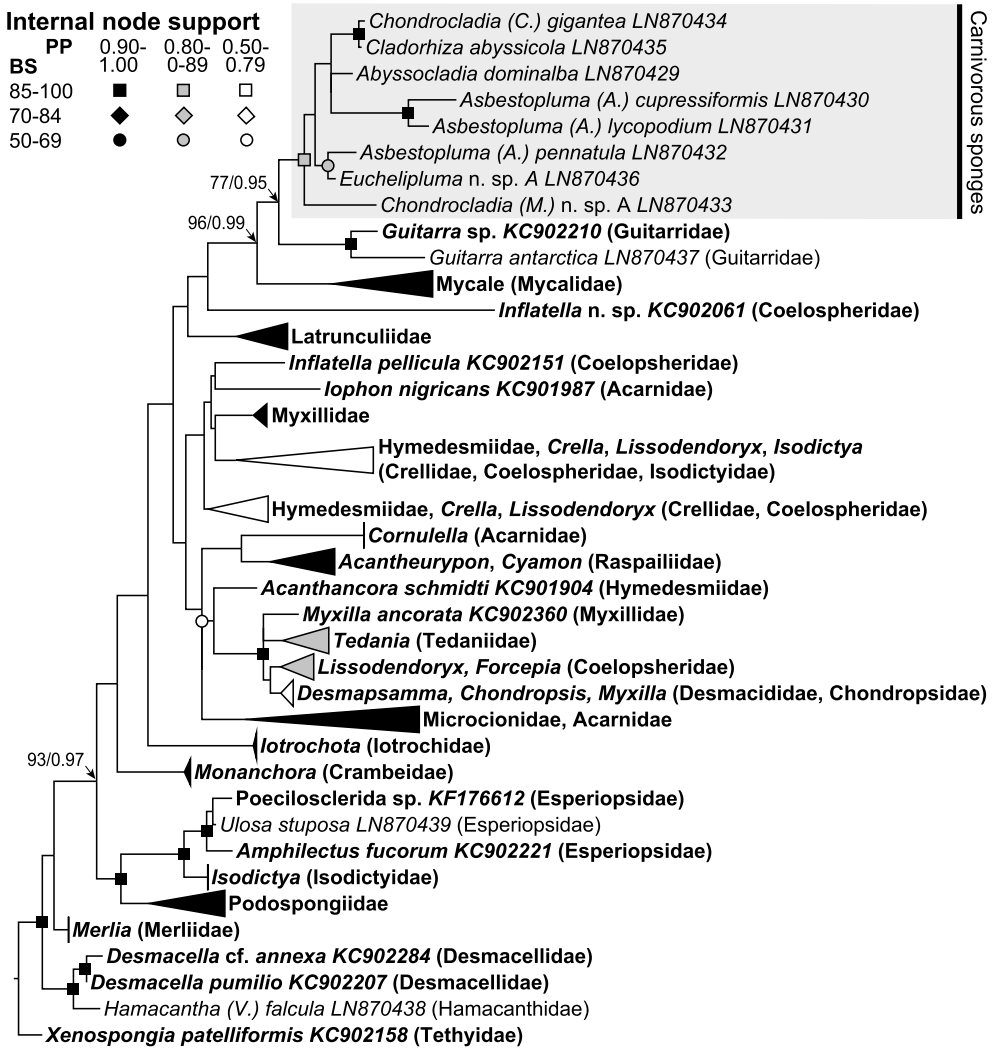

Fig. 1. ML best tree of the Poecilosclerida 18S rDNA phylogenetic dataset. Bootstrap values and posterior probabilities (from the Bayesian analysis) are indicated in full for main nodes and using symbols for internal nodes. The tree is rooted using *Xenospongia patelliformis* (Hadromerida). Species names in bold represent sequences downloaded from GenBank. The shaded area indicates the carnivorous sponge clade. High support defined as BS 85–100, PP 0.9–1; medium support as BS 70–84, PP 0.8–0.89; low support as BS 50–69, PP 0.5–0.79. Unmarked nodes have support values of either less than 50 (BS) or 0.5 (PP).

3.2. Combined 28S rDNA, COI and ALG11 tree

The results from the combined partial 28S rDNA, COI and ALG11 phylogenetic analyses (Fig. 2) recovers all carnivorous sponges, including Cladorhizidae, *Esperiopsis koltuni* Ereskovsky & Willenz, 2007 and genus *Eucheliplumа* Topsent, 1909, as a monophyletic group with high support (BS = 95; PP = 1), closely related to *Mycale* (BS = 73; PP = 0.81) and *Guitarra* (BS = 100; PP = 1). Contrary to the 18S rDNA analyses, *Mycale* rather than *Guitarra* is recovered as the closest sister group to the carnivorous sponges, and the precise relationship between these three families is thus not entirely clear. Similar to the 18S rDNA analysis the non-carnivorous Esperiopsidae species *Amphilectus fucorum* and *Ulosa stuposa* are recovered in a separate, more distant clade (BS = 79; PP = 0.99). Thus the analyses provide additional support for the inclusion of all carnivorous species into Cladorhizidae.

Asbestopluma species were recovered as a paraphyletic group (BS = 95–96; P = 1) sister to the remaining carnivorous sponges. Surprisingly, the species *A. (A.) lycopodium* is not monophyletic. The species *Cercicladia australis* Ríos, Kelly & Vacelet, 2011 and *Esperiopsis koltuni* were recovered within genus *Abyssocladia* Lévi, 1964 (BS = 68; PP = 0.72). *Eucheliplumа* was recovered as a sister group to *Abyssocladia* (BS = 45; PP = 0.9). *Chondrocladia* Thomson, 1873 and *Cladorhiza* Sars, 1872 are sister clades (BS = 85; PP = 1), and *Chondrocladia* subgenera *Meliiderma* Ridley and Dendy, 1887 and *Symmetrocladia* Lee et al., 2012 were both

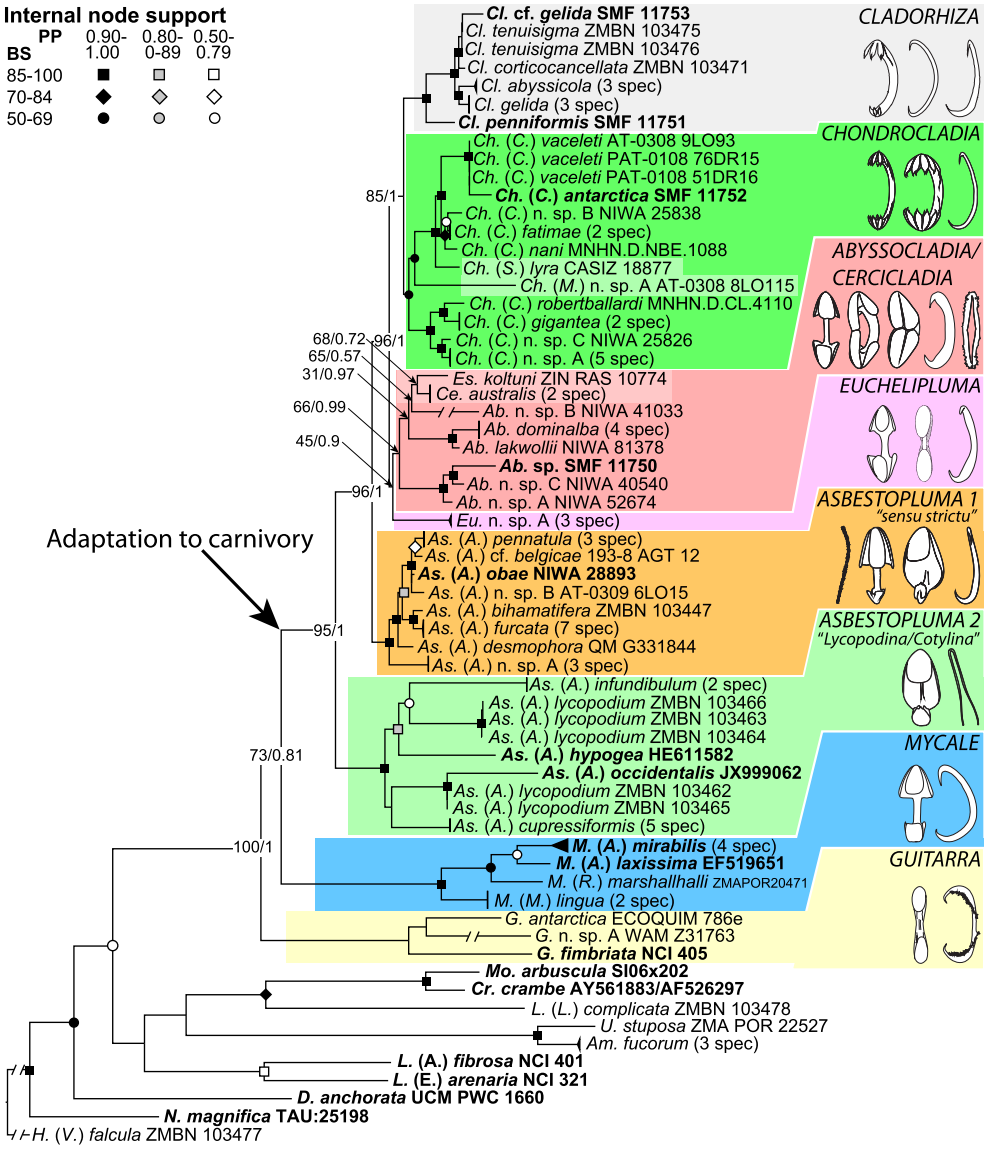

Fig. 2. ML best tree of the combined 28S rDNA, COI and ALG11 phylogenetic dataset. Bootstrap values and posterior probabilities (from the Bayesian analysis) are indicated in full for main nodes and nodes of special importance, and using symbols for other internal nodes. The tree is rooted using *Hamacantha (V.) falcula*. Species names in bold represent sequences collected from GenBank. High support defined as BS 85–100, PP 0.9–1; medium support as BS 70–84, PP 0.8–0.89; low support as BS 50–69, PP 0.5–0.79. Unmarked nodes have support values of either less than 50 (BS) or 0.5 (PP). The most common microscleres for each genus are indicated.

recovered within subgenus *Chondrocladia*, though the exact placement of *Meliiderma* is not entirely certain (BS = 54; PP = 0.92). Of the genes in the combined dataset COI is the most conservative while 28S rDNA and ALG11 are more variable. The combined 28S rDNA, COI and ALG11 analyses are able to provide strong support for the monophyly of carnivorous sponges, and resolved the phylogeny with robust support for almost all genus level clades.

4. Discussion

4.1. Systematic position of the carnivorous sponges

Almost all carnivorous species are currently assigned to Cladorhizidae. However, the family lacks a clear spicule-based synapomorphy and contains genera with different chela morphologies. Based on chela characters, certain carnivorous species have been

placed within Guitarridae and Esperiopsidae. Several authors have suggested that Cladorhizidae might be polyphyletic and that a carnivorous feeding mode has evolved several times in different poecilosclerid lineages as a response to oligotrophic deep-sea conditions (Lopes et al., 2011; Vacelet, 2006a, 2007).

In all molecular analyses, carnivorous sponges were recovered as a monophyletic assemblage closely associated with Mycalidae and the rest of Guitarridae. Based on spicule similarities, and given that the 18S rDNA dataset contains nearly all families within Poecilosclerida, this suggests that the latter two families are sister groups to the carnivorous sponges. However, the analyses were not able to completely resolve whether Guitarridae or Mycalidae is the closest sister group, or if they constitute their own separate clade. In contrast Esperiopsidae, excluding carnivorous species, was recovered in a different part of the Poecilosclerida tree (Fig. 1; Fig. 2; supplementary Figures A–J).

The molecular analyses thus support the hypothesis that a carnivorous habit has evolved only once within the sponges and that Cladorhizidae is monophyletic with the addition of *Eucheliplumа* and carnivorous species of *Esperiopsis* Carter, 1882. This result is congruent with earlier molecular data providing support for the abandonment of the chela-based suborders of the Poecilosclerida (Erpenbeck and Wörheide, 2007; Hajdu et al., 2013; Morrow and Cárdenas, 2015) and shows that differences in chela morphology represents evolutionary change within Cladorhizidae. The spicule complement of the carnivorous *Esperiopsis* species can be reinterpreted to fit into Cladorhizidae (see Section 4.4), and the close affinity of *Eucheliplumа* to *Abyssocladia* means that the presence of placochelae in *Guitarra*, *Eucheliplumа* and *Asbestopluma (A.) anisoplacochela* should be interpreted as a homoplasy.

A carnivorous feeding mode represents a radical transition from a filter-feeding sponge to a different body plan. *Chondrocladia* is unique among the carnivorous sponges in that the genus has retained a partial aquiferous system. A reasonable hypothesis has thus been that this genus represents an intermediate form between filter-feeding sponges and carnivory, implying an early divergence within the Cladorhizidae (e.g. Kübler and Barthel, 1999), a hypothesis also supported by evidence showing that this genus might have existed as early as the early Jurassic (Vacelet and Kelly, 2008). Surprisingly, *Chondrocladia* was recovered nested within the Cladorhizidae, suggesting the independent complete reduction of the aquiferous system in all preceding cladorhizid lineages.

4.2. Genera *Asbestopluma* Topsent, 1901 and *Lycopodina* Lundbeck, 1905

Asbestopluma is generally defined as Cladorhizidae with palmate anisochelae together with either sigma(ncistra)s, forceps spicules (which are only found in this genus among the Cladorhizidae), or microtylostyles (subgenus *Helophloeina* Topsent, 1929) (Hajdu and Vacelet, 2002; Lundbeck, 1905; Vacelet, 2006a, 2007). In recent years, several individual species have been described that, while overall conforming to the description above, depart from it in some way (e.g. presence of isochelae, anchorate anisochelae, anisoplacochelae) (Kelly and Vacelet, 2011; Lopes et al., 2011; Lopes and Hajdu, 2014).

Asbestopluma was originally erected by Topsent as a subgenus of *Cladorhiza* on the basis of an unpublished record by Lankester in 1882 (Topsent, 1901). Lundbeck (1905) elevated *Asbestopluma* to genus rank and erected three subgenera of *Asbestopluma* named *Asbestopluma sensu strictu* (containing the type species), *Lycopodina* and *Cotylina*. In Lundbeck's subgeneric classification *Asbestopluma s.s.* was defined as pinniform species with sigma(ncistra)s and a stalk coating containing acantho(tylo)styles or strongyles, *Lycopodina* as species with forceps spicules with an elongated body axis, and *Cotylina* as species also with forceps spicules but with a short, pedunculate body (Lundbeck, 1905). Hentschel (1914) cast doubt on the validity of the distinction between *Lycopodina* and *Cotylina*, and while de Laubenfels (1936) elevated *Lycopodina* to genus rank (not mentioning its relation to *Cotylina*, so his view of their relationship is unknown) these subgenera have generally not been considered valid as the diagnoses are partly based on habit (Hajdu and Vacelet, 2002; Vacelet and Boury-Esnault, 1996).

In the molecular results from our combined analysis *Asbestopluma* (*Asbestopluma*) was recovered in two separate clades with high support making the subgenus and also the genus itself clearly paraphyletic. The two clades recovered in the analysis correspond to Lundbeck's *Asbestopluma s.s.* and a separate clade containing both *Lycopodina* and *Cotylina* (Fig. 2).

In our opinion, this split is well-supported by morphological characters. The type species of *Asbestopluma* (*Asbestopluma*), *A. (A.) pennatula* (Schmidt, 1875), features two categories of palmate anisochela (with the larger type being more accurately described as intermediate between palmate and arcuate), sigmancistras, acanthotylostyles in a clearly separate basal coating, but never forceps spicules, which corresponds to Lundbeck's *Asbestopluma s.s.* subgenus (Fig. 3A–F). Other species of this type may vary in certain aspects, most often in regards to the large type of anisochela, which may be absent, transformed into isochelae or in one case anisoplacochelae. On the other hand the species of the second, more basal clade feature one type of palmate or arcuate anisochela only. There are no special coating spicules in the basal stalk, sigmas or sigmancistras are never present, and forceps spicules may be present though in many cases rare or missing as they seem connected to spermatic cysts (Riesgo et al., 2007) (Fig. 3G–L). This corresponds to both Lundbeck's *Cotylina* and *Lycopodina* subgenera, with the distinction between them being the habit (pedunculate, spherical or cup-shaped *vs.* elongated main body).

We thus propose to revive *Lycopodina* as a genus understood to include both subgenera *Lycopodina* and *Cotylina*, which removes the need to use the habit in the diagnosis. The first species described within this genus is *A. (A.) cupressiformis* (Carter, 1874). However de Laubenfels (1936) designated *A. (A.) lycopodium* (Levinsen, 1887) as the type species when elevating *Lycopodina* to genus rank and the affinity to *A. (A.) lycopodium* is also implied in the genus name, meaning that *A. (A.) lycopodium* should be considered as the type species of the genus.

The species *A. (A.) lycopodium* was recovered in two separate locations within the *Lycopodina* clade in the combined analysis (Fig. 2). These results were reproduced with more than one specimen in each clade and appear solid. While the specimens in both clades have a habit similar to *A. (A.) lycopodium* and no discernable differences in the spicule complements were found, the three specimens from the sister clade to *A. (A.) infundibulum* (Levinsen, 1887) have a thicker, stouter habit than those of the sister clade to *A. (A.) occidentalis* (Lambe, 1893). The habit and molecular differences imply that the specimens belong to two different species, though it is not possible to ascertain which of the two clades is actually *A. (A.) lycopodium* using the original species description (Levinsen, 1887). A possible interpretation is that this morphology represents the basal form within this clade (Fig. 3G), implying the existence of one or several cryptic species complexes, which is interesting in the light of the extreme depth range (70–8840 m) (Koltun, 1970; Lambe, 1893) reported for the morphologically very similar species *A. (A.) occidentalis*.

As it shares the palmate anisochelae of *Asbestopluma*, genus *Helophloeina* Topsent, 1929 has been considered a synonym of *Asbestopluma* (Hajdu and Vacelet, 2002). It has recently been resur-

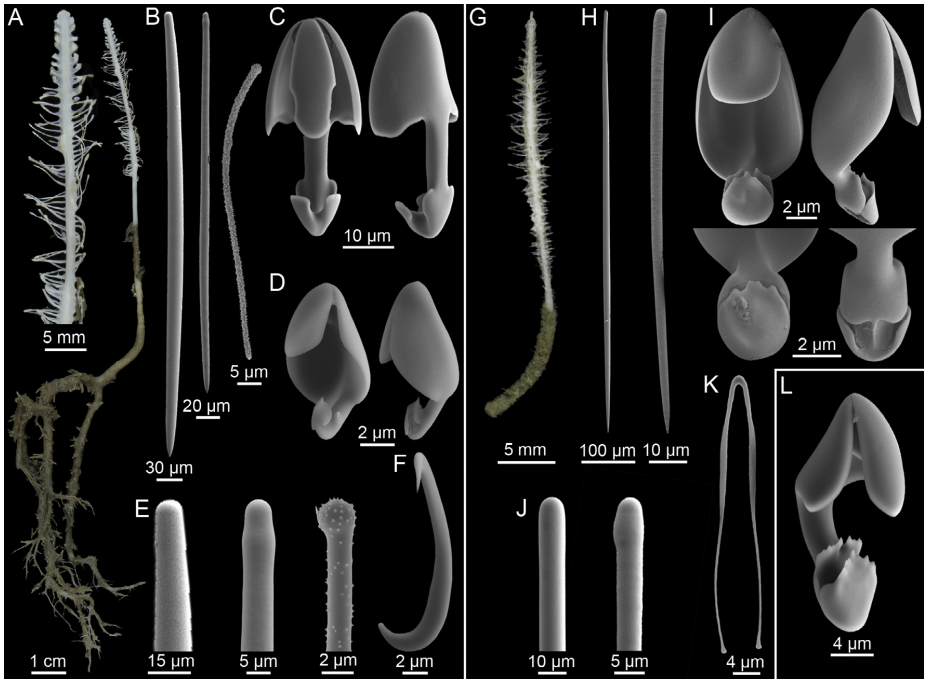

Fig. 3. Spicule complements of *A. (A.) pennatula* (left) and *A. (A.) lycopodium* (right) with chela of *A. (A.) infundibulum* (inset, right). Habit of *A. (A.) pennatula* (ZMBN 103468) with detail (A), mycalostyle, subtylostyle and basal acanthotylostyle (B), large palmate/arcuate anisochelae (C), small palmate anisochelae (D), detail of blunt ends of megascleres (E) and sigmancistra (F). Habit of *A. (A.) lycopodium* (ZMBN 103462) (G), style and tylostyle (H), palmate anisochelae with detail (I), with detail of blunt ends of megascleres (J) and forceps spicule (K). Arcuate chela from *A. (A.) infundibulum* (ZMBN 103460) (K), showing variation of chela shape within *Lycopodina*/*Cotylina*.

rected as a subgenus of *Asbestopluma* (Vacelet, 2006a) with other *Asbestopluma* species placed in subgenus *Asbestopluma* (*Asbestopluma*) by default. The vast majority of current *Asbestopluma* species are placed within subgenus *Asbestopluma*, with only three species currently recognized for subgenus *Helophloeina* (van Soest et al., 2015). Subgenus *Helophloeina* is not a part of the molecular dataset. As desmas and sigma(ncistra)s are found both within *Helophloeina* and the clade containing *Asbestopluma s.s.* we suggest to retain the current subgeneric classification for the remaining species within genus *Asbestopluma*.

4.3. Genus Eucheliplum *Topsent, 1909*

Eucheliplum contains four species that, based on habit and reports of partly digested crustacean debris (Vacelet, 2006b), are all known to be carnivorous. The genus shares placochelae with *Guitarra*, and is thus currently placed within Guitarridae. However, both general morphology and the presence of sigmancistras suggest a close affinity to other carnivorous sponges.

The combined analysis establishes genus *Eucheliplum* as belonging to Cladorhizidae (Fig. 2). It is recovered as a sister group to *Abyssocladia* with varying support (BS = 45; PP = 0.9) and the shape of the sigmancistras as well as presence of arcuate isochelae within *Eucheliplum* also suggests a close relationship between these genera. However, placochelae are not found within *Abyssocladia* and given that its exact position is ambiguous it is our view that *Eucheliplum* should be retained as an independent genus, but moved to the Cladorhizidae.

4.4. The carnivorous Esperiopsis species

A few species currently assigned to *Esperiopsis* Carter, 1882 based on the presence of palmate isochelae have a carnivorous habit, including *E. desmophora* Hooper & Lévi, 1989, *E. flagrum* Lehnert, Stone & Heimler, 2006, *E. koltuni* Ereskovsky & Willenz, 2007 and *E. symmetrica* Ridley & Dendy, 1886 (Lopes et al., 2011; Vacelet, 2006a, 2007). Given the presence of arcuate and palmate isochelae within certain recently described species in *Asbestopluma* and *Abyssocladia* (Lopes et al., 2011; Vacelet, 2006a) and the monophyly of carnivorous sponges including *E. koltuni* as shown by the molecular results (Fig. 2), these species should be reassigned to family Cladorhizidae.

Some morphological support for this reassignment is evident in the shape of the chelae themselves, as also noted by Lopes et al. (2011): While the isochelae within *Esperiopsis* and *Amphilectus* Vosmaer, 1880 are normally clearly palmate with broad frontal teeth and straight shafts (Fig. 4A), the chelae of the suspected carnivorous species are arcuate to palmate with arched shafts (Fig. 4B–D). The chelae of *E. symmetrica* are less developed than those of the other three species (Fig. 4B), however the presence of sigmancistras in this species (also found in *E. koltuni*) is an additional indicator for the inclusion of this species within Cladorhizidae, as sigmancistras are regarded as an apomorphic feature of carnivorous sponges (Vacelet, 2007).

While arcuate isochelae have also been reported in the *Asbestopluma* species *A. (A.) inexpectata* Lopes & Hajdu, 2014 this is in combination with smaller anisochelae. A smaller type of

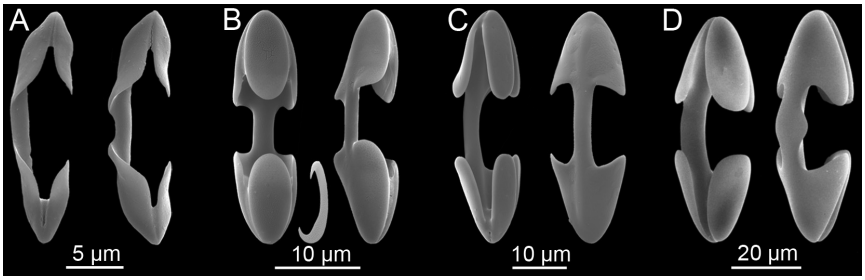

Fig. 4. Comparative view of the chelae of (A) *Amphilectus fucorum* (ZMA POR 19817), (B) *Esperiopsis symmetrica* (holotype: BMNH 87.5.2.179, sigmancistra also shown), (C) *Esperiopsis koltuni* (ZIN RAS 10774) and (D) *Abyssocladia faranauti* (MNHN.DJV.157).

chela is present in all described *s.s.* type *Asbestopluma*, but is not present in the carnivorous *Esperiopsis* species. Given the similarity in spiculation and the molecular results for *E. koltuni*, we thus propose to move *E. desmophora*, *E. flagrum*, *E. koltuni* and *E. symmetrica* to *Abyssocladia*.

4.5. Genus Abyssocladia Lévi, 1964

Abyssocladia was erected by Lévi (1964) to account for the presence of thaumatochelae, later renamed abyssochelae (van Soest Rob and Hajdu, 2002) in the type species of the genus, *Abyssocladia bruuni* Lévi, 1964. It was subsequently synonymized with *Phelloderma* (van Soest Rob and Hajdu, 2002). While the genus was resurrected within Cladorhizidae by Vacelet (2006a) on the basis of its apparent carnivorous habit, its precise status has been the subject of some debate (Lopes et al., 2011; Vacelet, 2007) before molecular results confirmed its affinity to the rest of the Cladorhizidae apart from *Phelloderma* (Vargas et al., 2013). The usage of the term abyssochelae was more stringently defined by Lopes et al. (2011) (frontal teeth touching or nearly touching; a height and width ratio close to one), recommending instead the term arcuate cleistochelae for chelae with frontal teeth nearly touching or touching, but with a larger height to width ratio.

Several of the species presently assigned to *Abyssocladia* do not feature abyssochelae *sensu* Lopes et al. (2011), with some species having neither abyssochelae nor cleistochelae, but rather only arcuate isochelae (e.g. *A. claviformis* Koltun, 1970; *A. faranauti* Hestetun et al., 2015), and which is reflected in the most current diagnosis of the genus (Lopes et al., 2011). The arcuate chelae–arcuate cleistochelae–abyssochelae transformation series proposed by Lopes et al. (2011) stresses the close affinity between these spicule types and provides a framework for understanding the genus. However, arcuate isochelae are also present in *Eucheliplum*a, and given the inclusion of that genus into Cladorhizidae the presence of arcuate chelae by itself cannot be used as a diagnostic character for *Abyssocladia*. This means that *Abyssocladia* currently lacks a clear synapomorphy, being instead defined by a negative character: its lack of placochelae. While this could be solved by the inclusion of *Eucheliplum*a as a subgenus of *Abyssocladia*, this is not unambiguously supported by our molecular results.

Confirming the results of Vargas et al. (2013) our analyses recovered *Abyssocladia* within Cladorhizidae in both analyses (Fig. 1; Fig. 2). Additionally *Esperiopsis koltuni* and *Cercicladia australis* were recovered as sister taxa nested within the genus (Fig. 2). Based on the molecular results we propose adding the carnivorous *Esperiopsis* species to *Abyssocladia*.

4.6. Genus Cercicladia Ríos, Kelly & Vacelet, 2011

Cercicladia australis was recovered as a sister species to *Esperiopsis koltuni* in the combined analyses (Fig. 2) suggesting the possible inclusion of the monotypic genus *Cercicladia* into *Abyssocladia*. However, this result is based on the 580 bp Erpenbeck COI marker only (Table 1), and *Cercicladia* lacks arcuate chelae or abyssochelae, instead being defined by the presence of cercichelae. At this point, given the lack of additional markers and conclusive support values, we have chosen to retain *Cercicladia* as an independent genus until more molecular data or the discovery of possible intermediate species can establish its position more firmly.

4.7. Genus Chondrocladia Thomson, 1873

Chondrocladia is defined as Cladorhizidae with anchorate (unguiferous) isochelae and typically retaining a remnant aquiferous system used for inflating terminal or subterminal branch swellings. The genus is currently divided into three subgenera: *Meliiderma* Ridley and Dendy, 1887, *Symmetrocladia* Lee et al., 2012 and *Chondrocladia*. *Meliiderma*, also featuring trochirhabds or subtrochirhabds, has been considered a synonym of *Chondrocladia* (Hajdu and Vacelet, 2002), but is now considered a valid subgenus to *Chondrocladia* (Vacelet et al., 2009). Recently, the monotypic subgenus *Symmetrocladia* was erected on the basis of the description of *Chondrocladia (S.) lyra*, understood as *Chondrocladia* with a radial symmetry of multiple harp-shaped structured termed vanes, composed of basal horizontal stolons supporting unilateral rows of vertical branches, but without a central stalk, and with special rostriform subtylote spicules (Lee et al., 2012). The subgenus *Chondrocladia* (*Chondrocladia*) contains the vast majority of the species in the genus, and is defined as *Chondrocladia* lacking the diagnostic characters of *Meliiderma* and *Symmetrocladia*.

The species currently assigned to subgenus *Chondrocladia* were historically divided into two separate subgenera: *Chondrocladia sensu strictu* and *Crinorhiza* (Topsent, 1930). Also referred to more informally as the "*concrescens* group" (e.g. Lévi, 1993; Tendal, 1973), *Chondrocladia s.s.* was understood by Topsent to contain *Chondrocladia* with elongated morphology, branches arranged in a spiral or a series of whorls with terminal swellings, and a coating layer covering the stalk containing special (acantho)(sub)(tylo) styles. In contrast, *Crinorhiza* was characterized by condensed branching around a compact spherical or subspherical pedunculate body, with no special coating spicules, and with no obvious branch swellings. *Crinorhiza* was originally used as genus assignment for *Chondrocladia amphactis* (Schmidt, 1880), and following the

example set by Ridley and Dendy (1886, 1887), has also confusingly been used as an informal habit description designating compact, pedunculated species of both *Cladorhiza* and *Chondrocladia*. Thus the current recommendation is to avoid the use of these subgenera, especially in a formal systematic context (Hajdu and Vacelet, 2002). More recently Lee et al. (2012) presented another informal arrangement grouping *Chondrocladia* (including all subgenera) into six alphabetical categories according to general body form (A–F), though these groups should be regarded as practical rather than phylogenetically accurate.

In our results, genus *Chondrocladia* was recovered as a monophyletic clade in the combined analysis (Fig. 2). The subgenus *Chondrocladia* was recovered as polyphyletic, with one clade of *s. s.* species (*C. (C.) robertballardi*, *C. (C.) gigantea* as well as two undescribed species) and one clade of *Crinorhiza* type species (*C. (C.) fatimae*, *C. (C.) nani*, *C. (C.) antarctica*, *C.(C.) vaceleti* as well as *C. (C.)* n. sp. B). While the exact position of subgenus *Meliiderma* is unclear (*C. (M.)* n. sp. A; BS = 54; PP = 0.92), *Symmetrocladia* is sister to the clade corresponding to *Crinorhiza*-type species (*C. (S.) lyra*; BS = 97; PP = 1).

The polyphyly of the subgenus *Chondrocladia* could be resolved by excluding the group of species corresponding to *Crinorhiza* as a fourth subgenus of *Chondrocladia*. However, while it is possible to get a general idea of the differences between *s.s.* and *Crinorhiza* forms of *Chondrocladia*, re-erecting the subgenus *Crinorhiza* with consistent diagnostic characters is more challenging. The habit, especially including more recently described species, is often more complex than the elongated *vs.* stalked dichotomy would suggest and is in any case not recommended as a formal diagnostic character. The presence of a covering layer on the lower stem or peduncle of the sponge is consistent in several *s.s.* species, but as many species are described based on fragments missing the lower stem the extent of this feature is not precisely known. *Crinorhiza*-type species tend to have large type anchorate isochelae with three teeth in each end (compared to 6–9 for other *Chondrocladia*), but exceptions exist. Thus solving the intra-genus polyphyly within *Chondrocladia* would require careful study of a wider range of *Chondrocladia* species. As such we do not propose any changes to the current systematics of the genus at this time, but would stress that this is a topic that should be subject to further study.

4.8. Genus *Cladorhiza* Sars, 1872

Cladorhiza is defined by the presence of anchorate anisochelae. The combined analysis recovers *Cladorhiza* as a monophyletic genus within the Cladorhizidae and sister to genus *Chondrocladia* (Fig. 2). It should be noted that the species included in the analysis, with the exception of *C. penniformis* Göcke & Janussen, 2013, are all branching *Cladorhiza* from the North Atlantic. Some Pacific, South Atlantic and Southern Ocean species of *Cladorhiza* have a pedunculate habit with a body reminiscent of an umbrella or crown in combination with tridentate anchorate anisochelae. Examples include several of the *Cladorhiza* species from the "Challenger" Expedition (Ridley and Dendy, 1886, 1887), *C. flosabyssi* Topsent, 1909, *C. ephyrula* Lévi, 1964 and *C. corona* Lehnert, Watling & Stone, 2005. While these species probably belong in the same genus based on other morphological similarities, this hypothesis needs to be assessed using additional molecular data.

4.9. Genera *Neocladia* Koltun, 1970 and *Lollipocladia* Vacelet, 2008

Specimens from the monotypic genera *Neocladia* and *Lollipocladia* were not available for this study. The genera are defined by the presence of highly arched birotula-like isochelae and sigmancistras for *Neocladia* (Koltun, 1970; Vacelet, 2008) and highly arched multidentate anchorate unguiferous isochelae together with palmate isochelae and sigmancistras for *Lollipocladia* (Vacelet, 2008). Their pedunculate disc-like habits are reminiscent of the common *Abyssocladia* form (Vacelet, 2008) and molecular data is needed to ascertain their exact affinities.

Neocladia Koltun, 1970 is a junior synonym of *Neocladia* Perkins, 1906, type species *Neocladia howardi* Perkins, 1906: 251 (Insecta: Hymenoptera: Chacidoidea: Encyrtidae), and a replacement name is needed. We propose the replacement name *Koltunicladia* (nom. nov.). The new name is a combination derived from Professor V. M. Koltun† and from *Neocladia*.

5. Conclusions

This study presents the first major molecular analysis of carnivorous sponges, adding 11 18S rDNA sequences, 78 partial 28S rDNA sequences, 91 partial COI sequences and 60 partial ALG11 sequences to sequences already in GenBank. With the exception of the monotypic genera *Neocladia* and *Lollipocladia* and the small *Asbestopluma* subgenus *Helophloeina*, all taxa with known carnivorous species at the subgenus level or higher were included, making the study a comprehensive analysis of known carnivorous sponges. Most importantly, this study provides strong support to the hypothesis that carnivory within the sponges has appeared only once. With the inclusion of *Eucheliplum*a and the carnivorous *Esperiopsis* species, Cladorhizidae, encompassing all carnivorous sponges, is retained as a clade inside order Poecilosclerida with Guitarridae and Mycalidae as closest sister groups and non-carnivorous Esperiopsidae more distantly related.

Except for the revisions proposed in this article, current carnivorous genera were found to be monophyletic, meaning that spicule characters in most cases are diagnostic at the genus level. However, the differences in spiculation between genera represents evolution within family Cladorhizidae rather than evolution of carnivory in separate lineages, and in the case of the carnivorous genus *Eucheliplum*a and the species formerly assigned to *Esperiopsis*, conflicting spicule characters can be reinterpreted to fit an inclusion into Cladorhizidae.

For future taxonomic studies, this result means that a carnivorous feeding habit should be regarded as the main diagnostic character for incorporation of new species into Cladorhizidae, and that conflicting spicule characters can usually be reinterpreted to support such an inclusion. This result is in accordance with other molecular studies showing that spicule characters are often more plastic than previously accounted for in general, and more specifically within the chela morphologies of order Poecilosclerida.

6. Diagnoses

Based on the results and discussion above we propose the following revised systematics for Cladorhizidae, containing all current cladorhizid genera and species, with the additional inclusion of *Esperiopsis* spp. and *Eucheliplum*a, encompassing all presently known carnivorous sponges. A list of changes to the current systematic classification is given in Table 3. Diagnostic characters and a key to the genera and subgenera of Cladorhizidae are given in Tables 4 and 5.

6.1. Family *Cladorhizidae* Dendy, 1922

Synonymy. Cladorhizeae Dendy, 1922:58; Cladorhizidae de Laubenfels, 1936:122.

Diagnosis. Carnivorous sponges adapted to feeding on small, typically crustacean, prey. Adaptations to carnivory include partial or complete reduction of the aquiferous system, erect habit with radiating processes with either a basal disc or root (rhizoid)

Table 3
List of species systematic reassignments in Cladorhizidae.

Species name	Reassignment
Cladorhizidae	**Cladorhizidae**
Asbestopluma (A.) bilamellata Lévi, 1993	*Lycopodina bilamellata* (Lévi, 1993)
Asbestopluma (A.) callithrix Hentschel, 1914	*Lycopodina callithrix* (Hentschel, 1914)
Asbestopluma (A.) calyx Hentschel, 1914	*Lycopodina calyx* (Hentschel, 1914)
Asbestopluma (A.) comata Lundbeck, 1905	*Lycopodina comata* (Lundbeck, 1905)
Asbestopluma (A.) communis Lopes & Hajdu, 2014	*Lycopodina communis* (Lopes and Hajdu, 2014)
Asbestopluma (A.) cupressiformis (Carter, 1874)	*Lycopodina cupressiformis* (Carter, 1874)
Asbestopluma (A.) ecoprof Lopes & Hajdu, 2014	*Lycopodina ecoprof* (Lopes and Hajdu, 2014)
Asbestopluma (A.) globularis Lévi, 1964	*Lycopodina globularis* (Lévi, 1964)
Asbestopluma (A.) gracilis Koltun, 1955	*Lycopodina gracilis* (Koltun, 1955)
Asbestopluma (A.) hydra Lundbeck, 1905	*Lycopodina hydra* (Lundbeck, 1905)
Asbestopluma (A.) hypogea Vacelet & Boury-Esnault, 1996	*Lycopodina hypogea* (Vacelet and Boury-Esnault, 1996)
Asbestopluma (A.) lebedi Koltun, 1962	*Lycopodina lebedi* (Koltun, 1962)
Asbestopluma (A.) lycopodium (Levinsen, 1887)	*Lycopodina lycopodium* (Levinsen, 1887)
Asbestopluma (A.) occidentalis (Lambe, 1893)	*Lycopodina occidentalis* (Lambe, 1893)
Asbestopluma (A.) hadalis Lévi, 1964	*Lycopodina hadalis* (Lévi, 1964)
Asbestopluma (A.) infundibulum (Levinsen, 1887)	*Lycopodina infundibulum* (Levinsen, 1887)
Asbestopluma (A.) infundibulum orientalis Koltun, 1970	*Lycopodina infundibulum orientalis* (Koltun, 1970)
Asbestopluma (A.) microstrongyla Lopes, Bravo & Hajdu, 2011	*Lycopodina microstrongyla* (Lopes et al., 2011)
Asbestopluma (A.) minuta (Lambe, 1900)	*Lycopodina minuta* (Lambe, 1900)
Asbestopluma (A.) parvula Hestetun et al., 2015	*Lycopodina parvula* (Hestetun et al., 2015)
Asbestopluma (A.) rastrichela Hestetun et al., 2015	*Lycopodina rastrichela* (Hestetun et al., 2015)
Asbestopluma (A.) vaceleti van Soest & Baker, 2011	*Lycopodina vaceleti* (van Soest and Baker, 2011)
Asbestopluma (A.) versatilis (Topsent, 1890)	*Lycopodina versatilis* (Topsent, 1890)
Neocladia flabelliformis Koltun, 1970	*Koltunicladia flabelliformis* (Koltun, 1970)
Esperiopsidae	
Esperiopsis desmophora Hooper & Lévi, 1989	*Abyssocladia desmophora* (Hooper and Lévi, 1989)
Esperiopsis flagrum Lehnert, Stone & Heimler, 2006	*Abyssocladia flagrum* (Lehnert et al., 2006)
Esperiopsis koltuni Ereskovsky & Willenz, 2007	*Abyssocladia koltuni* (Ereskovsky and Willenz, 2007)
Esperiopsis symmetrica Ridley & Dendy, 1886	*Abyssocladia symmetrica* (Ridley and Dendy, 1886)
Guitarridae	
Eucheliplura pristina Topsent, 1909	*Eucheliplura pristina* Topsent, 1909
Eucheliplura arbuscula (Topsent, 1928)	*Eucheliplura arbuscula* (Topsent, 1928)
Eucheliplura congeri de Laubenfels, 1936	*Eucheliplura congeri* de Laubenfels, 1936
Eucheliplura elongata Lehnert, Stone & Heimler, 2006	*Eucheliplura elongata* Lehnert et al., 2006

processes for anchoring in soft sediment. Axial or abaxial skeleton composed of monactinal or diactinal megascleres, from which extend extra-axial branches. Microscleres include palmate, arcuate and anchorate (an)isochelae and their derivatives, including placochelae and cercichelae; sigmas, forceps or micro(subtylo)styles (microspined, and also spear-shaped in a few cases), and trochirhabds (modified from Lee et al., 2012).

Type species. *Cladorhiza abyssicola* Sars, 1872:65.

6.2. Genus *Abyssocladia* *Lévi, 1964*

Diagnosis. Cladorhizidae most often pedunculate, carrying a disciform or flabelliform body with a radial architecture, in other cases pinnate or branching. Microscleres are a combination of abyssochelae, cleistochelae, arcuate chelae and/or sigmancistras, but not placochelae (modified from Lopes et al., 2011).

Type species. *Abyssocladia bruuni* Lévi, 1964 (by original designation).

Species. *A. atlantica* Lopes and Hajdu, 2014; *A. bruuni* Lévi, 1964; *A. carcharias* Kelly and Vacelet, 2011; *A. claviformis* Koltun, 1970; *A. desmophora* (Hooper and Lévi, 1989); *A. diegoramirezensis* Lopes, Bravo & Hajdu, 2011; *A. dominalba* Vacelet, 2006; *A. faranauti* Hestetun, Fourt, Vacelet, Boury-Esnault & Rapp, 2015; *A. flagrum* (Lehnert, Stone & Heimler); *A. huitzilopochtli* Vacelet, 2006; *A. inflata* Vacelet, 2006; *A. koltuni* (Ereskovsky and Willenz, 2007); *A. lakwollii* Vacelet and Kelly, 2014; *A. myojinensis* Ise and Vacelet, 2010; *A. natushimae* Ise and Vacelet, 2010; *A. naudur* Vacelet, 2006; *A. oxeata* Koltun, 1970; *A. symmetrica* (Ridley and Dendy, 1886); *A. tecta* Hestetun, Fourt, Vacelet, Boury-Esnault & Rapp, 2015; *A. umbellata* Lopes, Bravo & Hajdu, 2011.

6.3. Genus *Asbestopluma* *Topsent, 1901*

Synonymy. [*Cometella*] Schmidt, 1870:49 (nomen oblitum); [*Asbestopluma*] Lankester, 1882:478 (nomen nudum); *Asbestopluma* Topsent, 1901:23; *Helophloeina* Topsent, 1929:8; not *Lycopodina* Lundbeck, 1905:58; *Cotylina* Lundbeck, 1905:68.

Diagnosis. Cladorhizidae with at least one type of palmate, or in one case anchorate unguiferate, anisochela. Usually with a second larger type of palmate to arcuate anisochela that may in some cases be modified to isochela, anisoplacochela or tridentate anchorate chela. Sigmancistras and basal acantho(sub)(tylo)styles are also present with a few exceptions. Never forceps spicules (modified from Lopes and Hajdu, 2014).

Type species. *Cladorhiza pennatula* Schmidt, 1875 (by subsequent designation; Topsent, 1901).

6.4. Subgenus *Asbestopluma* *Topsent, 1901*

Diagnosis. *Asbestopluma* without spear-shaped microtylostyles (from Lopes et al., 2011).

Type species. *Cladorhiza pennatula* Schmidt, 1875 (by subsequent designation; Topsent, 1901).

Species. *A. (A.) agglutinans* Vacelet, 2006; *A. (A.) anisoplacochela* Kelly and Vacelet, 2011; *A. (A.) belgicae* (Topsent, 1901); *A. (A.) bihamatifera* (Carter, 1876); *A. (A.) biserialis* (Ridley and Dendy, 1886); *A. (A.) biserialis californiana* de Laubenfels, 1935; *A. (A.) bitri-*

Table 4
List of diagnostic characters defining genera and subgenera of Cladorhizidae.

Genus/subgenus	Microscleres	Typical habit	Distribution	Known species	Other
Abyssocladia	A combination of arcuate isochelae, cleistochelae and/or abyssochelae. Sigma (ncistra)s	Pedunculate disc-shaped with radiating filaments or pinnate with rows of filaments	Pacific, Atlantic	19	
Asbestopluma (Asbestopluma)	Most commonly two types of palmate anisochelae. The larger type is palmate to arcuate and may in some species be absent or modified. Sigma(ncistra)s	Pinnate with two or more rows of filaments, in some cases branching	Worldwide	21	Desmas present in a few species
Asbestopluma (Helophloeina)	Like subgenus *Asbestopluma*, with the addition of spear-like microtylostyles. Sigma(ncistra)s	Branching	Pacific, Atlantic	3	Desmas present in one species
Cercicladia	Cercichelae only. Sigma(ncistra)s	Pinnate with two rows of filaments	Pacific, SW Atlantic	1	
Chondrocladia (Chondrocladia)	Anchorate isochelae and commonly sigma (ncistra)s	Most commonly either pedunculate spherical body with projections or elongated with projections in whorls. Projections often carry inflatable spheres that deflate on collection	Worldwide	32	
Chondrocladia (Meliiderma)	Anchorate isochelae, either subtrochirhabds or trochirhabds and commonly sigma(ncistra)s	Small (most <50 mm), pedunculate with spherical body and typically without projections	Worldwide	5	
Chondrocladia (Symmetrocladia)	Anchorate isochelae and sigma(ncistra)s	Three to five harp-like vanes radiating from the center, composed of horizontal stolons and vertical branches	Eastern Pacific	1	
Cladorhiza	Anchorate anisochelae and sigma(ncistra)s	Branching with filaments in all directions or pedunculate with either cup-shaped or umbrella-like body terminating in filaments	Worldwide	40	
Euchelipluma	Arcuate isochelae and isoplacochelae. sigma(ncistra)s commonly present	Pinnate with rows of filaments or branching	Worldwide	5	Desmas present in one species
Koltunicladia	Arched, birotula-like chelae, anchorate chelae and sigma(ncistra)s	Pedunculate, disc-shaped body with radiating filaments	NW Pacific	1	
Lollipocladia	Palmate/arcuate isochelae and strongly arched anchorate chelae	Pedunculate, disc-shaped body with radiating filaments	NE Pacific	1	
Lycopodina	Single category of palmate or arcuate anisochela. No sigmas or sigmancistras. Commonly forceps spicules	Pedunculate, cup-shaped, spherical or elongated main body with filaments in all directions	Worldwide	23	

Table 5
Key to genera and subgenera of Cladorhizidae.

1. a. Anchorate chelae are present (2)
   b. No anchorate chelae present (7)
2. a. Anchorate chelae are strongly arched or found together with strongly arched birotula-like chelae or palmate/arcuate isochelae (3)
   b. Chelae are anchorate only (4)
3. a. Chelae are a combination of arched birotula-like chelae and anchorate chelae. Pedunculate, disc-shaped body. One known species (genus *Koltunicladia*)
   b. Chelae are a combination of palmate/arcuate isochelae and strongly arched anchorate chelae. Pedunculate, disc-shaped body. One known species (genus *Lollipocladia*)
4. a. Chelae are anchorate anisochelae (genus *Cladorhiza*)
   b. Chelae are anchorate isochelae (5, genus *Chondrocladia*)
5. a. Subtrochirhabds or trochirhabds are present, pedunculate body typically a few centimeters in length (subgenus *Meliiderma*)
   b. Meliiderm spicules or trochirhabds are absent (6)
6. a. Rostriform subtylostyles, body composed of stolons radiating from the center of the sponge supporting numerous long vertical branches. One known species (subgenus *Symmetrocladia*)
   b. No rostriform subtylostyles. Body typically either pedunculate with projections in all directions or elongated with projections in whorls but other forms exist. Projections may be reduced to knob-like structures (subgenus *Chondrocladia*)
7. a. Chelae are palmate or arcuate (8)
   b. Chelae are cercichelae. One known species (genus *Cercicladia*)
8. a. Chelae are of a single type of palmate or arcuate anisochela. Sigmas or sigmancistras are absent; forceps spicules may be present (genus *Lycopodina*)
   b. Sigmas or sigmancistras are present (9)
9. a. Chelae are larger palmate/arcuate anisochelae and smaller palmate anisochelae. The larger type may be absent or modified into isochelae, more arcuate forms or anisoplacochelae (10, genus *Asbestopluma*)
   b. Chelae include arcuate isochelae, cleistochelae and/or abyssochelae (11)
10. a. Spear-shaped microtylostyles and microstrongyles present, habit typically branching (subgenus *Helophloeina*)
    b. No spear-shaped microtylostyles or microstrongyles. Habit often pinnate with filaments in rows, or branching (subgenus *Asbestopluma*)
11. a. Isoplacochelae present (genus *Eucheliplumа*)
    b. Chelae a combination of arcuate isochelae, cleistochelae and/or abyssochelae with no placochelae (genus *Abyssocladia*)

chela Lopes, Bravo & Hajdu, 2011; *A. (A.) desmophora* Kelly and Vacelet, 2011; *A. (A.) flabellum* Koltun, 1970; *A. (A.) furcata* Lundbeck, 1905; *A. (A.) gracilior* (Schmidt, 1870); *A. (A.) inexpectata* Lopes and Hajdu, 2014; *A. (A.) magnifica* Lopes, Bravo & Hajdu, 2011; *A. (A.) monticola* Lundsten, Reiswig & Austin, 2014; *A. (A.) obae* Koltun, 1964; *A. (A.) pennatula* (Schmidt, 1875); *A. (A.) quadriserialis* Tendal, 1973; *A. (A.) ramosa* Koltun, 1958; *A. (A.) rickettsi* Lundsten, Reiswig & Austin, 2014; *A. (A.) voyager* Lopes and Hajdu, 2014; *A. (A.) wolffi* Lévi, 1964.

6.5. Subgenus Helophloeina *Topsent, 1929*

Diagnosis. *Asbestopluma* with a basal sheath of spear-shaped microtylostyles and microstrongyles (from Lopes et al., 2011).

Type species. *Helophloeina stylivarians* Topsent, 1929 (by monotypy).

Species. *A. (H.) delicata* Lopes, Bravo & Hajdu, 2011: *A. (H.) formosa* Vacelet, 2006: *A. (H.) stylivarians* (Topsent, 1929).

6.6. Genus Chondrocladia *Thomson, 1873*

Synonymy. *Chondrocladia* Thomson, 1873:188; *Crinorhiza* Schmidt, 1880:83; *Meliiderma* Ridley and Dendy 1887:102; not *Neocladia* Koltun, 1970:193; Vacelet et al., 2009:59.

Diagnosis. Cladorhizidae with anchorate isochelae (from Lee et al., 2012).

Type species. *Chondrocladia virgata* Thomson, 1873 (by monotypy).

6.7. Subgenus Chondrocladia *Thomson, 1873*

Diagnosis. *Chondrocladia* without a layer of special spicules (subtrochirhabds or trochirhabds), lacking special rostriform (snoutlike) subtylostyles in filaments or terminal balls, and without planar vanes formed of evenly spaced upright branches (from Lee et al., 2012).

Type species. *Chondrocladia virgata* Thomson, 1873 (by monotypy).

Species. *C. (C.) albatrossi* Tendal, 1973; *C. (C.) amphactis* (Schmidt, 1880); *C. (C.) antarctica* Hentschel, 1914; *C. (C.) arenifera* Brøndsted, 1929; *C. (C.) asigmata* Lévi, 1964; *C. (C.) burtoni* Tendal, 1973; *C. (C.) clavata* Ridley and Dendy, 1886; *C. (C.) concrescens* (Schmidt, 1880); *C. (C.) crinita* Ridley and Dendy, 1886; *C. (C.) dichotoma* Lévi, 1964; *C. (C.) fatimae* Boury-Esnault and van Beveren, 1982; *C. (C.) gigantea* (Hansen, 1885); *C. (C.) grandis* (Verrill, 1879); *C. (C.) gracilis* Lévi, 1964; *C. (C.) guiteli* Topsent, 1904; *C. (C.) koltuni* Vacelet, 2006; *C. (C.) lampadiglobus* Vacelet, 2006; *C. (C.) levii* Cristobo, Urgorri & Rios, 2005; *C. (C.) magna* Tanita, 1965; *C. (C.) michaelsarsi* Arnesen, 1920; *C. (C.) multichela* Lévi, 1964; *C. (C.) nani* Boury-Esnault and van Beveren, 1982; *C. (C.) nicolae* Cristobo, Urgorri & Rios, 2005; *C. (C.) pulvinata* Lévi, 1993; *C. (C.) robertballardi* Cristobo, Rios, Pomponi & Xavier, 2014; *C. (C.) schlatteri* Lopes, Bravo & Hajdu, 2011; *C. (C.) scolionema* Lévi, 1993; *C. (C.) vaceleti* Cristobo, Urgorri & Rios, 2005; *C. (C.) verticillata* Topsent, 1920; *C. (C.) virgata* Thomson, 1873; *C. (C.) yatsui* Topsent, 1930.

6.8. Subgenus Meliiderma *Ridley and Dendy, 1887*

Diagnosis. *Chondrocladia* bearing a dense encrustation of special spicules (subtrochirhabds or trochirhabds) packed in a single layer around the stalk and projecting vertically outward, lacking special rostriform (snoutlike) subtylostyles in filaments or terminal balls, and without planar vanes formed of evenly spaced upright branches (from Lee et al., 2012).

Type species. *Meliiderma stipitata* Ridley and Dendy, 1887 (by monotypy).

Species. *C. (M.) latruncul ioides* Lopes, Bravo & Hajdu, 2011; *C. (M.) occulta* (Lehnert, Stone & Heimler, 2006); *C. (M.) stipitata* (Ridley and Dendy, 1886); *C. (M.) tasmaniensis* Vacelet, Kelly, Schlacher-Hoenlinger, 2009; *C. (M.) turbiformis* Vacelet, Kelly, Schlacher-Hoenlinger, 2009.

6.9. Subgenus Symmetrocladia *Lee et al., 2012*

Diagnosis. *Chondrocladia* with biradial, triradial, tetraradial, or pentaradial symmetry of right triangles (vanes) formed of vertically aligned branches arising unilaterally from basal stolons. Without a stalk. Spicules are styles, rostriform (snoutlike) subtylostyles in the filaments and on the terminal balls, and unguiferous anchorate isochelae and sigmas. Trochirhabds and forceps are absent (from Lee et al., 2012).

Type species. *Chondrocladia* (*Symmetrocladia*) *lyra* Lee et al., 2012 (by original designation).

Species. *C. (S.) lyra* Lee, Reiswig, Austin & Lundsten, 2012.

6.10. Genus Cercicladia *Ríos, Kelly & Vacelet, 2011*

Diagnosis. Cladorhizidae with cercichelae, microxeas, and rare toxas, in addition to the usual mycalostyles and sigmancistras.

Acanthosubtylostyles may be present. Body feather-shaped (pinnate), with a long, thick fleshy pedicle, and a long flattened blade at the apex. Long fine filaments extend on either side of the blade forming an incurved feather-like structure (from Ríos, Kelly and Vacelet, 2011).

Type species. *Cercicladia australis* Ríos, Kelly and Vacelet, 2011 (by original designation).

Species. *C. australis* Ríos, Kelly & Vacelet, 2011.

6.11. Genus *Cladorhiza* Sars, 1872

Synonymy. *Cladorhiza* Sars, 1872:65; [*Trochoderma*] Ridley and Dendy, 1886:344 (preoccupied); *Axoniderma* Ridley and Dendy, 1886:493; *Exaxinata* de Laubenfels, 1936:122; *Raoa* de Laubenfels, 1936:123.

Diagnosis. Cladorhizidae with only anchorate/unguiferate anisochelae (from Lopes and Hajdu, 2014).

Type species. *Cladorhiza abyssicola* Sars, 1872 (by monotypy).

Species. *C. abyssicola* Sars, 1872; *C. acanthoxea* Hestetun, Fourt, Vacelet, Boury-Esnault & Rapp, 2015; *C. arctica* Koltun, 1959; *C. bathycrinoides* Koltun, 1955; *C. caillieti* Lundsten, Reiswig & Austin, 2014; *C. corona* Lehnert, Watling & Stone, 2005; *C. corticocancellata* Carter, 1876; *C. depressa* Kieschnick, 1896 (nomen nudum); *C. diminuta* Lopes and Hajdu, 2014; *C. ephyrula* Lévi, 1964; *C. evae* Lundsten, Reiswig & Austin, 2014; *C. flosabyssi* Topsent, 1909; *C. fristedti* (Lambe, 1900); *C. gelida* Lundbeck, 1905; *C. grimaldii* Topsent, 1909; *C. iniquidentata* Lundbeck, 1905; *C. inversa* Ridley and Dendy, 1886; *C. linearis* Ridley and Dendy, 1886; *C. longipinna* Ridley and Dendy, 1886; *C. mani* Koltun, 1964; *C. methanophila* Vacelet and Boury-Esnault, 2002; *C. microchela* Lévi, 1964; *C. mirabilis* (Ridley and Dendy, 1886); *C. moruliformis* Ridley and Dendy, 1886; *C. nematophora* Lévi, 1964; *C. oxeata* Lundbeck, 1905; *C. penniformis* Göcke and Janussen, 2013; *C. pentacrinus* Dendy, 1887; *C. pteron* Reiswig and Lee, 2007; *C. rectangularis* Ridley and Dendy, 1886; *C. schistochela* Lévi, 1993; *C. segonzaci* Vacelet, 2006; *C. septemdentalis* Koltun, 1970; *C. similis* Ridley and Dendy, 1886; *C. tenuisigma* Lundbeck, 1905; *C. thomsoni* Topsent, 1909; *C. tridentata* Ridley and Dendy, 1886.

6.12. Genus *Eucheliplura* Topsent, 1909

Synonymy. *Eucheliplura* Topsent, 1909:18; *Desmatiderma* Topsent, 1928:308.

Diagnosis: Cladorhizidae pinnate or branching, with placochelae, smooth arcuate/palmate isochelae and sigmancistras (modified from Hajdu and Lerner, 2002).

Type species. *Eucheliplura pristina* Topsent, 1909 (by monotypy).

Species. *E. pristina* Topsent, 1909; *E. arbuscula* (Topsent, 1928); *E. congeri* de Laubenfels, 1936; *E. elongata* Lehnert, Stone & Heimler, 2006.

6.13. Genus *Koltunicladia nom. nov.*

Synonymy. [*Neocladia*] Koltun, 1970:193 (preoccupied).

Diagnosis. Body pedunculate, with a flattened disciform body. Main skeleton is of the axial type. The microscleres are characteristic birotula-like chelae with numerous teeth and a strongly curved shaft lacking fimbriae, and sigmancistras (modified from Koltun, 1970; English translation 1972 and Vacelet, 2008).

Type species. *Neocladia flabelliformis* Koltun, 1970 (by monotypy).

Species. *K. flabelliformis* (Koltun, 1970).

6.14. Genus *Lollipocladia* Vacelet, 2008

Diagnosis. Cladorhizidae devoid of aquiferous system, with anchorate isochelae and palmate isochelae (from Vacelet, 2008).

Type species. *Lollipocladia tiburoni* Vacelet, 2008 (by original designation).

Species. *L. tiburoni* Vacelet, 2008.

6.15. Genus *Lycopodina* Lundbeck, 1905

Synonymy. *Lycopodina* Lundbeck, 1905:58; de Laubenfels, 1936:122; *Cotylina* Lundbeck, 1905:68.

Diagnosis. Cladorhizidae pedunculate with body either in the form of an erect stem or sphere with filaments in all directions, or cup-shaped. Megascleres are mycalostyles and commonly shorter (tylo)styles. Microscleres are one type of arcuate or palmate anisochela where the smaller end is in the shape of a central plate and two rudimentary, flat, lateral teeth, all with serrated edges toward the middle. To this forceps spicules are often added, but may be rare or absent in particular species or specimens of a single species. Never sigmas or sigmancistras.

Type species. *Esperella cupressiformis* var. *lycopodium* Levinsen, 1887 (by subsequent designation de Laubenfels, 1936).

Species. *L. bilamellata* (Lévi, 1993); *L. callithrix* (Hentschel, 1914); *L. calyx* (Hentschel, 1914); *L. comata* (Lundbeck, 1905); *L. communis* (Lopes and Hajdu, 2014); *L. cupressiformis* (Carter, 1874); *L. ecoprof* (Lopes and Hajdu, 2014); *L. globularis* (Lévi, 1964); *L. gracilis* (Koltun, 1955); *L. hydra* (Lundbeck, 1905); *L. hypogea* (Vacelet & Boury-Esnault, 1996); *L. lebedi* (Koltun, 1962); *L. lycopodium* (Levinsen, 1887); *L. occidentalis* (Lambe, 1893); *L. hadalis* (Lévi, 1964); *L. infundibulum* (Levinsen, 1887); *L. infundibulum* orientalis (Koltun, 1970); *L. microstrongyla* (Lopes, Bravo & Hajdu, 2011); *L. minuta* (Lambe, 1900); *L. parvula* (Hestetun, Fourt, Vacelet, Boury-Esnault & Rapp, 2015); *L. rastrichela* (Hestetun, Fourt, Vacelet, Boury-Esnault & Rapp, 2015); *L. vaceleti* (van Soest and Baker, 2011); *L. versatilis* (Topsent, 1890).

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Appendix A. Supplementary material

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References

Aguilar, R., Correa, M.L., Calcinai, B., Pastor, X., De la Torriente, A., Garcia, S., 2011. First records of *Asbestopluma hypogea* Vacelet and Boury-Esnault, 1996 (Porifera, Demospongiae Cladorhizidae) on seamounts and in bathyal settings of the Mediterranean Sea. Zootaxa 2925, 33–40.

Arnesen, E., 1920. Spongia. Report on the Scientific Results of the "Michael Sars" North Atlantic Deep-Sea Expedition, 1910, vol. III, Part 2, pp. 1–29.

Bakran-Petricioli, T., Vacelet, J., Zibrowius, H., Petricioli, D., Chevaldonné, P., 2007. New data on the distribution of the 'deep-sea' sponges *Asbestopluma hypogea* and *Oopsacas minuta* in the Mediterranean Sea. Mar. Ecol. 28 (s1), 10–23. http://dx.doi.org/10.1111/j.1439-0485.2007.00179.x.

Belinky, F., Szitenberg, A., Goldfarb, I., Feldstein, T., Wörheide, G., Ilan, M., Huchon, D., 2012. ALG11 – a new variable DNA marker for sponge phylogeny: comparison of phylogenetic performances with the 18S rDNA and the COI gene. Mol. Phylogenet. Evol. 63 (3), 702–713. http://dx.doi.org/10.1016/j.ympev.2012.02.008.

Bergquist, P.R., 1978. Sponges. University of California Press, Berkeley and Los Angeles, p. 268.

Borchiellini, C., Chombard, C., Manuel, M., Alivon, E., Vacelet, J., Boury-Esnault, N., 2004. Molecular phylogeny of Demospongiae: implications for classification and scenarios of character evolution. Mol. Phylogenet. Evol. 32 (3), 823–837. http://dx.doi.org/10.1016/j.ympev.2004.02.021.

Boury-Esnault, N., van Beveren, M., 1982. Les démosponges du plateau continental de Kerguellen-Heard. Comité national français des recherches antarctiques, no 52.

Bowerbank, J.S., 1874. A Monograph of the British Spongiadae. Ray Society, London, p. 367.

Brøndsted, H.V., 1929. Neue Schwämme aus Amoy an der Formosa-Strasse. Zool. Anz. 81 (7–10), 224–229.

Cárdenas, P., Pérez, T., Boury-Esnault, N., 2012. Advances in sponge science: phylogeny, systematics, ecology. Chapter 2: Sponge systematics facing new challenges. Adv. Mar. Biol. 61, 79–209. http://dx.doi.org/10.1016/b978-0-12-387787-1.00010-6.

Carter, H.J., 1874. Descriptions and figures of deep-sea sponges and their spicules from the Atlantic Ocean, dredged up on board H.M.S. 'Porcupine', chiefly in 1869; with figures and descriptions of some remarkable Spicules from the Agulhas Shoal and Colon, Panama. Ann. Mag. Nat. Hist. (4) 14 (79), 207–221, 245–257 (pls. XIII–XV).

Carter, H.J., 1876. Descriptions and figures of deep-sea sponges and their Spicules, from the Atlantic Ocean, dredged up on board H.M.S. 'Porcupine', chiefly in 1869 (concluded). Ann. Mag. Nat. Hist. (4) 18 (105–108), 226–240, 307–324, 388–410, 458–479 (pls. XII–XVI).

Carter, H.J., 1882. Some sponges from the West Indies and Acapulco in the Liverpool Free Museum described, with general and classificatory remarks. Ann. Mag. Nat. Hist. (5) 9 (52), 266–301, 346–368 (pls. XI–XII).

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17 (4), 540–552.

Chevaldonné, P., Perez, T., Crouzet, J., Bay-Nouailhat, W., Bay-Nouailhat, A., Fourt, M., Almon, B., Perez, J., Aguilar, R., Vacelet, J., 2014. Unexpected records of 'deep-sea' carnivorous sponges *Asbestopluma hypogea* in the shallow NE Atlantic shed light on new conservation issues. Mar. Ecol., 1–10 http://dx.doi.org/10.1111/maec.12155.

Chombard, C., Boury-Esnault, N., Tillier, A., Vacelet, J., 1997. Polyphyly of "sclerosponges" (Porifera, Demospongiae) supported by 28S ribosomal sequences. Biol. Bull. 193 (3), 359–367.

Cristobo, F.J., Ríos, P., Pomponi, S.A., Xavier, J.R., 2015. A new carnivorous sponge, *Chondrocladia robertballardi* sp. nov. (Porifera: Cladorhizidae) from two northeast Atlantic seamounts. J. Mar. Biol. Assoc. UK 95 (7), 1345–1352.

Cristobo, F.J., Urgorri, V., Ríos, P., 2005. Three new species of carnivorous deep-sea sponges from the DIVA-1 expedition in the Angola Basin (South Atlantic). Org. Divers. Evol. 5, 203–213. http://dx.doi.org/10.1016/j.ode.2004.11.004.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8). http://dx.doi.org/10.1038/nmeth.2109, 772.

de Laubenfels, M.W., 1935. Some sponges of lower California (Mexico). Am. Mus. Novit. 779, 1–14.

de Laubenfels, M.W., 1936. A Discussion of the Sponge Fauna of the Dry Tortugas in Particular and the West Indies in General, with Material for a Revision of the Families and Orders of the Porifera. Carnegie Institute of Washington Publication 467.

Dendy, A., 1887. On a remarkable new species of Cladorhiza obtained from H.M.S. 'Challenger'. Ann. Mag. Nat. Hist. (5) 20, 279–282, pl. 215.

Dendy, A., 1922. The Percy Sladen Trust Expedition to the Indian Ocean in 1905, under the leadership of Mr. J. Stanley Gardiner, M.A. Volume VII. No. I.—Report on the Sigmatotetraxonida collected by H.M.S. "Sealark" in the Indian Ocean. Trans. Linn. Soc. Lond. 2nd Ser.: Zool. 18 (1), 1–164 (pls. 161–118).

Dendy, A., 1924. Porifera. Part I. Non-Antarctic sponges. Natural history report. British Antarctic (Terra Nova) Expedition, 1910. Zoology 6 (3), 269–392 (pls I–XV).

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29 (8), 1969–1973.

Dupont, S., Carre-Mlouka, A., Domart-Coulon, I., Vacelet, J., Bourguet-Kondracki, M.-L., 2014. Exploring cultivable Bacteria from the prokaryotic community associated with the carnivorous sponge *Asbestopluma hypogea*. FEMS Microbiol. Ecol. 88 (1), 160–174. http://dx.doi.org/10.1111/1574-6941.12279.

Dupont, S., Corre, E., Li, Y., Vacelet, J., Bourguet-Kondracki, M.-L., 2013. First insights into the microbiome of a carnivorous sponge. FEMS Microbiol. Ecol. 86 (3), 520–531. http://dx.doi.org/10.1111/1574-6941.12178.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797. http://dx.doi.org/10.1093/nar/gkh340.

Ereskovsky, A.V., Willenz, P., 2007. *Esperiopsis koltuni* sp. nov. (Demospongiae: Poecilosclerida: Esperiopsidae), a carnivorous sponge from deep water of the Sea of Okhotsk (North Pacific). J. Mar. Biol. Assoc. UK 87 (6), 1379–1386. http://dx.doi.org/10.1017/S0025315407058109.

Erpenbeck, D., List-Armitage, S., Alvarez, B., Degnan, B.M., Wörheide, G., Hooper, J.N.A., 2007a. The systematics of Raspailiidae (Demospongiae: Poecilosclerida: Microcionina) re-analysed with a ribosomal marker. J. Mar. Biol. Assoc. UK 87 (6), 1571–1576. http://dx.doi.org/10.1017/S0025315407058201.

Erpenbeck, D., Nichols, S.A., Voigt, O., Dohrmann, M., Degnan, B.M., Hooper, J.N.A., Wörheide, G., 2007b. Phylogenetic analyses under secondary structure-specific substitution models outperform traditional approaches: case studies with diploblast LSU. J. Mol. Evol. 64 (5), 543–557. http://dx.doi.org/10.1007/s00239-006-0146-3.

Erpenbeck, D., Wörheide, G., 2007. On the molecular phylogeny of sponges (Porifera). Zootaxa 1668, 107–126.

Esper, E.J.C., 1794. Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet, nebst Beschreibungen. Zweyter Theil, Nürnberg, Raspe, p. 303.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294.

Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27 (2), 221–224.

Gray, J.E., 1858. Description of a new genus of sponge (*Xenospongia*) from Torres Strait. Proc. Zool. Soc. Lond. 1858, 229–230 (pl. 212).

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52 (5), 696–704. http://dx.doi.org/10.1080/10635150390235520.

Göcke, C., Janussen, D., 2013. Demospongiae of ANT XXIV/2 (SYSTCO I) expedition—Antarctic Eastern Weddell Sea. Zootaxa 3692 (1), 28–101. http://dx.doi.org/10.11646/zootaxa.3692.1.5.

Hajdu, E., de Paula, T.S., Redmond, N.E., Cosme, B., Collins, A.G., Lôbo-Hajdu, G., 2013. Mycalina: another crack in the Poecilosclerida framework. Integr. Comp. Biol. 53 (3), 462–472. http://dx.doi.org/10.1093/icb/ict074.

Hajdu, E., Lerner, C., 2002. Family Guitarridae Dendy, 1924. In: Hooper, J.N., van Soest, R.W. (Eds.), Systema Porifera. A Guide to the Classification of Sponges, vol. 1. Kluwer Academic/Plenum, New York, Boston, Dordrecht, London, Moscow, pp. 651–655.

Hajdu, E., Vacelet, J., 2002. Family Cladorhizidae Dendy, 1922. In: Hooper, J.N., van Soest, R.W. (Eds.), Systema Porifera. A Guide to the Classification of Sponges, vol. 1. Kluwer Academic/Plenum, New York, Boston, Dordrecht, London, Moscow, pp. 636–641.

Hajdu, E., van Soest, R.W.M., Hooper, J.N.A., 1994. Proposal for a phylogenetic subordinal classification of poecilosclerid sponges. In: van Soest, R.W., van Kempen, T.M., Braekman, J.-C. (Eds.), Sponges in Time and Space. Balkema, Rotterdam, pp. 123–139.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Proceedings of the Nucleic Acids Symposium Series, pp. 95–98.

Hansen, G.A., 1885. Spongiadæ. The Norwegian North-Atlantic expedition 1876–1878. Zoology 13, 1–26 (pls. I–VII).

Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27 (3), 570–580.

Hentschel, E., 1914. Monaxone Kieselschwämme und Hornschwämme der Deutschen Südpolar-Expedition 1901–1903. Deutsche Südpolar-Expedition, 1901–1903, vol. XV-1, pp. 35–141 (pls. IV–VIII).

Hentschel, E., 1923. Erste Unterabteilung der Metazoa: Parazoa, Porifera-Schwämme. In: Kükenthal, W., Krumbach, T. (Eds.), Handbuch der Zoologie. Eine Naturgeschichteder Stämme des Tierreiches. Protozoa, Porifera, Coelenterata, Mesozoa, vol. 1, pp. 307–418, (figs. 288–377). Walter de Gruyter und Co., Berlin and Leipzig.

Hestetun, J.T., Fourt, M., Vacelet, J., Boury-Esnault, N., Rapp, H.T., 2015. Cladorhizidae (Porifera, Demospongiae, Poecilosclerida) of the deep Atlantic collected during Ifremer cruises, with a biogeographic overview of the Atlantic species. J. Mar. Biol. Assoc. UK 95 (7), 1311–1342. http://dx.doi.org/10.1017/s0025315413001100.

Hooper, J.N.A., Lévi, C., 1989. *Esperiopsis desmophora* n. sp. (Porifera: Demospongiae): a desma-bearing Poecilosclerida. Mem. Queensl. Mus. 27 (2), 437–441.

Hooper, J.N.A., van Soest, R.W.M., 2002. Order Poecilosclerida Topsent, 1928. In: Hooper, J.N., van Soest, R.W. (Eds.), Systema Porifera. A Guide to the Classification of Sponges, vol. 1. Kluwer Academic/Plenum, New York, Boston, Dordrecht, London, Moscow, pp. 403–408.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294 (5550), 2310–2314. http://dx.doi.org/10.1126/science.1065889.

Ise, Y., Vacelet, J., 2010. New carnivorous sponges of the genus *Abyssocladia* (Demospongiae, Poecilosclerida, Cladorhizidae) from Myojin Knoll, Izu-Ogasawara Arc, southern Japan. Zool. Sci. 27 (11), 888–894. http://dx.doi.org/10.2108/zsj.27.888.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30 (4), 772–780. http://dx.doi.org/10.1093/molbev/mst010.

Kelly, M., Samaai, T., 2002. Suborder Latrunculina subord. nov. incertae sedis. In: Hooper, J.N., van Soest, R.W. (Eds.), Systema Porifera. A Guide to the Classification of Sponges, vol. 1. Kluwer Academic/Plenum, New York, Boston, Dordrecht, London, Moscow, p. 707.

Kelly, M., Vacelet, J., 2011. Three new remarkable carnivorous sponges (Porifera, Cladorhizidae) from deep New Zealand and Australian (Macquarie Island) waters. Zootaxa 2976, 55–68.

Kieschnick, O., 1896. Silicispongiae von Ternate nach den Sammlungen von Herrn Prof. Dr. W. Kükenthal. Zool. Anz. 19 (520), 526–534.

Kirkpatrick, R., 1908. On two new genera of recent pharetronid sponges. Ann. Mag. Nat. Hist. (8) 2 (12), 503–514 (pls. XIII–XV).

Koltun, V.M., 1955. Sponges. Atlas of the invertebrate fauna of the far-Eastern seas of the USSR. Akad. Nauk SSSR, 45–50 (pls. IV–VI) (in Russian).

Koltun, V.M., 1958. Cornacuspongia of sea waters washing the South Sakhalin and the South Kurile Island region. Issled. Dal'nevostochnykh Morei SSSR 5, 42–77 (figs. 41-25) (in Russian).

Koltun, V.M., 1959. Siliceous horny sponges of the northern and fareastern seas of the U.S.S.R. Opredeliteli po faune SSR, izdavaemye Zoologicheskim muzeem Akademii nauk, 67, pp. 1–236 (in Russian).

Koltun, V.M., 1962. Four rayed and siliceous horny sponges from the Pacific shallow waters of Paramushir and Shumshu Islands. Issledovaniya dal'nevostochnykh morei SSSR 8, 181–199 (in Russian).

Koltun, V.M., 1964. Sponges of the Antarctic. 1. Tetraxonida and Cornacuspongida. In: Pavlovskii, E., Andriyashev, A., Ushakov, P. (Eds.), Biological Reports of the Soviet Antarctic Expedition (1955–1958), Academy of Sciences of the U.S.S.R. [English translation, Israel Program for Scientific Translations Ltd., 1966, Jerusalem, pp. 1–372 (pls I–VIII)].

Koltun, V.M., 1970. Sponge fauna of the northwestern Pacific from the shallows to the hadal depths. In: Bogorov, V. (Ed.), Fauna of the Kurile–Kamchatka Trench and Its Environment, Institute of Oceanology of the Academy of Sciences of the U.S.S.R., vol. 86, [English translation, Israel Program for Scientific Translations Ltd., 1972, Jerusalem, pp. 1–372].

Kübler, B., Barthel, D., 1999. A carnivorous sponge, *Chondrocladia gigantea* (Porifera: Demospongiae), the giant deepsea clubsponge from the Norwegian Trench. Mem. Queensl. Mus. 44, 289–298.

Lambe, L.M., 1893. Sponges from the Pacific coast of Canada. Proc. Trans. Roy. Soc. Canada 11 (4), 25–43 (pls II–IV).

Lambe, L.M., 1900. Catalogue of the recent marine sponges of Canada and Alaska. Ottawa Nat. 14 (9), 153–172.

Lambert, S.M., Reeder, T.W., Wiens, J.J., 2015. When do species-tree and concatenated estimates disagree? An empirical analysis with higher-level scincid lizard phylogeny. Mol. Phylogenet. Evol. 82, 146–155.

Larkin, M.A., Blackshields, G., Brown, N., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23 (21), 2947–2948.

Lavrov, D.V., Wang, X., Kelly, M., 2008. Reconstructing ordinal relationships in the Demospongiae using mitochondrial genomic data. Mol. Phylogenet. Evol. 49 (1), 111–124. http://dx.doi.org/10.1016/j.ympev.2008.05.014.

Lee, W.L., Reiswig, H.M., Austin, W.C., Lundsten, L., 2012. An extraordinary new carnivorous sponge, *Chondrocladia lyra*, in the new subgenus *Symmetrocladia* (Demospongiae, Cladorhizidae), from off of northern California, USA. Invertebr. Biol. 131 (4), 259–284. http://dx.doi.org/10.1111/Ivb.12001.

Lehnert, H., Stone, R., Heimler, W., 2006. New species of deep-sea demosponges (Porifera) from the Aleutian Islands (Alaska, USA). Zootaxa 1250, 1–35.

Lehnert, H., Watling, L., Stone, R., 2005. *Cladorhiza corona* sp. nov. (Porifera: Demospongiae: Cladorhizidae) from the Aleutian islands (Alaska). J. Mar. Biol. Assoc. UK 85 (6), 1359–1366. http://dx.doi.org/10.1017/S0025315405012531.

Lévi, C., 1964. Spongiaires des zones bathyale, abyssale et hadale. Galathea Report. Scientific Results of The Danish Deep-Sea Expedition Round the World, 1950–52, vol. 7, pp 63–112 (pls II–XI).

Lévi, C., 1993. Porifera Demospongiae: Spongiaires bathyaux de Nouvelle-Calédonie, récoltés par le «Jean Charcot» Campagne BIOCAL, 1985. Mém. Mus. Natl. d'Hist. Nat. 158, 9–87.

Levinsen, G.M.R., 1887. Kara-Havets Svampe (Porifera). Dijmphna-Togtets zoologisk-botaniske Udbytte, vol. 1, pp. 339–372 (pls XXIX–XXXI).

Lopes, D.A., Bravo, A., Hajdu, E., 2011. New carnivorous sponges (Cladorhizidae: Poecilosclerida: Demospongiae) from off Diego Ramírez Archipelago (south Chile), with comments on taxonomy and biogeography of the family. Invertebr. Syst. 25 (5), 407–443. http://dx.doi.org/10.1071/is11015.

Lopes, D.A., Hajdu, E., 2014. Carnivorous sponges from deep-sea coral mounds in the Campos Basin (SW Atlantic), with the description of six new species (Cladorhizidae, Poecilosclerida, Demospongiae). Mar. Biol. Res. 10 (4), 329–356. http://dx.doi.org/10.1080/17451000.2013.797587.

Lundbeck, W., 1905. Porifera. (Part II.) Desmacidonidae (pars.). In: The Danish Ingolf-Expedition 6(2). BiancoLuno, Copenhagen, pp. 1–219 (pls I–XX).

Lundsten, L., Reiswig, H.M., Austin, W.C., 2014. Four new species of Cladorhizidae (Porifera, Demospongiae, Poecilosclerida) from the Northeast Pacific. Zootaxa 3786 (2), 101–123. http://dx.doi.org/10.11646/zootaxa.3786.2.1.

Morrow, C.C., Cárdenas, P., 2015. Proposal for a revised classification of the Demospongiae (Porifera). Front. Zool. 12 (7), 1–27. http://dx.doi.org/10.1186/s12983-015-0099-8.

Morrow, C.C., Picton, B.E., Erpenbeck, D., Boury-Esnault, N., Maggs, C.A., Allcock, A.L., 2012. Congruence between nuclear and mitochondrial genes in Demospongiae: a new hypothesis for relationships within the G4 clade (Porifera: Demospongiae). Mol. Phylogenet. Evol. 62 (1), 174–190. http://dx.doi.org/10.1016/j.ympev.2011.09.016.

Perkins, R.C.L., 1906. Leaf-hoppers and their natural enemies (Pt. VIII. Encyrtidae, Eulophidae, Trichogrammidae). Bulletin of the Hawaiian Sugar Planters' Association Experiment Station (Entomology Series), No. 1, pp. 239–267.

Redmond, N.E., Morrow, C.C., Thacker, R.W., Diaz, M.C., Boury-Esnault, N., Cárdenas, P., Hajdu, E., Lôbo-Hajdu, G., Picton, B.E., Pomponi, S.A., Kayal, E., Collins, A.G., 2013. Phylogeny and systematics of Demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. Integr. Comp. Biol. 53 (3), 388–415. http://dx.doi.org/10.1093/icb/ict078.

Redmond, N.E., van Soest, R.W.M., Kelly, M., Raleigh, J., Travers, S.A.A., McCormack, G.P., 2007. Reassessment of the classification of the Order Haplosclerida (Class Demospongiae, Phylum Porifera) using 18S rRNA gene sequence data. Mol. Phylogenet. Evol. 43 (1), 344–352. http://dx.doi.org/10.1016/j.ympev.2006.10.021.

Reiswig, H.M., Lee, W.L., 2007. A new species of Cladorhiza (Porifera: Cladorhizidae) from S California (USA). In: Porifera Research: Biodiversity, Innovation, Sustainability. Série Livros, vol. 28, pp. 517–523.

Ridley, S.O., Dendy, A., 1886. Preliminary report on the Monaxonida collected by HMS 'Challenger'. Ann. Mag. Nat. Hist. 5 (18), 325–351, 470–493.

Ridley, S.O., Dendy, A., 1887. Report on the Monaxonida Collected by H.M.S. 'Challenger' During the Years 1873–1876. Report on the Scientific Results of the Voyage of H.M.S. 'Challenger', 1873–1876. Zoology 20(59): i–lxviii. Neill and Company, Edinburgh, pp. 1–275 (pls I–LI).

Riesgo, A., Novo, M., Sharma, P.P., Peterson, M., Maldonado, M., Giribet, G., 2014. Inferring the ancestral sexuality and reproductive condition in sponges (Porifera). Zool. Scr. 43 (1), 101–117. http://dx.doi.org/10.1111/Zsc.12031.

Riesgo, A., Taylor, C., Leys, S.P., 2007. Reproduction in a carnivorous sponge: the significance of the absence of an aquiferous system to the sponge body plan. Evol. Dev. 9 (6), 618–631. http://dx.doi.org/10.1111/j.1525-142X.2007.00200.x.

Ríos, P., Kelly, M., Vacelet, J., 2011. *Cercicladia australis*, a new carnivorous sponge with novel chelae from the Tasman Basin and the Argentine Patagonian Margin (Porifera, Cladorhizidae). Zootaxa 3131, 52–62.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19 (12), 1572–1574.
Rot, C., Goldfarb, I., Ilan, M., Huchon, D., 2006. Putative cross-kingdom horizontal gene transfer in sponge (Porifera) mitochondria. BMC Evol. Biol. 6 (1), 71. http://dx.doi.org/10.1186/1471-2148-6-71.
Sars, G.O., 1872. On Some Remarkable Forms of Animal Life from the Great Deeps Off the Norwegian Coast. Part 1, Partly from Posthumous Manuscripts of the Late Professor Dr. Michael Sars. University Program for the 1st Half-year 1869. Brøgger & Christie, Christiania.
Schmidt, O., 1870. Grundzüge einer Spongien-Fauna des atlantischen Gebietes. Wilhelm Engelmann, Leipzig, p. 88.
Schmidt, O., 1875. Spongien. Die Expedition zur physikalisch-chemischen und biologischen Untersuchung der Nordsee im Sommer 1872. Jahresbericht der Commission zur Wissenschaftlichen Untersuchung der Deutschen Meere in Kiel, vols. 2–3, pp. 115–120 (pl I).
Schmidt, O., 1880. Die Spongien des Meerbusen von Mexico (Und des caraibischen Meeres). Heft II. Abtheilung II. Hexactinelliden. Abtheilung III. Tetractinelliden. Monactinelliden und Anhang. Nachträge zu Abtheilung I (Lithistiden). Reports on the Dredging under the Supervision of Alexander Agassiz, in the Gulf of Mexico, by the USCSS 'Blake'. Gustav Fischer, Jena, pp. 33–90 (pls V–X).
Stamatakis, A., 2014a. The RAxML v8.0.X Manual. Heidelberg Institute for Theoretical Studies.
Stamatakis, A., 2014b. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9), 1312–1313. http://dx.doi.org/10.1093/bioinformatics/btu033.
Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56 (4), 564–577. http://dx.doi.org/10.1080/10635150701472164.
Tanita, S., 1965. Porifera. In: Okada, Y., Uchida, S., Uchida, T. (Eds.), New Illustrated Encyclopedia of the Fauna of Japan, vol. 1. Hokuryukan, Tokyo, pp. 138–166 (in Japanese).
Tendal, O.S., 1973. Sponges collected by the Swedish deep sea expedition. Zool. Scr. 2 (1), 33–38.
Thacker, R.W., Hill, A.L., Hill, M.S., Redmond, N.E., Collins, A.G., Morrow, C.C., Spicer, L., Carmack, C.A., Zappe, M.E., Pohlmann, D., Hall, C., Diaz, M.C., Bangalore, P.V., 2013. Nearly complete 28S rRNA gene sequences confirm new hypotheses of sponge evolution. Integr. Comp. Biol. 53 (3), 373–387. http://dx.doi.org/10.1093/icb/ict071.
Thomson, W.C., 1873. The Depths of the Sea. Macmillan and Co, London, p. 527.
Topsent, E., 1890. Notice préliminaire sur les spongiaires recueillis durant les campagnes de l'Hirondelle. Bulletin de la Société zoologique de France 15, 26–32, 65–71.
Topsent, E., 1901. Spongiaires. Résultats du voyage du S.Y. 'Belgica'en 1897–99 sous le commandement de A. de Gerlache de Gomery. Expédition antarctique belge. Zoologie 4, 1–54 (pls I–VI).
Topsent, E., 1904. Spongiaires des Açores. Résultats des campagnes scientifiques accomplies sur son yacht par le Prince Albert I, Monaco. Govt. of Monaco, Monaco.
Topsent, E., 1909. Etude sur quelques Cladorhiza et sur Eucheliplura pristina n. g. et n. sp. Bulletin de l'Institut Océanographique de Monaco, vol. 151, pp. 1–23.
Topsent, E., 1920. Spongiaires du Musée Zoologique de Strasbourg. Monaxonides. Bulletin de l'Institut océanographique, Monaco, vol. 381, pp. 1–36.
Topsent, E., 1928. Une Mycaline productrice de desmes Desmatiderma arbuscula, n. g., n. sp. Bulletin de l'Institut Océanographique de Monaco, vol. 519, pp. 1–8.
Topsent, E., 1929. Notes sur Helophloeina stylivarians, n. g., n. sp., Mycaline à desmes des Canaries. Bulletin de l'Institut Océanographique de Monaco, vol. 533, pp. 1–8.
Topsent, E., 1930. *Chondrocladia yatsui* n. sp., de la Baie de Sagami. Annot. Zool. Japon. 12, 421–432.
Vacelet, J., 1996. Deep-sea sponges in a Mediterranean cave. In: Uiblein, F., Ott, J., Stachowitsch, M. (Eds.), Deep-sea and Extreme Shallow-water Habitats: Affinities and Adaptations. Biosystematics and Ecology Series 11. Österreichische Akademie der Wissenschaften, pp. 299–312.
Vacelet, J., 2006a. New carnivorous sponges (Porifera, Poecilosclerida) collected from manned submersibles in the deep Pacific. Zool. J. Linn. Soc. 148 (4), 553–584. http://dx.doi.org/10.1111/j.1096-3642.2006.00234.x.
Vacelet, J., 2006b. Porifera. In: Desbruyères, D., Segonzac, M., Bright, M. (Eds.), Handbook of Deep-Sea Hydrothermal Vent Fauna. Second completely revised edition, p. 544 (Denisia, No. 18).
Vacelet, J., 2007. Diversity and evolution of deep-sea carnivorous sponges. In: Porifera Research: Biodiversity, Innovation and Sustainability. Série Livros, vol. 28, pp. 107–115.
Vacelet, J., 2008. A new genus of carnivorous sponges (Porifera: Poecilosclerida, Cladorhizidae) from the deep N–E Pacific, and remarks on the genus *Neocladia*. Zootaxa 1752, 57–65.
Vacelet, J., Boury-Esnault, N., 1996. A new species of carnivorous sponge (Demospongiae: Cladorhizidae) from a Mediterranean cave. Bulletin van het Koninklijk Belgisch Instituut voor Natuurwetenschappen. Biologie/Bulletin de l'Institut Royal des Sciences Naturelles de Belgique. Biologie, 66(Suppl.).
Vacelet, J., Boury-Esnault, N., 2002. A new species of carnivorous deep-sea sponge (Demospongiae: Cladorhizidae) associated with methanotrophic bacteria. Cah. Biol. Mar. 43 (2), 141–148.
Vacelet, J., Boury-Esnault, N., Fiala-Medioni, A., Fisher, C.R., 1995. A methanotrophic carnivorous sponge. Nature 377 (6547). http://dx.doi.org/10.1038/377296a0, 296.
Vacelet, J., Duport, E., 2004. Prey capture and digestion in the carnivorous sponge *Asbestopluma hypogea* (Porifera: Demospongiae). Zoomorphology 123 (4), 179–190. http://dx.doi.org/10.1007/s00435-004-0100-0.
Vacelet, J., Fiala-Medioni, A., Fisher, C.R., Boury-Esnault, N., 1996. Symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous cladorhizid sponge. Mar. Ecol. Prog. Ser. 145 (1–3), 77–85. http://dx.doi.org/10.3354/Meps145077.
Vacelet, J., Kelly, M., 2008. New species from the deep Pacific suggest that carnivorous sponges date back to the Early Jurassic. Nat. Preced.
Vacelet, J., Kelly, M., 2014. A new species of *Abyssocladia* (Porifera, Demospongiae, Poecilosclerida, Cladorhizidae) and other carnivorous sponges from the far eastern Solomon Islands. Zootaxa 3815, 386–396. http://dx.doi.org/10.11646/zootaxa.3815.3.4.
Vacelet, J., Kelly, M., Schlacher-Hoenlinger, M., 2009. Two new species of *Chondrocladia* (Demospongiae: Cladorhizidae) with a new spicule type from the deep south Pacific, and a discussion of the genus *Meliiderma*. Zootaxa 2073, 57–68.
van Soest Rob, W.M., Hajdu, E., 2002. Family Phellodermidae fam. nov. In: Hooper, J. N., van Soest, R.W. (Eds.), Systema Porifera. A Guide to the Classification of Sponges, vol. 1. Kluwer Academic/Plenum, New York, Boston, Dordrecht, London, Moscow, pp. 621–624.
van Soest, R.W.M., Baker, B.J., 2011. A new carnivorous shallow-water sponge from McMurdo Sound, Antarctica (Porifera, Poecilosclerida). Mar. Biodivers. 41 (4), 495–501. http://dx.doi.org/10.1007/s12526-010-0076-6.
van Soest, R.W.M., Boury-Esnault, N., Hooper, J.N.A., Rützler, K., de Voogd, N.J., Alvarez, B.d.G., Hajdu, E., Pisera, A.B., Manconi, R., Schoenberg, C., Janussen, D., Tabachnick, K.R., Klautau, M., Picton, B., Kelly, M., Vacelet, J., Dohrmann, M., Cristina Díaz, M., Cárdenas, P., 2015. World Porifera Database (accessed 15.08.24).
Vargas, S., Erpenbeck, D., Göcke, C., Hall, K.A., Hooper, J.N., Janussen, D., Wörheide, G., 2013. Molecular phylogeny of *Abyssocladia* (Cladorhizidae: Poecilosclerida) and *Phelloderma* (Phellodermidae: Poecilosclerida) suggests a diversification of chelae microscleres in cladorhizid sponges. Zool. Scr. 42 (1), 106–116. http://dx.doi.org/10.1111/j.1463-6409.2012.00560.x.
Verrill, A.E., 1879. Notice of recent additions to the marine invertebrates of the northeastern coast of America, with descriptions of new genera and species, and critical remarks. Proc. U.S. Natl. Mus. 2, 165–205.
Voigt, O., Erpenbeck, D., Wörheide, G., 2008. Molecular evolution of rDNA in early diverging Metazoa: first comparative analysis and phylogenetic application of complete SSU rRNA secondary structures in Porifera. BMC Evol. Biol. 8 (1), 69.
Vosmaer, G.C.J., 1880. The sponges of the Leyden Museum. 1. The family of the Desmacidinae. Notes Leyden Mus. 2, 99–164.
Xia, X., 2013. DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. Mol. Biol. Evol. 30 (7), 1720–1728. http://dx.doi.org/10.1093/molbev/mst064.