Phylogeny and taxonomy of Polymastiidae (Porifera: Demospongiae)

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out from the seafloor, explained that it was Polymastia and offered to study its life history. Five years later I defended a master thesis under his supervision. I am very grateful to Alexander who sparked my interest in sponges and taught me the fundamentals of their biology.

In the years preceding my PhD-studentship I was actively collaborating on the deep-sea Antarctic sponges with Dorte Janussen (Senckenberg Forschungsinstitutt und Naturmuseum, Frankfurt am Main). I am very grateful to Dorte for I learnt a lot in her laboratories and together we published two papers on the Antarctic polymastiids, which made a very useful background for my PhD-project.

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My project for the most part focused on molecular phylogenies. When I came to UiB, I was just a dummy in the DNA techniques. But thanks the staff of the Biodiversity Laboratories, Kenneth Meland, Solveig Thorkildsen and Louise Lindblom, I learnt these methods, first through the group introductory course and then through their individual consulting.

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When all morphological material was examined and the DNA sequences were obtained it was time to prepare the manuscripts. I would like to heartily thank the co-authors of my papers, Oliver Voigt (Ludwig-Maximilians-Universität München, Paleontology and Geobiology) and Christine Morrow (Department of Zoology, Ryan Institute, National University of Ireland, Galway). Despite being overloaded with many other projects and teaching activity, Oliver enthusiastically agreed to work together on the polymastiid phylogeny. It was him who adapted the secondary structure of 28S rDNA reconstructed from other sponge families (but still unpublished) to the Polymastiidae. Furthermore, he taught me PHASE,

the software specially designed for the RNA specific substitution models and, when a fairly new algorithm of RNA model selection became available, he suggested trying it. Christine kindly accepted my suggestion to work together on Sphaerotylus and came with a new species discovered by her in the Irish Coast many years ago and awaiting publishing. She also provided me with the successful primer sequences for 28S rDNA before they became publicly available.

Last, but not least, I am definitely much obliged to Elena Gerasimova. Elena is not only a co-author of three of my papers presented in this thesis. She was with me from the very beginning of my carrier and studies, assisting at all stages. I appreciate deeply that she carried all my failures and troubles on this long way on her shoulders. Her steadfast believe in my success was decisive in the completion of this project.

Abstract

This thesis focuses on the family Polymastiidae, one of the key taxa of sponges (phylum Porifera), an important component of marine benthos in the polar and temperate seas. The current taxonomy of this family is based on rather few unstable morphological characters. Molecular data were previously obtained from a relatively small number of polymastiid species and the phylogeny of the polymastiids has been never studied. The main aim of the present research was to fill the gap in our knowledge on the Polymastiidae by taxonomic revision and phylogenetic reconstructions based on novel morphological and molecular data.

Applying multiple morphological characters, we have revised the polymastiid genera, which were so far distinguished exclusively by the shape of extraordinary cortical spicules (exotyles). A new genus *Koltunia* and three new species of *Sphaerotylus* have been established. We have also proposed resurrection of *Suberitechinus*, previously synonymized with *Trachyteleia*, and transferring of two species of *Polymastia*, one to *Sphaerotylus* and the other to *Proteleia*.

Based on morphological characters of 21 species representing most of the polymastiid genera, we have recovered three possible scenarios for character evolution in the family. Non-monophyly of the Polymastiidae and its largest genus *Polymastia* has been revealed. The most parsimonious scenario implies three synapomorphies of the polymastiid clade: loss of oscula on the main body surface, acquisition of the oscula-bearing papillae, and acquisition of the regular choanosomal skeleton. Consistency of the skeleton architecture, being radial or reticulate, within the polymastiid clade has been demonstrated.

The phylogenies based on two molecular markers, the 5'-end barcoding region of cytochrome oxidase I (COI) and a fragment of the large ribosomal subunit DNA (28S rDNA), have challenged the hypotheses on the relationships between the polymastiid species based on morphology , indicating homoplasy of most morphological characters, except for the presence of oscula-bearing papillae and the absence of oscula on the main body surface. Particularly, a secondary loss of the regular choanosomal skeleton has been suggested and inconsistency of the skeleton architecture has been revealed in the polymastiid clade. Non-monophyly of four genera, *Polymastia*, *Radiella*, *Sphaerotylus* and *Tentorium*, has been demonstrated with the molecular data. The molecular phylogenies strongly support three clades, each including the type species of the respective genera *Polymastia*, *Sphaerotylus* and *Spinularia*. However, no morphological

synapomorphies can be defined for these clades and, accordingly, no satisfactory classification of Polymastiidae can be proposed for now. Nevertheless, based on the molecular phylogenies, we have proposed one change in the current classification, the abandonment of *Radiella*, with two species previously placed in this genus transferred to *Spinularia* and one species transferred to *Polymastia*.

Some inconsistencies between the estimated 28S rDNA and CO1 trees were found. They may result from unequal evolutionary rates and different genealogical histories of the mitochondrial and nuclear genes studied. The other factor leading to the inconsistencies is the lower resolution of the CO1 tree in comparison with the 28S rDNA tree, indicating insufficiency of the phylogenetic signal in the standard 5'-end barcoding region for reconstruction of sponge phylogenies.

Another problem revealed in our study is an intragenomic polymorphism of 28S rDNA in three closely related species of *Polymastia*, with some identical gene versions observed in individuals of different species. Since these species are otherwise clearly distinguished in CO1, we assume that the polymorphism in the nuclear gene may result from incomplete lineage sorting, or from a gene flow through hybridization between the species.

Based on both morphological and molecular data, we have revised the polymastiid fauna of the Nordic and Siberian Seas. Twenty species, of which two are new to science and three are new for the area of the study, have been documented. We assume an Atlantic origin of all polymastiid species recorded in the Arctic, with ten species distributed in a wide area from Canada to the Siberian Seas and four species limited to the northern-east sector of this area. Furthermore, we have questioned the allegedly cosmopolitan distribution of two species.

Our study emphasizes once again the advantages of the integrative approach based on multiple morphological characters and datasets of several genes for natural classification of organisms.

Sammendrag

Denne avhandlingen fokuserer på familien Polymastiidae, som er en av nøkkeltaksonene av svamper (rekke Porifera) og en viktig komponent av bunndyrsamfunnene i polare og tempererte hav. Taksonomien til denne familien har så langt vært basert på svært få og ustabile morfologiske trekk. Molekylære data har vært tilgjengelig for kun et relativt lite antall arter og polymastiidenes fylogeni har aldri blitt studert. Målet med denne avhandlingen er å fylle viktige kunnskapshull om Polymastiidae gjennom taksonomisk revisjon og fylogenetiske analyser basert på nye morfologiske og molekylære data.

Ved bruk av multiple morfologiske trekk har vi revidert polymastiideslektene som fram til nå har vært adskilt på bakgrunn av formen på ekstraordinære cortikale spikler (exotyler). En ny slekt (*Koltunia*) og tre nye arter av *Sphaerotylus* er opprettet. Vi foreslår også gjenopprettelse av slekten *Suberitechinus*, som tidligere ble synonymisert med *Trachyteleia*, og overføring av to arter fra *Polymastia*, én til *Sphaerotylus* og den andre til *Proteleia*.

Fylogenetiske analyser basert på morfologiske trekk hos 21 arter indikerer at Polymastiidae og dens største slekt *Polymastia* ikke er naturlige monofyletiske grupper. Det mest parsimoniske scenariet viser tre sannsynlige synapomorfier for cladden Polymastiidae: tap av utstrømningsåpninger (osculi) på svampens overflate, utvikling av papiller med osculi og utvikling av et regelmessig choanosomalskjelett. Den radiære eller retikulære skjelettarkitekturen synes å være konsistent i hele Polymastiidae.

Fylogeniene basert på de to molekylære markørene, 5'-end-strekkodefragmentet av cytochrome oxidase I (COI) og et fragment av den store ribosom-subenheten (28S rDNA) tyder imidlertid på at det er stor grad av homoplasi i de fleste morfologiske trekk hos polymastiider bortsett fra de oskulabærende papillene og mangel på oskuli på kroppsoverflaten. Blant annet antydes mangelen på et regelmessig choanosomalskjelett å være et sekundært tap og det er observert inkonsistens av skjelettarkitekturen innen Polymastiidae. Analysene indikerer også at de fire slektene *Polymastia*, *Radiella*, *Sphaerotylus* og *Tentorium* ikke er monofyletiske.

De fylogenetiske analysene basert på molekylære data gir tre godt definerte clader, hver med de respektive typeartene av slektene *Polymastia*, *Sphaerotylus* og *Spinularia* inkludert, men ettersom ingen morfologiske synapomorfier kan defineres for disse cladene, kan det for øyeblikket ikke foreslås en ny og tilfredsstillende klassifikasjon av

Polymastiidae. Ut fra disse analysene kan man imidlertid foreslå en mindre endring i den nåværende klassifikasjonen. Slekten *Radiella* legges ned og to arter som tidlegere var plassert i denne slekten flyttes, én art overføres til *Spinularia* og den andre flyttes til *Polymastia*.

De individuelle gentrærne estimerte fra 28S rDNA og CO1 viser noen enkelte motsetninger. De kan skyldes ulike evolusjonstempoer og forskjellige genealogier for mitochondrielle og nukleæregener. Lavere oppløsning i CO1-treet i sammenligning med 28S rDNA-treet tyder på at variasjonen i det vanlige 5'-end-strekkodefragmentet alene er utilstrekkelig for rekonstruering av svampenes fylogeni.

Et annet problem avslørt ved studiet vårt er en intragenomisk polymorfisme i tre nært beslektede *Polymastia*-arter, med noen identiske genversjoner funnet i individer av forskjellige arter. Siden disse artene ellers er klart skilte i CO1, antar vi at polymorfismen i nukleæregenet kan forårsakes av ufullstendig linjesortering eller av genflyt gjennom hybridisering mellom artene.

Basert på både morfologiske og molekylære data har vi revidert polymastiidfaunaen i nordiske og sibirske havområder. Tjue arter, hvorav to er nye for vitenskap og tre er nye for området, har blitt dokumentert. Vi antar en atlantisk opprinnelse av alle arter av polymastiider registrert i Arktis, med ti arter utbredt i et stort område fra Kanada til Sibir og fire arter begrenset til den nordøstlige sektoren av dette området. To arter som tidligere var regnet som kosmopolitiske har nå vist seg å ha en langt mer begrenset utbredelse.

Vårt studium understreker tydelig viktigheten av å kombinere morfologiske og genetiske data for å komme fram til en i størst mulig grad naturlig klassifikasjon av organismer.

List of publications

I. Plotkin, A., Gerasimova, E., & Rapp, H.T. (2012): Phylogenetic reconstruction of Polymastiidae (Demospongiae: Hadromerida) based on morphology. Hydrobiologia, 687(1): 21–41.

II. Plotkin, A., Morrow, C., Gerasimova, E., & Rapp, H.T. (2016): Polymastiidae (Demospongiae: Hadromerida) with ornamented exotyles: a review of morphological affinities and description of a new genus and three new species. Journal of the Marine Biological Association of the United Kingdom, on-line early view, available at http://dx.doi.org/10.1017/S0025315416000655

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Contents

1. Introduction

1.1. Introduction to the phylum Porifera

1.1.1. Sponges as the oldest metazoans and an important component of benthic ecosystems

Phylum Porifera Grant, 1836 (sponges) comprises aquatic sessile metazoans commonly characterized by the possession of an aquiferous system with flagellated cells, the choanocytes, producing water current through the body, the lack of a tissue grade of construction and the presence of a highly totipotent population of cells (Hooper et al. 2002). These traits distinguishing the sponges from other metazoans suggest the ancientness of Porifera that is confirmed by the fossil records (Li et al. 1998) and molecular clock analyses (Savolainen et al. 2005; Sperling et al. 2010) dating the origin of this phylum to Precambrian. For the time being almost 9000 extant poriferan species, of which 98% are marine and others exist in freshwaters, are recognized as valid (Van Soest et al. 2016). This number apparently represents only a small fraction of the sponge species that have ever lived (Hooper & Van Soest 2002a) and just a half of the estimated extant sponge biodiversity considering that the shelf areas in the tropics and around South America as well as the spacious deep-water seabed areas elsewhere are poorly explored (Van Soest 2007).

Sponges are an important component of many benthic ecosystems (Maldonado et al. 2016). World-famous sponge reefs having survived from the Late Jurassic Period occupy more than 800 km^2 along the Pacific Coast of Canada (Conway et al. 1991; 2005; Krautter et al. 2001). Other well-known habitats are the deep-sea sponge grounds in the North Atlantic, where the biomass of these animals may exceed 500 kg per hectare (Klitgaard et al. 1997; Klitgaard & Tendal 2004; Murillo et al. 2012; 2016; Kutti et al. 2013), sponge aggregations on coral reefs and mangroves in tropics and sponge fields in the Antarctic (Maldonado et al. 2016). Large sponges serve as nursery and breeding areas for plenty of small invertebrates and fishes living both on the sponge surface and inside the aquiferous system. Sponges lack the nervous and muscular systems, but produce an extremely powerful chemical defence against parasites and predators. During the last decades extensive studies of the secondary metabolites produced by sponges have proved their strong antiviral and antibacterial activity with a large potential in pharmaceutical industry (Sipkema et al. 2005; Perdicaris et al. 2013). Furthermore, the sponges are promising indicators of the environmental health state used for estimation

of the impact caused by the seafloor drilling (Kutti et al. 2015; Edge et al. 2016) and possible climate changes (Kahn et al. 2012; Bell et al. 2013).

1.1.2. Basics of sponge morphology and physiology

Sponge body is composed of three main components, an outer layer of exopinacocytes, a gel-like inner mass, the mesohyl, and an aquiferous system, and reinforced by organic and, in most species, mineral skeleton (Boury-Esnault & Rützler, 1997; Hooper et al. 2002). The organic skeleton comprises relatively thin fibres of collagen and thicker fibres of spongin, a compound protein-chitinous substance (Ehrlich et al. 2007a, b). The mineral skeleton is calcareous or siliceous. Some sponges produce exoskeletons, but most possess endoskeletons often composed of separate or partially fused elements of specific shape, the spicules. The mesohyl is an extracellular matrix of collagen fibres incorporating cells of various functions, collencytes synthesizing collagen, spongocytes producing spongin, sclerocytes synthesizing mineral skeleton, different cells producing defensive metabolites, gametes and archeocytes, the totipotent cells capable to differentiate to any other type. The aquiferous system comprises inhalant (afferent) and exhalant (efferent) canals lined with endopinacocytes and connected to internal cavities lined with choanocytes and to apertures at the surface, through which the water enters the body (ostia) and comes out (oscula). Synchronized movements of the choanocyte flagella produce unidirectional water current. Contraction/expansion of the oscula and large canals is performed by sphincter-like structures composed of elongated contractile cells, the actinocytes (Boury-Esnault & Rützler 1997).

Most sponges are filter-feeders. Bacteria-sized food particles pass through the canals and are consumed by the choanocytes, while larger particles may be consumed by the endopinacocytes after being trapped into the ostia (Bergquist 2001). Transfer of nutrients to other cells is performed by the archeocytes. Meanwhile, some sponge species are carnivorous and lack choanocytes (Vacelet & Boury-Esnault 1995). They capture tiny planktonic animals with their spicules and digest them extracellularly. Reproduction modes in sponges are various, though most of them are hermaphrodites. The reproduction starts with spawning when sperm is released into the water. Most species are ovoviviparous (Ereskovsky 2010). The sperm is trapped into the ostia and transferred to oocytes. Embryogenesis following fertilization takes place inside the body of maternal individuals. Mature larvae are released and, after a short period of free-swimming, attach to substrata and undergo metamorphosis. Some sponges are oviparous (Ereskovsky 2010). Their oocytes, after being fertilized inside the body, are

released and the embryonic development takes place externally. In the life histories of several oviparous species there is no swimming larva stage. Their eggs undergo a direct development to sessile organisms. In few oviparous sponges both the sperm and the eggs are released before the fertilization, which occurs outside maternal individuals.

1.1.3. Classification of Porifera based on morphology

Taxonomy of Porifera was traditionally based on the architecture of aquiferous system and the traits of skeleton, its chemical nature, general architecture, shape and size of spicules and organic fibres, e.g. classifications by Schmidt (1862; 1870), Gray (1867), Carter (1875), Sollas (1885), von Lendenfeld (1887; 1889), Vosmaer (1885a; 1887), Hanitsch (1894) and Arndt (1935). Extensive development of morphological techniques provided more characters including the cytological traits, reproductive modes and types of larvae, e.g. classifications by Lévi (1953; 1957; 1973), Bergquist (1978), Hartman (1980; 1982) and Simpson (1984), while appearance of numerical phylogenetic concepts induced the application of such approaches for poriferan taxonomy (e.g. Van Soest 1987; Hajdu 1994; Sará & Burlando 1994; Rosell & Uriz 1997; Alvarez et al. 2000; Manuel et al. 2003). The knowledge on morphological taxonomy of sponges accumulated during more than two centuries of studies was summarized in Systema Porifera (Hooper & Van Soest 2002a), which acknowledged the traditional classification of this phylum into three classes, Calcarea Bowerbank, 1864, Hexactinellida Schmidt, 1870 and Demospongiae Sollas, 1885. Since this benchmark publication the definition and contents of the first two classes have not changed, although the classification of lower taxa within each class has been always debatable because of a high plasticity of morphological characters and a large number of anomalies and exceptions between otherwise closely allied groups of species. On the contrary, the class Demospongiae has been radically reconsidered (Cárdenas et al. 2012; Gazave et al. 2012; Morrow & Cárdenas 2015).

Calcarea representing ca. 9% of all sponges is distinguished by the presence of calcareous spicules, a great variety of aquiferous systems, from asconoid and syconoid to sylleibid and leuconoid, and an exclusively ovivivipарous reproduction resulting in formation of blastula larvae (Manuel et al. 2002). Hexactinellida (ca. 7% of all species) is defined as sponges with siliceous spicules of triaxonic symmetry or their derivates, exclusively leuconoid aquiferous system, a syncytial architecture of pinacyte and choanocyte layers and a ovoviviparous reproduction with formation of trichimella larvae, although documented only in one species (Reiswig 2002). Demospongiae comprises the overwhelming majority of sponge species and, consequently, exhibits a great variety of

traits. According to Systema Porifera (Hooper & Van Soest 2002b) most demosponges possess siliceous spicules, but some species have aspicular solid calcareous skeletons in addition to or instead of the siliceous component, while several demosponge taxa lack any mineral skeleton. Aquiferous system is leuconoid or sylleibid. Reproduction may be ovoviviparous or oviparous, while larvae are blastulae or parenchymellae.

In Systema Porifera three demosponge subclasses, Tetractinomorpha Lévi, 1953, Ceractinomorpha Lévi, 1953 and Homoscleromorpha Bergquist, 1978, were recognized based on general skeleton architecture, spicule symmetry, content of organic skeleton and the type of larvae (Hooper & Van Soest 2002b). Tetractinomorpha comprising four orders (Table 1) was defined as demosponges with predominantly radial or axially compressed skeletons, tetraxonic and/or monaxonic megascleres (main spicules), asterose or asterose-derivative microscleres (auxiliary spicules) if present, predominantly weakly developed organic fibres and larvae of parenchymella or blastula type. Ceractinomorpha encompassing nine orders (Table 1) was defined as demosponges with skeletons of various architectures (e.g. plumose, reticulate or confused) except for the radial one, exclusively monaxonic megascleres and diverse microscleres which, however, never included asterose spicules, with some orders lacking spicules, predominantly well-developed spongin skeleton and parenchymella larvae. In early studies Tetractinomorpha and Ceractinomorpha were also discriminated based on the reproduction mode, oviparity in the former subclass and ovoviviparity in the latter (e.g. Lévi 1953; 1957). However, later oviparity was recorded in four orders, which were otherwise clearly allied with Ceractinomorpha based on their skeleton architectures, spicule shape and type of larvae (Hooper & Van Soest 2002b). Monophyly of several tetractinomorph and ceractinomorph orders caused doubt and, moreover, the allocation of many demosponge families to Tetractinomorpha or Ceractinomorpha was not fixed. Therefore Hooper and Van Soest (2002b) regarded the classification of demosponges into Tetractinomorpha and Ceractinomorpha only as a working hypothesis and admitted a possible non-monophyly of these subclasses.

Unlike the two large demosponge subclasses reviewed above the third subclass recognized in Systema Porifera, Homoscleromorpha, containing a single order and family (Table 1) was well-defined and its monophyly was very clear (Muricy & Diaz 2002). Homoscleromorphs are primarily distinguished by the presence of flagellated exo- and endopinacocytes, a basement membrane lining both choanoderm and pinacoderm, leuconoid or sylleibid aquiferous system, spicules not differentiated to mega- and microscleres in contrast to other demosponges, with some species lacking

any spicules, a weakly developed organic skeleton, ovoviviparous reproduction and a unique type of larva, cinctoblastula. Spicule assortment of homoscleromorphs comprises calthrops or their derivates. Tetraxonic symmetry of the calthrops induced allocation of homoscleromorphs to Tetractinomorpha in some early studies (e.g. Vosmaer 1887). However, in more recent studies the calthrops were no longer regarded as homologous to the tetraxonic megascleres (Hooper & Van Soest 2002b). The uniqueness of Homoscleromorpha was also confirmed by the discovery of type IV collagen in their basement membranes (Boute et al. 1996), which is absent in other sponges, but is typical of other Metazoa (Placozoa, Cnidaria, Ctenophora and Bilateria). This discovery led to the assumption that the pinacoderm and choanoderm in Homoscleromorpha were true epithelia and, consequently, questioned the affiliation of this taxon with Demospongiae and even with the phylum Porifera, although the cell layers in homoscleromorphs (as well as in other sponges) were found to lack belt desmosomes, a special type of cell-to-cell junctions present in epithelia of all other metazoans (Leys & Riesgo 2012). Thus, already in the early 2000-s, when the gross classification of Demospongiae was still mainly based on morphological characters, the monophyly of this class was questioned, with subclass Homoscleromorpha presumably presenting another evolutionary lineage, while the consistency of two other subclasses and their orders also caused doubt. The necessity of credible phylogenetic studies based on molecular data became evident.

1.1.4. Classification of Porifera based on molecular phylogenies

Pioneer molecular studies on sponges were based on allozyme polymorphism and aimed at detection of sibling species (Solé-Cava & Thorpe 1986). Rapid development of DNA sequencing techniques inspired molecular-phylogenetic studies on higher sponge taxa like families and orders. Among the first phylogenetic markers used, the genes coding the RNA of the small ribosomal subunit (18S rDNA) and the large ribosomal subunit (28S rDNA) were most common (e.g. Kelly-Borges et al. 1991; Lafay et al. 1992; Kelly-Borges & Pomponi 1994; Chombard et al. 1997; 1998; Alvarez 1998; Chombard 1998; Zrzavy et al. 1998; Borchiellini et al. 2001; Erpenbeck et al. 2007d). Later studies also involved the mitochondrial genes. Particularly, the barcoding region of cytochrome c oxydase I (COI) was extensively used (e.g. Erpenbeck et al. 2007b; 2012a; Pöppe et al. 2010) and for some taxa complete mitochondrial genomes were sequenced (e.g. Lavrov et al. 2005; 2008; Erpenbeck et al. 2007f; 2009; Belinky 2008). Further additional phylogenetic markers were proposed, e.g. heat-shock proteins (Koziol et al. 1996; 1997; 1998; Borchiellini et al. 1998), seven nuclear housekeeping proteins

(7NHP) (e.g. Peterson & Butterfield 2005; Peterson et al. 2005; Sperling et al. 2009) and a nuclear gene coding asparagine-linked glycosylation 11 homolog (ALG11) (Belinky et al. 2012). The increasing amount of data induced the usage of multi-gene datasets for reconstruction of sponge phylogenies (e.g. Peterson et al. 2005; Erwin et al. 2011; Morrow et al. 2013). The most recent trend is phylogenomics, an integrative approach based on the datasets comprising many tens or even hundreds of genes evolving at different rates and performing different functions (e.g. Phillipe et al. 2009; Schierwater et al. 2009; Pick et al. 2010; Nosenko et al. 2013).

All molecular phylogenies confirmed that sponges were the first animals that diverged from the main metazoan lineage. At the same time many phylogenies based on single-gene or few-gene datasets challenged the monophyly of Porifera, with Calcarea more closely related to the metazoan phyla Placozoa, Ctenophora and Cnidaria than to Demospongiae and Hexactinellida (e.g. Zrzavy et al. 1998; Borchiellini et al. 2001; Peterson & Eernise 2001; Peterson & Butterfield 2005; Peterson et al. 2005). Moreover, when the molecular data on Homoscleromorpha, the taxon of dubious allocation in Demospongiae, became available, its sister relationships with the clade Placozoa + Ctenophora + Cnidaria + Bilateria were revealed and this superclade was designated as Epitheliozoa referring to the possession of true epithelia, while Calcarea, in its turn, appeared to be the sister to Epitheliozoa (Sperling et al. 2007; 2009). However, in another phylogeny Homoscleromorpha and Calcarea were sisters and this pair was, in its turn, the sister to Placozoa + Cnidaria + Bilateria (Erwin et al. 2011). On the contrary, the studies engaging large multi-gene datasets argued for the monophyly of Porifera by demonstrating the sister relationships between the two strongly supported pairs Homoscleromorpha + Calcarea and Demospongiae (excluding Homoscleromorpha) + Hexactinellida (Schierwater et al. 2009; Philippe et al. 2009; Pick et al. 2010; Nosenko et al. 2013).

Critical analyses of the conflicting phylogenetic hypotheses, i.e. monophyletic vs. paraphyletic Porifera (Philippe et al. 2011; Wörheide et al. 2012; Dohrmann & Wörheide 2013; Nosenko et al. 2013), suggested several reasons for this conflict: 1) Datasets of different genes and proteins evolving at unequal rates could support different phylogenies. Specifically, most phylogenies demonstrating paraphyly of sponges were based on ribosomal genes and non-translational proteins with a relatively high level of substitutional saturation, whereas the datasets of slowly evolving proteins involved in translation and exhibiting a much lower saturation level conversely supported the monophyly of Porifera. 2) Data on some genes could provide a strong

non-phylogenetic signal (e.g. undetected homoplasies) resulting from the invoking of oversimplified evolutionary models under phylogenetic computing that distorted the natural synapomorphies. 3) Use of sequences from poorly studied taxa, for which no or few reference molecular data existed, increased the risk of potential taxonomic misidentification or undetected contamination of the samples (Philippe et al. 2011). 4) Outgroups used in the phylogenetic analyses of Metazoa could be considerably distant from the ingroup as compared to the genetic distances between the metazoan taxa. This might reduce the resolution within the ingroup resulting in an inadequately weak support for the deep tree branches and distortion of the natural relationships between different metazoans. Thus, the advantages of the multi-gene datasets were demonstrated and the phylogenies recovered by Philippe et al. (2009), Pick et al. (2010) and Nosenko et al. (2013) were recognized as the most credible (Wörheide et al. 2012; Dohrmann & Wörheide 2013). Consequently, for the time being the phylum Porifera is regarded as monophyletic, with four valid classes, Hexactinellida, Demospongiae, Calcarea and Homoscleromorpha (Van Soest et al. 2016). The latter was nominated by the rank elevation of the former demosponge subclass (Gazave et al. 2012).

Table 1. Comparison between the classifications of the class Demospongiae proposed in Systema Porifera (Hooper & Van Soest 2002) and by Morrow & Cárdenas (2015)

Subclass/order in Systema Porifera (Hooper & Van Soest 2002)	Number of families included in Systema Porifera (Hooper & Van Soest 2002)	Status in the new classification (Morrow & Cárdenas 2015)	Number of families included in the new classification (Morrow & Cárdenas 2015)
Subclass Homoscleromorpha Bergquist, 1978	1	elevated to class	1
Homosclerophorida Dendy, 1905	1	retained without emendations	2 (the single family is split in two)
Subclass Tetractinomorpha Lévi, 1953	22	abandoned	
Astrophorida Sollas, 1888	5	relegated to suborder and emended	16
Chondrosida Boury-Esnault & Lopés, 1985	1	retained with emendations	1 (the single family is split in two, of which one is transferred to another order)

Table 1 (continued)

Subclass/order in Systema Porifera (Hooper & Van Soest 2002)	Number of families included in Systema Porifera (Hooper & Van Soest 2002)	Status in the new classification (Morrow & Cárdenas 2015)	Number of families included in the new classification (Morrow & Cárdenas 2015)
Hadromerida Topsent, 1894	13	abandoned	
Spirophorida Bergquist & Hogg, 1969	3	relegated to suborder and emended	6
Subclass Ceractinomorpha Lévi, 1953	57	abandoned	
Agelasida Hartman, 1980	2	retained with emendations	3
Dendroceratida Minchin, 1900	2	retained without emendations	
Dictyoceratida Minchin, 1900	4	retained with emendations	5
Halichondrida Gray, 1867	5	abandoned	
Halisarcida Bergquist, 1996	1	abandoned	
Haplosclerida Topsent, 1928	13	retained with emendations	6
Poecilosclerida Topsent, 1928	25	retained with emendations	20
Verongida Bergquist, 1978	4	retained without emendations	
Verticillitida Termier & Termier in Termier et al., 1977	1	abandoned	

1.1.5. Classification of the class Demospongiae based on molecular phylogenies

The most common markers applied for reconstruction of demosponge phylogenies are 18S rDNA (e.g. Redmond et al. 2007; 2013; Redmond & McCormack 2008; Gazave et al. 2010), 28S rDNA (e.g. Alvarez et al. 2000; McCormack et al. 2002; Erpenbeck et al. 2005; 2007c, d) and barcoding regions of CO1 (Erpenbeck et al. 2002; 2007b; Pöppe et al. 2010). Phylogenies based on complete mitochondrial genomes are also developing (e.g. Erpenbeck et al. 2007f; Lavrov et al. 2008) and an application of multi-gene datasets has become a usual practice (e.g. Addis & Peterson 2005; Nichols 2005;

Sperling et al. 2009; Erpenbeck et al. 2012b; Morrow et al. 2012; 2013). The numerous studies of the deep phylogeny of Demospongiae preceded by an intensive accumulation of molecular data on single genera and families have revealed the polyphyly of subclasses Tetractinomorpha and Ceractinomorpha as well as the non-monophyly of their several orders recognized in Systema Porifera. Here only the decisive studies will be briefly reviewed. Building of a natural classification for demosponges was started by Borchiellini et al. (2004) who recovered four strongly supported clades, designated as G1 to G4, in the 18S rDNA and 28S rDNA phylogenies. Further the monophyly of these clades was confirmed and the relationships between them were better resolved in the phylogenies based on complete mitochondrial genomes (Lavrov et al. 2008). The first proposal to nominate these clades as new demosponge subclasses provided with clear definitions was done by Cárdenas et al. (2012). At the same time fourteen strongly supported subclades, designated as C1 to C14, were recovered within G4, the largest of the four main clades, in the phylogenies based on 28S rDNA and barcoding region of CO1 (Morrow et al. 2012). Further studies confirmed the monophyly of these subclades with only few emendations and shed more light on their relationships (Morrow et al. 2013; Redmond et al. 2013).

Based on the main clades recovered by Borchiellini et al. (2004) and the subclades revealed by Morrow et al. (2012) as the prototypes for new higher taxa and considering the phylogenies recovered by other authors during the last fifteen years Morrow and Cárdenas (2015) have finally proposed a novel classification of Demospongiae, which is commonly accepted nowadays (Van Soest et al. 2016). Of the four subclasses proposed by Cárdenas et al. (2012), only three subclasses, Keratosa Grant, 1861 with two orders, Verongimorpha Erpenbeck et al., 2012 with three orders and Heteroscleromorpha Cárdenas et al., 2012 with 17 orders, are recognized (Table 2). The former subclass Haploscleromorpha Cárdenas et al., 2012 (corresponding to clade G3 in Borchiellini et al. 2004) was merged with Heteroscleromorpha (corresponding to clade G4) based on the sister relationships between with these two formerly segregated subclasses recovered in most molecular phylogenies (Lavrov et al. 2008; Redmond et al. 2013; Thacker et al. 2013) and on their synapomorphy, the possession of megascleres (Morrow & Cárdenas 2015). Of thirteen demosponge orders (excluding Homoscleromorpha nominated to a class, see above) recognized in Systema Porifera (Hooper & Van Soest 2002b) only seven orders are retained, with radical emendations in five of them, two orders are relegated to suborders and four orders are abandoned (Tables 1 and 2). Five orders established in early studies, but disclaimed in Systema Porifera, are resurrected.

Table 2. Currently accepted classification of the class Demospongiae (following Morrow & Cárdenas 2015)

Subclass/Order	Nomenclatural origin	Number of families included	Corresponding clades in Borchiellini et al. (2004)	Corresponding subclades in Morrow et al. (2012)
Subclass Heteroscleromorpha Cárdenas, Perez & Boury-Esnault, 2012	recently established	84	G3 + G4	
Agelasida Hartman, 1980	inherited from Systema Porifera (Hooper & Van Soest, 2002)	3		C6
Axinellida Lévi, 1953	resurrected	4		C7 + C8 + C9
Biemnida Morrow et al., 2013	recently established	2		smaller part of C12
Bubarida Morrow & Cárdenas, 2015	recently established	3		C10
Clionaida Morrow & Cárdenas, 2015	recently established	4		C4 appended with two families
Desmacellida Morrow & Cárdenas, 2015	recently established	1		larger part of C12
Haplosclerida Topsent, 1928	inherited from Systema Porifera (Hooper & Van Soest, 2002)	6	G3	
Merliida Vacelet, 1979	resurrected	2		
Poecilosclerida Topsent, 1928	inherited from Systema Porifera (Hooper & Van Soest, 2002)	20		C5
Polymastida Morrow & Cárdenas, 2015	recently established	1		C2
Scopalinida Morrow & Cárdenas, 2015	recently established	1		C14
Sphaerocladina Schrammen, 1924	resurrected	1		
Spongillida Manconi & Pronzato, 2002	rank elevation of a former haplosclerid suborder	7		C13
Suberitida Chombard & Boury-Esnault, 1999	resurrected	3		C1
Tethyida Morrow & Cárdenas, 2015	recently established	3		larger part of C3

Table 2 (continued)

Subclass/Order	Nomenclatural origin	Number of families included	Corresponding clades in Borchiellini et al. (2004)	Corresponding subclades in Morrow et al. (2012)
Tetractinellida Marshall, 1876	resurrected by remerging of former Astrophorida and Spirophorida	22		C11
Trachycladida Morrow & Cárdenas, 2015	recently established	1		smaller part of C3
Subclass Keratosa Grant, 1861	resurrected	7	G1	
Dendroceratida Minchin, 1900	inherited from Systema Porifera (Hooper & Van Soest, 2002)	2		
Dictyoceratida Minchin, 1900	inherited from Systema Porifera (Hooper & Van Soest, 2002)	5		
Subclass Verongimorpha Erpenbeck et al., 2012	recently established	7	G2	
Chondrillida Redmond et al., 2013	recently established	2		
Chondrosiida Boury-Esnault & Lopés, 1985	inherited from Systema Porifera (Hooper & Van Soest, 2002)	1		
Verongiida Bergquist, 1978	inherited from Systema Porifera (Hooper & Van Soest, 2002)	4		

One more order results from the rank elevation of a former suborder. Seven new orders are established since the publication of Systema Porifera.

The new classification of Demospongiae, despite reflecting the natural relationships between the taxa definitely better than the older classifications, has faced at least two serious challenges: 1) Because of a high level of homoplasy of currently used morphological characters (e.g. as demonstrated by Morrow et al. 2013), it is impossible to define clear and stable non-molecular synapomorphies for the subclasses and orders recognized (Cárdenas et al. 2012; Morrow & Cárdenas 2015). 2) Phylogenies based on different molecular markers are inconsistent regarding the relationships between

the orders as well as between the families inside each order (e.g. the inconsistencies between 18S rDNA and 28S rDNA phylogenies in Borchiellini et al. 2004; between these both and the NHP7 phylogenies in Sperling et al. 2009 as well as between the 28S rDNA and CO1 phylogenies in Morrow et al. 2012). The problem of homoplasy may be resolved by application of characters from other sources, e.g. cytological, biochemical and embryological data. The inconsistencies between different molecular datasets may be overcome by the engaging of large multi-gene datasets and invoking of adequate substitution models for the genes of different evolutionary rates under phylogenetic computing as recommended by Philippe (2011), Wörheide et al. (2012) and Dohrmann & Wörheide (2013) (see more detailed discussion on these inconsistencies in section 1.1.4 above).

1.1.6. Comments on the rank-based and phylogenetic nomenclatures

All classifications of the phylum Porifera and its lower taxa preceding the era of molecular phylogenies were based on the widely accepted rank-based, or "Linnean", nomenclature governing the application of names to taxa and constraining the assignment of taxa to categorical ranks. The names of metazoan taxa from family-group to species-group are regulated by the International Code of Zoological Nomenclature (ICZN, the latest version – Anonymous 1999). The ICZN is based on the principles of priority and typification. A taxonomic name is regarded as available only if a definition of one or more traits of physical or chemical origin is provided for the respective taxon in the publication or a reference to a previously published definition is given (Articles 12.1 and 13.1 of the ICZN). Once available, a name attributed with the notation on its author and year of publication remains unchangeable (Article 10) and linked to a unique type, which is a specimen or a group of specimens if the name is applied to a taxon of a species group, or a taxon of the next lower rank if the name is applied to a supraspecific taxon (Article 61). The typification secures stability and continuity of taxonomy. The valid name of a taxon is the oldest available name applied to it (Article 23.1). The name formation is standardized, with the family-group names formed by adding a proper suffix to the stem of the name or the entire name of the type genus (Article 29.1). When a taxon of a family-group name is raised or lowered in rank, the suffix of its name must be changed correspondingly (Article 34.1). This standardization secures the mutual exclusion of the names of taxa in different ranks and makes them universal and clear for all scientists, who can get brief information about the coverage of a given taxon directly from its name, without possessing deep knowledge on its classification and relationships.

Inflexibility of the rank-based nomenclature seriously hinders the classification of multiple nested clades of taxa recovered in rapidly produced, but often conflicting, molecular phylogenies. The number of main ranks is limited and even the engaging of the intermediate ranks is unable to cope with the continually complexifying hierarchies of the clades (Pleijel & Rouse 2003). Assignment of a certain rank to a taxon is usually biased and its criteria vary from one group of organisms to another (de Queiroz & Donoghue 1988; Stevens 1994; Minelli 2000). This results in the incomparability of taxa classified in the same rank, but belonging to different higher taxonomic groups. The requirement to provide a definition of traits, or synapomorphies in terms of the phylogenetics, for each newly established taxon prohibits the application of names to many clades recovered under adequate evolutionary models, but lacking synapomorphies (de Queiroz & Gauthier 1990; de Queiroz 1992; 1997). Furthermore, a potential secondary loss of traits during the evolution is ignored, and the nomenclature based on the character similarities and distinctions of taxa rather than on their evolutionary histories risks to apply names to non-monophyletic taxa (de Queiroz 1988; 1992).

In order to overcome the shortcomings listed above a concept of phylogenetic nomenclature has been elaborated (de Queiroz & Gauthier 1990; 1992; 1994) and the International Code of Phylogenetic Nomenclature, the PhyloCode, has been launched (first version by Cantino & de Queiroz 2000, the latest version, 4c, by Cantino & de Queiroz 2010). The PhyloCode governs the application of names to the clades recovered in the computed trees, while the species names are not regulated and categorical ranks are not used. Consequently, a name does not change spelling when the clade, to which this name is applied, becomes more inclusive or less inclusive (Article 3.1 of the PhyloCode 4c). A clade is defined as an entity comprising both a common ancestor and all its descendants, which may be organisms, populations or species (Article 2.1). The names of clades are established through conversion of the pre-existing names available from the rank-based nomenclature or introduction of new names (Article 9.1). In order to be established a clade name must be provided with a phylogenetic definition, which may be node-based, branch-based, apomorphy-based, branch-modified node-based and apomorphy-modified node-based (Article 9.3). A definition must refer to at least two specifiers. The specifiers are species, specimens, or apomorphies (Article 11.1). Each clade name is attributed with a notation on the nominal author, the definitional author and the respective years of publications. The nominal author is the person who first published the name, regardless whether it was phylogenetically defined. The definitional author is the person who established that name and provided its phylogenetic definition

in accordance with the rules of the PhyloCode (Article 19.1). The launch of the PhyloCode has caused a harsh critique (e.g. Forey 2002; Pickett 2005; Benton 2007; Platnick 2011). Association of each clade name with at least two specifiers, of which one may be a bare abstraction (apomorphy-specifiers) able to alter from one phylogeny to another, leads to instability. Disordered treatment of the names adopted from the rank-based nomenclature, lack of any rules for the formation of new names and attribution of double authorship to the converted names break the continuity of taxonomy and make the nomenclature confused and difficult in use.

Nevertheless, the PhyloCode is nowadays actively applied in the classification of some groups, particularly in the classification of sponges, both at the level of genera and families (e.g. Cárdenas et al. 2010; Gazave et al. 2010) and for higher ranks (e.g. Manuel et al. 2003; Borchiellini et al. 2004; Cárdenas et al. 2012), that was criticized by other researchers (e.g. Hooper & Van Soest 2010). Fortunately, the listed studies presented both the phylogenetic and rank-based classifications. It is no doubt that the alternative nomenclature concepts will exist concurrently. It seems, however, rational to continue presenting traditional rank-based classifications in all taxonomic studies in order to keep them compatible with the existing public biodiversity databases (e.g. the World Porifera Database, Van Soest et al. 2016) and understandable for a broad range of biologists and other people concerned with biodiversity, that is done in the present study. At the same time the application of the PhyloCode remains optional.

1.2. Presentation of the family Polymastiidae

1.2.1. General information

The present study focuses on a common demosponge family Polymastiidae Gray, 1867 comprising 122 species distributed in various regions of the World Ocean, but most common in the temperate waters of both hemispheres (Boury-Esnault 2002; Van Soest et al. 2016). Polymastiids are recorded in a wide range of depths, from the intertidal zone in South Africa (Stephens 1915a) to the hadal zone in some North Pacific trenches (Koltun 1970). The polymastiids never reach large sizes usually occupying not more than several dm2 of the substrate (Boury-Esnault 1987), but may occur in large quantities in some benthic habitats. Particularly, they are subdominants of shallow-water hard bottom communities along the Brazilian Coast (Bakus et al. 2004), in some Norwegian fjords (Svensen, personal communication), in the White Sea (Plotkin et al. 2005) and Laptev Sea (Golikov et al. 1990). In the deep-waters of the Nordic Seas and Arctic Ocean the

common polymastiids *Tentorium semisuberites* (Schmidt, 1870) and *Radiella* spp. are often the most frequently recorded macrobenthic species (Barthel & Tendal 1993; Witte 1996). Furthermore, Polymastiidae is one of the pivotal families in the classification of Demospongiae. In the most recent classification based on molecular phylogenies the order Polymastiida was established for this family (Morrow & Cárdenas 2015). Finally, numerous sterols obtained from several polymastiid species and demonstrating a strong antibacterial, antiviral and antitumor activity are regarded as perspective for pharmaceutical industry (Kong & Andersen 1993; 1996; Xu & Zeng 2000; Santafé et al. 2002; Da Frota et al. 2008).

1.2.2. Historical review of taxonomy

Polymastiidae was established by Gray (1867) for two genera, *Polymastia* Bowerbank, 1862 (misspelled as *Polymastica* in Gray's paper) and *Pencillaria* Gray, 1867, and placed in the order Leiospongia Gray, 1867 of the superorder section Spiculospongiae Gray, 1867. In fact *Pencillaria* appeared to be an objective synonym of *Polymastia* because they both were erected for the same species, *Spongia mamillaris* Müller, 1806 (Fristedt 1885). Gray (1867) defined Polymastiidae as massive sponges with numerous "open-mouthed erect tubes" (now called "papillae", Boury-Esnault & Rützler 1997) and a skeleton comprising fascicules of "pin-shaped or needle-shaped" spicules, divergent at the sponge base and lying longitudinally and transversally in "the tubes". Three years later Schmidt (1870) established a family Suberitidae for *Suberites* Nardo, 1833 and six other genera possessing a spicule assortment like that in *Polymastia*. Among these genera four, *Papillina* Schmidt, 1862, *Radiella* Schmidt, 1870, *Rinalda* Schmidt, 1870 and *Thecophora* Schmidt, 1870, resembled *Polymastia* also by the possession of papillae. Gray (1872) placed Polymastiidae and Suberitidae in two different orders, Keratospongia and Suberispongia respectively, which he established within Leiospongia elevated to a superorder group. Moreover, he appended Polymastiidae with *Quasillina* Norman, 1869. But Carter (1875) abandoned Polymastiidae and allocated *Polymastia*, along with *Radiella*, *Rinalda*, *Suberites*, *Thecophora* and a mixture of several other genera, to "Donatina", a subfamily group within Suberitidae placed in his new order Holorhaphidota. However, a year later Carter (1876) separated from "Donatina" a subfamily group "Polymastina" for *Polymastia*. Schmidt (1880) agreed upon the allocation of *Polymastia* to Suberitidae, although did not accept the classification of this family into the subfamily groups.

In the following twenty years the status of Polymastiidae and its relationship with Suberitidae were actively debated, while the allocation of these families was altering depending on changes in the classification of higher taxa. Most authors acknowledged the abandonment of Polymastiidae and the allocation of *Polymastia* and *Quasillina* to Suberitidae (Fristedt 1885; 1887; Ridley & Dendy 1886; 1887; von Lendenfeld 1887; 1898; Carter 1886; Topsent 1892; Hanitsch 1894; Lambe 1896). On the contrary, Vosmaer (1885b; 1887) and Levinsen (1887) recognized Polymastiidae and Suberitidae as separate families. Vosmaer (1885b) presenting his own classification erected within the order Spiculispongiae Gray, 1867 a new suborder, Clavulina, where he placed Polymastiidae, Suberitidae and Clionaidae D'Orbigny, 1851. In Polymastiidae Vosmaer (1885b; 1887) included five genera, *Osculina* Schmidt, 1868, *Papillella* Vosmaer, 1885, *Polymastia*, *Raphyrus* Bowerbank, 1866, *Tentorium* Vosmaer, 1887 and *Weberella* Vosmaer, 1885. *Papillella* replaced the name *Papillina* Schmidt, 1870 preoccupied by a mollusk genus *Papillina* Conrad, 1855, while *Tentorium* replaced *Thecophora* Schmidt, 1870 preoccupied by an insect genus *Thecophora* Róndani, 1845. Regarding *Quasillina* Vosmaer (1885b; 1887) decided to keep it in Suberitidae. The suborder Clavulina established by Vosmaer was also acknowledged by most supporters of the merging of Polymastiidae with Suberitidae, but they appended this suborder with more families and proposed alternative classifications of the higher taxa (Ridley & Dendy 1886; 1887; von Lendenfeld 1887; 1889; Hanitsch 1894). Carter (1886) stuck to his own earlier classification (Carter 1876) retaining the subfamily Polymastina within Suberitidae. Topsent (1892) acknowledged this subfamily and recognized four genera in it, *Polymastia*, *Quasillina*, *Tentorium* and *Trichostemma* Sars, 1872. But later (Topsent 1900) he resurrected Polymastiidae in the rank of family and, applying his new classification, placed it, along with Suberitidae, Clionaidae and Spirastrellidae Ridley & Dendy, 1886, to a section Clavulida of the suborder Hadromerina Topsent, 1894 within the order Monaxonida Sollas, 1885. In the same paper he appended Polymastiidae with four genera, *Proteleia* Dendy & Ridley, 1886, *Rhaphidorus* Topsent, 1898, *Sphaerotylus* Topsent, 1898 and *Tylexocladus* Topsent, 1898. Since then and until now the validity of Polymastiidae as a family has been acknowledged by the overwhelming majority of authors (e.g. Whiteaves 1901; Wilson 1904; 1925; Swarczewsky 1906; Kirkpatrick 1908; Lundbeck 1909; Topsent 1913; 1928; Brøndsted 1914; Hentschel 1914; 1929; Stephens 1915b; de Laubenfels 1932; Arndt 1935; Alander 1942; Koltun 1966 and all subsequent authors) except for Dendy (1922), Burton (1930a, b; 1959a, b) and de Laubenfels (1936; 1949). In different studies Polymastiidae was placed in one or another order, until Lévi (1953) acknowledged Demospongiae Sollas, 1885 as a class, where Hadromerina

Topsent, 1894 including Polymastiidae, Suberitidae and some other families was elevated to the suborder Hadromerida placed in the new subclass Tetractinomorpha. This classification was widely recognized until recently (Boury-Esnault 2002). However, the generic content of Polymastiidae remained unstable. Many new genera were erected within the family, several genera were shuttling between Polymastiidae and Suberitidae, some genera were transferred to Polymastiidae from other families and the status of few polymastiid taxa was dubious.

The taxonomic histories of *Halicnemia* Bowerbank, 1864, *Radiella*, *Spinularia* Gray, 1867 and *Trichostemma* were the most confusing. These four genera are characterized by very similar external morphology, a discoid body usually bearing one or several papillae and a marginal spicule fringe. *Halicnemia* was established by Bowerbank (1864a) for his new species *H. patera*. Gray (1867) allocated *Halicnemia* to his new family Xenospongiidae. In the same paper he established *Spinularia* for a new species name *Spinularia tetheoides* for unknown reason proposed as a replacement for *Tethea Spinularia* Bowerbank, 1866 and allocated this genus to his other new family Halichondriidae. Schmidt (1870) erected *Radiella* for two species, his new species *R. sol* and *Tethea Spinularia* Bowerbank, 1866, and placed this genus in a new family Suberitidae. Carter (1875) allocated *Halicnemia* and *Radiella* to "Donatina", a subfamily group within Suberitidae. *Trichostemma* was first mentioned by Sars (1869) as a new genus for his new species *T. hemisphaericum* in a list of the Norwegian sponges. However, the description of these taxa was published three years later (Sars 1872). Von Marenzeller (1878) regarded *Trichostemma* as a junior synonym of *Halicnemia*. Schmidt (1880) stated that *Trichostemma hemisphaericum* was a synonym *Radiella sol* and, consequently, *Trichostemma* was a synonym of *Radiella*. Furthermore, he reconsidered the status of *Radiella spinularia* (ex *Tethea spinularia*) acknowledging that it was conspecific with *Halicnemia patera* Bowerbank, 1864 and admitted that *Radiella* might be a synonym of *Halicnemia* Bowerbank, 1864. Meanwhile, Carter (1882) followed Gray (1867) and placed *Halicnemia* along with *Xenospongia* Gray, 1858 in a subfamily group Xenospongina within Suberitidae. Fristedt (1885; 1887) preferred to retain *Tethea spinularia* in *Radiella* as *R. spinularia*, while Vosmaer (1885a, b; 1887) and Levinsen (1887) regarded *Halicnemia*, *Radiella* and *Trichostemma* as junior synonyms of *Polymastia*. However, Topsent (1897) noted that *Halicnemia* was distinguished from *Polymastia*, *Radiella*, *Spinularia* and *Trichostemma* by a quite different skeleton architecture and the possession of acanthose megascleres. Later (Topsent 1928) he placed *Halicnemia* in Astraxinellidae Dendy, 1905. For now *Halicnemia* is placed in

Stelligeridae von Lendenfeld, 1898 (Van Soest et al. 2016). *Spinularia* was resurrected as a valid genus in Polymastiidae by Stephens (1915b) based on its distinction from other polymastiids, the possession of raphides, tiny filiform spicules, grouped in dense packs, trichodragmata. Moreover, Stephens put *Rhaphidorus* Topsent, 1898 in synonymy with *Spinularia* and since then the status of the latter has not changed (Van Soest et al. 2016).

Meanwhile, the status of *Radiella* and *Trichostemma* remained dubious for a long time. Following Schmidt (1880) all subsequent authors regarded them synonymous, but there was a disagreement on what name took precedence. Most authors encouraged the precedence of *Trichostemma* referring to its first record in Sars (1869) (Whiteaves 1874; 1901; Ridley & Dendy 1886; 1887; von Lendenfeld 1887; Lambe 1896; Topsent 1904; 1913; 1928; Lundbeck 1909; Wilson 1925; Boury-Esnault 1987; Uriz & Rosell 1990; Boury-Esnault et al. 1994). Conversely, Hansen (1885), Burton (1930a; 1959a), Vacelet (1961) and Koltun (1964a) considered *Radiella* as the senior synonym. Rezvoj (1924) regarded Trichostemma as a junior synonym of *Polymastia*, while Koltun (1966) following Levinsen (1887) and Vosmaer (1887) considered both *Radiella* and Trichostemma as junior synonyms of *Polymastia*. The final decision was done by Boury-Esnault (2002) who regarded the record of Trichostemma in Sars (1869) as nomen nudum and acknowledged *Radiella* as a valid genus based on the principle of priority and defining the different architecture of its upper and basal surface as a trait separating this genus from other polymastiids.

Besides *Halicnemia*, six genera were allocated to Polymastiidae, mainly due to the possession of papillae, at one time or another, but later transferred to other families based on the substantial differences between their skeleton architectures and spicule assortments and those in the acknowledged polymastiid genera. Allocation of *Latrunculia* du Bocage, 1869 to Polymastiidae proposed by Hentschel (1929) and Arndt (1935) was not supported by any other authors. Now *Latrunculia* is widely acknowledged as the type genus of Latrunculiidae Topsent, 1922, a poecilosclerid family distinguished by the peculiar chessman-shaped microscleres (Samaai & Kelly 2002; Van Soest et al. 2016). *Rhizaxinella* Keller, 1880 placed in Polymastiidae by Koltun (1966) is commonly regarded as a suberitid based on its skeleton architecture (Van Soest 2002). *Osculina*, *Papillella* and *Raphyrus* placed in Polymastiidae by Vosmaer (1885b; 1887) were regarded as synonyms of *Cliona* Grant, 1826 (in the Clionaidae) by Topsent (1900) based on the possession of spiraster microscleres. *Vosmaeria* Fristedt, 1885 regarded as a polymastiid genus by Burton (1930a), Arndt (1935), Alander (1942) and Koltun (1966) was transferred to Halichondriidae by Borojevic et al. (1968) due to its skeleton architecture

1.2.3. Morphology and currently accepted classification

(see Glossary of polymastiid morphology in Table 3)

The most recent morphological review and classification of Polymastiidae were presented by Boury-Esnault (2002). Of thirty nominal genera ever placed in this family only fourteen genera were recognized as valid (Table 4), while nine genera were regarded as junior synonyms and seven were transferred to other families (see the paragraph above). Since then Polymastiidae was appended with one more genus, *Astrotylus* Plotkin & Janussen, 2007. According to Boury-Esnault (2002) Polymastiidae are sponges with a massive, encrusting, globular, discoid or pedunculate body (Figures 1A–G) often equipped with a fringe of extra long spicules at the edge (Figures 1F–G), a choanosomal skeleton constituted by radial tracts of megascleres (here defined as the main choanosomal skeleton, Figure 2A) and free-scattered megascleres or microscleres (here defined as the auxiliary choanosomal skeleton), and a cortical skeleton composed of at least a palisade of megascleres (Figure 3A). In many species the superficial cortical palisade is appended with one or several inner layers, which may be additional spicule palisades (Figure 3B), tangentially or confusedly arranged spicules (Figures 3B–D), or collagen fibres. Spicule assortment of the polymastiids comprises two or more size categories of monaxonic megascleres, which may be monactines (strongyloxeas, styles, subtylostyles, tylostyles – Figure 4 or exotyles – Figure 5) or diactines (oxeas). The megascleres of the choanosomal tracts (principal megascleres) are larger than the megascleres composing the cortical palisade, whereas the free-scattered choanosomal spicules and the spicules constituting the inner cortical layers may be of various size categories. In addition to the megascleres, some species possess monactinal microscleres (smooth centrotylote microxeas, acanthose microxeas or raphides in trichodragmata – Figure 6). Most polymastiids have papillae, the protuberances of the cortex where the aquiferous canals ascend to. A sponge has at least one or few exhalant papillae, and in many species there are also numerous inhalant papillae. In exhalant papilla a single exhalant canal terminating with an osculum at the summit usually runs in the middle, while several inhalant canals (if present) are located in the periphery and open with ostia in the wall. Inhalant papillae are imperforate at the summits, with a single or several inhalant canals connected to the ostia in the walls. The skeleton of the papilla wall is composed of at least the superficial palisade of small megascleres stretching from the cortex and the inner tracts of principal megascleres ascending from the choanosome. Sometimes the wall skeleton is reinforced with additional spicule layers located between the outer palisade and the innermost tracts. The bulkheads separating the canals may be

reinforced by the spicule tracts and/or free-scattered spicules. In some polymastiids the ostia open directly on the surface of the main body, while in few taxa lacking papillae (all species of *Pseudotrachya* Hallmann, 1914 and some species from other genera) both ostia and oscula open on the surface of the main body.

Table 3. Glossary of polymastiid morphology (emended from Boury-Esnault & Rützler 1997)

Term	Definition	Figure
astrotylostyle	monactinal microsclere with a tyle on the proximal tip and a aster-like ornament on the distal tip	6C
centrotylote	possessing a median tyle	6A
choanosome	internal region of sponge body including the choanocyte chambers	
cladotylostyle	exotyle with a tyle on the proximal tip and a denticulate ornament on the distal tip	5G–I
cortex	bark-like superficial region of sponge body distinct from the choanosome and reinforced with a special skeleton	3
diactine	monaxonic spicule with both tips acerate or blunt	6A, B
exhalant	refers to all elements of the aquiferous system through which the water runs from the choanocyte chambers to the oscula	
exotyle	cortical monactine differing from other spicules in shape and/or in size, with the distal tip (acerate or ornamented) projecting above the body surface	5
inhalant	refers to all elements of the aquiferous system through which the water runs from the ostia to the choanocyte chambers	
megasclere	spicule of main skeleton, relatively large in size	4, 5
microsclere	auxiliary spicule usually distinguishing from megascleres in shape and/or by smaller size	6
monactine	monaxonic spicule with dissimilar tips, e.g. one acerate, the other blunt, or one with a tyle, the other smooth etc.	4
monaxon	linear, non-radiate spicule	
osculum	aperture through which the water leaves the sponge body	
ostium	aperture through which the water enters the sponge body	
oxea	diactine with both tips acerate	6A, B
papilla	protuberance of the cortex bearing either osculum at the summit, or ostia in the walls, or both	
polytylote	possessing several tyles scattered along the spicule shaft	
raphides	extremely thin, filiform monaxonic microscleres, often grouped in dense packs (see trichodragma)	6D–F
spherotylostyle	exotyle with a tyle on the proximal tip and a larger spherical knob on the distal tip	5D–F
strongyloxea	extremely fusiform style with a tapering proximal tip	4A

Table 3 (continued)

Term	Definition	Figure
style	monactine with one (proximal) tip blunt and the other (distal) acerate	4B–D
subtylostyle	style with a weakly developed tyle often displaced along the shaft	4E–G
trichodragma	dense pack of raphides	3A, 6D
tyle	rounded or oval swelling in the proximal tip or in the middle of a spicule	4F, I
tylostyle	style with a well-developed tyle	4H–J

The traits discriminating Polymastiidae from other demosponge families are in fact unclear. Several genera currently allocated to Suberitidae, e.g. *Aaptos* Gray, 1867, bear all features stated above in the diagnosis of Polymastiidae: a spicule assortment comprising two or more size categories of monactines, radial tracts of principal monactines constituting the main choanosomal skeleton and a superficial palisade of small monactines in the cortical skeleton (Kelly-Borges & Bergquist 1994). Furthermore, at least one species of *Aaptos*, *A. papillata* (Keller, 1880), possesses well-developed papillae, which anatomically do not distinguish from the polymastiid papillae. This led to a misidentification of *A. papillata* as a new species of *Polymastia*, *P. gleneni*, by Descatoire (1966), that was revealed by Boury-Esnault (1987).

Discrimination between the polymastiid genera proposed by Boury-Esnault (2002) is based on the architecture of the choanosomal and cortical skeleton and the presence/absence of the spicules other than ordinary monactinal megascleres (tylostyles, subtylostyles, styles and strongyloxeas) (Table 4, see also the schematic body plans of the polymastiid genera in **Paper I**: Figures 1–2). Three genera, *Quasillina*, *Ridleia* Dendy, 1888 and *Weberella*, do not in fact fit in the above-stated diagnosis of Polymastiidae, because their main choanosomal skeletons are not radial. *Weberella* is distinguished by a reticulate arrangement of the choanosomal megasclere tracts (Figure 2B), while in *Quasillina* and *Ridleia* the megasclere tracts are located in the cortex and underlie the outer cortical layers (Figures 3E, F). Such architecture of the cortex in these two genera actually resembles the architecture of the papilla walls in other polymastiids. In *Quasillina* the choanosomal skeleton is just an unordered mass of megascleres corresponding to the auxiliary choanosomal skeletons in other taxa (Figure 2C), while in *Ridleia* the choanosomal skeleton is represented just by a subcortical layer of criss-cross

small spicules (Figures 3F). Five polymastiid genera are distinguished by the possession of extraordinary spicules in the choanosome: *Acanthopolymastia* Kelly-Borges & Bergquist, 1997 with acanthose microxeas (Figure 6B), *Astrotylus* with tiny tylostyles bearing star-like distal ornaments (astrotylostyles, Figure 6C), *Atergia* Stephens, 1915 with smooth centrotylote microxeas, *Pseudotrachya* with smooth oxeas of larger size and *Spinularia* with raphides in trichodragmata (Figures 3A, 6D–F). Of these genera two are distinguished also by the presence of extraordinary spicules in the cortex. In *Acanthopolymastia* the acanthose microxeas constitute an inner cortical layer, while in *Pseudotrachya* the smooth oxeas form a superficial palisade unlike all other polymastiids with cortical palisades composed exclusively of monactines (tylostyles, subtylostyles or exotyles). Some polymastiids possess exotyles, the cortical megascleres differing from the ordinary monactines in size and/or in shape. The exotyles may constitute a cortical palisade, but usually just reinforce the palisade composed of ordinary tylostyles and protrude forming a surface hispidation. In all *Radiella* spp. and some species of *Polymastia* the exotyles differing from the choanosomal monactines just by larger size compose a marginal fringe preventing the immersion of sponge body in the soft bottom sediments (Figures 1F–G). Four genera are distinguished by the possession of exotyles with distal ornamentations: *Proteleia* with grapnel-like ornaments on the exotyles (Figures 5A–C), *Sphaerotylus* with spherotylostyles (exotyles with spherical knobs, Figures 5D–F), *Trachyteleia* Topsent, 1928 with the distal parts of extra-large tylostyles ornamented with tiny spines and *Tylexocladus* with cladotylostyles (exotyles with denticulate ornaments, Figures 5G–I). Two genera, *Radiella* and *Tentorium*, are characterized by a heterogeneous cortex. *Radiella* spp. live predominantly on soft bottom and attach to tiny hard substrates only with central basal points, while the most part of the basal surface remains free. The skeleton of their basal cortex is mainly constituted by the tracts of principal megascleres running parallel to the surface, while the skeleton of the upper cortex has usual polymastiid architecture with a superficial palisade of small monactines (Figure 3H). *Tentorium* is distinguished by its lateral cortex with a skeleton constituted by densely packed, tangentially arranged megascleres, whereas its upper cortex has usual polymastiid architecture.

Table 4. Classification of the family Polymastiidae following Boury-Esnault (2002) and appended with the data from Ilan et al. (2003), Lehnert et al. (2005), Samaai & Gibbons (2005), Boury-Esnault & Bézac (2007), Plotkin & Janussen (2007; 2008) and Austin et al. (2014)

Genus	Number of species	Main synapomorphies	Inconsistencies
Acanthopolymastia Kelly-Borges & Bergquist, 1997	3	acanthose microxeas in choanosome and cortex	
Astrotylus Plotkin & Janussen, 2007	1	astrotylostyles in choanosome	
Atergia Stephens, 1915	3	smooth centrotylote microxeas in choanosome	
Polymastia Bowerbank, 1864	74	absence of any traits typical of other genera	*P. boletiformis* and *P. corticata* (reticulate main choanosomal skeleton as in *Weberella*); *P. grimaldii* (heterogeneous cortex with basal skeleton as in *Radiella*), *P. invaginata* (single-layered cortex as in *Astrotylus*); *P. tapetum* (exotyles with grapnel-like distal ornaments as in *Proteleia*); *P. umbraculum* (reticulate main choanosomal skeleton as in *Weberella*, exotyles with grapnel-like ornaments as in *Proteleia*, smooth centrotylote oxeas as in *Atergia*)
Proteleia Dendy & Ridley, 1886	2	exotyles with grapnel-like distal ornaments in cortex	

Table 4 (continued)

Genus	Number of species	Main synapomorphies	Inconsistencies
Pseudotrachya Hallmann, 1914	2	smooth oxeas in choanosome and cortex	
Quasillina Norman, 1869	4	choanosomal skeleton is an unordered mass of small megascleres, tracts of principal megascleres in the cortex	
Radiella Schmidt, 1870	9	heterogeneous cortex with basal cortical skeleton mainly composed of tracts of principal megascleres lying parallel to the surface	
Ridleia Dendy, 1888	2	choanosomal skeleton limited to the tangentially arranged dense packs of small megascleres in subcortical area, tracts of principal megascleres in the cortex	
Sphaerotylus Topsent, 1898	9	exotyles with spherical distal knobs (spherotylostyles) in cortex	*S. antarcticus* and *S. borealis* (exotyles with grapnel-like ornaments as in *Proteleia*)
Spinularia Gray, 1867	2	raphides in trichodragmata in choanosome	*S. australis* (heterogeneous cortex with basal skeleton as in *Radiella*)

Table 4 (continued)

Genus	Number of species	Main synapomorphies	Inconsistencies
Tentorium Vosmaer, 1887	3	heterogeneous cortex with lateral cortical skeleton of tangentially arranged dense packs of intermediary spicules	*T. papillatum* (homogeneous cortex)
Trachyteleia Topsent, 1928	2	extra-large tylostyles (exotyles) with spiny distal parts in cortex	*T. hispida* (ex *Suberitechinus hispidus*) (extra-large tylostyles without spines)
Tylexocladus Topsent, 1898	2	exotyles with denticulate distal ornaments (cladotylostyles) in cortex	
Weberella Vosmaer, 1885	4	reticulate main choanosomal skeleton	

Figure 1. Body shapes in Polymastiidae. **A.** Pedunculate (*Quasillina brevis*, ZMBN 098067, original photo). **B.** Columnar (*Tentorium semisuberites*, original photo). **C.** Encrusting (*Polymastia svenseni* sp. nov., photo from **Paper IV**: Figure 14B, courtesy of E. Svensen, OceanPhoto / Dalane Tidende). **D.** Massive (*Polymastia boletiformis*, photo from **Paper IV**: Figure 5A, courtesy of E. Svensen). **E.** Globular (*Polymastia thielei*, photo from **Paper IV**: Figure 15I, courtesy of P. Leopold, University of Tromsø). **F** (view from above) and **G** (bottom view). Discoid (*Polymastia hemisphaerica*, holotype NHMUO B862, photos from **Paper IV**: Figures 8A–B). Note the marginal spicule fringe.

Figure 2. Choanosomal skeletons in Polymastiidae (view on histological sections). **A.** Radial (*Spinularia njordi* sp. nov., ZMBN 098038, original photo). **B.** Reticulate (*Weberella bursa*, ZIN RAS ocwb016, original photo). **C.** Unordered (*Quasillina brevis*, ZMBN 098084, photo from **Paper IV**: Figure 21E).

The most problematic genus is *Polymastia*, the type genus of Polymastiidae encompassing more than 60 % of all polymastiid species. The widely accepted definition of *Polymastia* (Boury-Esnault 2002) is rather obscure. According to this definition *Polymastia* comprises the sponges of thickly encrusting, spherical or cushion-like shape, always with papillae. The main choanosomal skeleton is radial, while the cortical skeleton consists of at least two layers, a superficial palisade of small tylostyles and an inner layer of intermediary monactines located tangentially to the surface. Spicule

assortment comprises monactines in at least three size categories, the principal spicules of the choanosomal tracts and the cortical spicules in two categories. Besides *Polymastia*, at least two genera, *Proteleia* and *Sphaerotylus*, perfectly fit in this definition. *Radiella* also exhibits all these traits, but differs by the presence of a differentiated basal cortex. Consequently, *Polymastia* can in fact be defined only by the absence of any traits typical of other polymastiid genera, i.e. no extraordinary choanosomal spicules, no ornamented exotyles, no difference between the upper, lateral and basal cortex, rather than by the possession of any characteristic features (Table 4). This is the result of that *Polymastia* was for years used as a taxonomic "dump" for numerous species, which did not fit in the diagnoses of other polymastiid genera. Moreover, many species not completely fitting in the above-stated definition of *Polymastia* are still retained in this genus predominantly based on the opinions of the original authors or long-lived traditions (Plotkin & Janussen 2008; Table 4 in this study). For instance, *Polymastia boletiformis* (Lamarck, 1815) and *P. corticata* Ridley & Dendy, 1886 are characterized by a reticulate main choanosomal skeleton as in *Weberella* spp. *P. grimaldii* (Topsent, 1913) living on soft bottoms has a differentiated basal cortex constituted by tracts of principal megascleres running parallel to the surface as in *Radiella* spp. *P. invaginata* Kirckpatrick, 1907 possesses a single-layered cortex as in *Astrotylus*. *P. tapetum* Kelly-Borges & Bergquist, 1997 possesses exotyles with grapnel-like distal ornaments that are typical of *Proteleia*. The most remarkable example is *P. umbraculum* Kelly-Borges & Bergquist, 1997, which bears the features of at least four genera, absence of papillae (*Pseudotrachya*), a reticulate choanosomal skeleton (*Weberella*), exotyles with grapnel-like ornaments in the cortex (*Proteleia*) and smooth centrotylote oxeas free-scattered in the choanosome (*Atergia*).

Inconsistencies are also found in other polymastiid genera (Table 4). Contrary to the definition of *Sphaerotylus*, two species currently allocated to this genus, *S. antarcticus* Kirckpatrick, 1907 and *S. borealis* (Swarczewsky, 1906), possess exotyles with grapnel-like ornaments as in *Proteleia*. In defiance to the diagnosis of *Tentorium*, *T. papillatum* (Kirkpatrick, 1908), has a homogeneous cortex. Some individuals of the type species of *Tylexocladus*, *T. joubini* Topsent, 1898, have choanosomal centrotylote oxeas typical of *Atergia*. *Suberitechinus* de Laubenfels, 1949 currently regarded as a synonym of *Trachyteleia* (Boury-Esnault 2002; Van Soest et al. 2016) lacks the main distinguishing feature of the latter, the spines on the exotyles. Hence, the taxonomy of Polymastiidae needs a radical revision based on the characters other than architecture of skeleton and spicule shape and on a solid phylogenetic background.

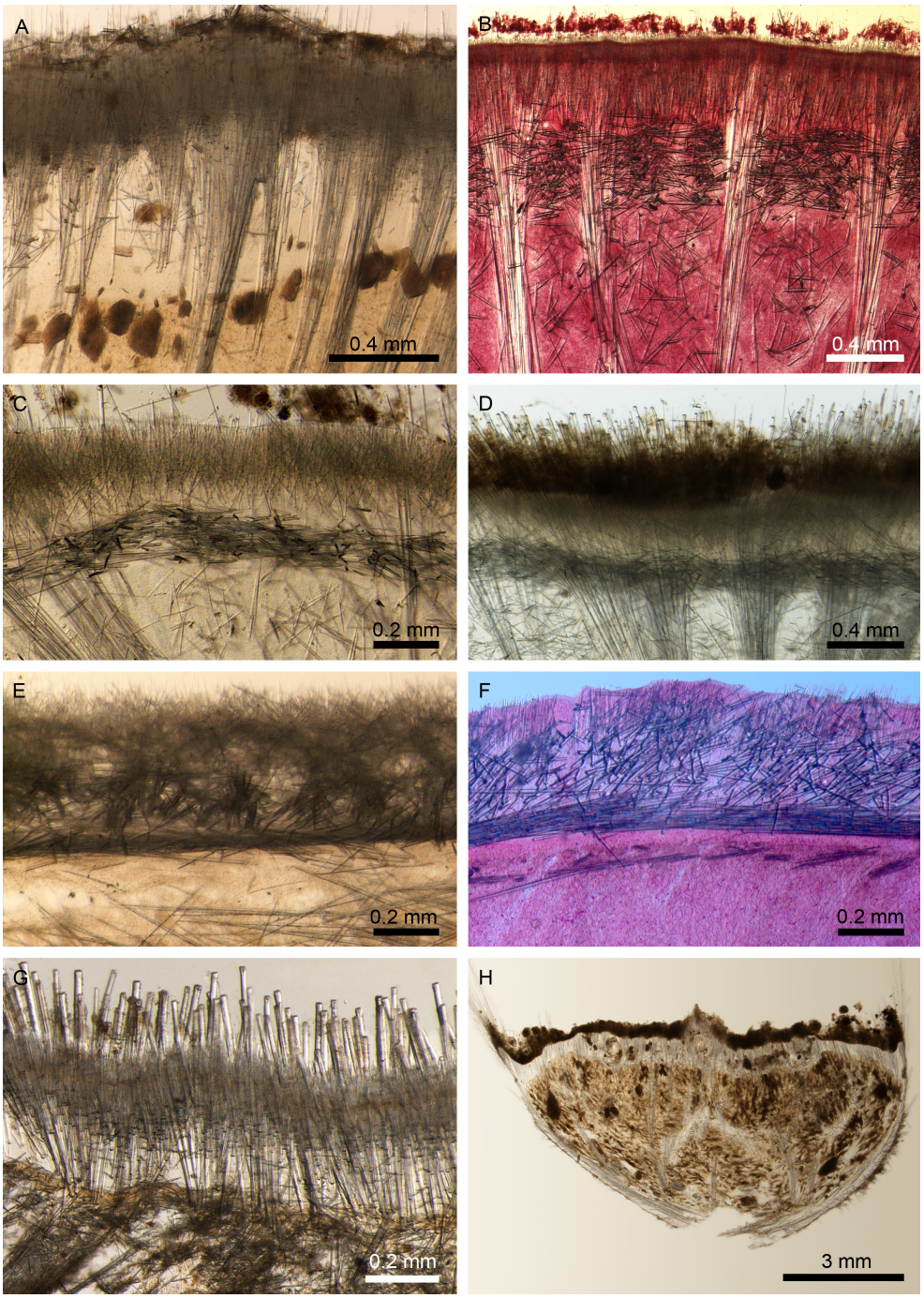
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Figure 3. Cortical skeletons in Polymastiidae (view on histological sections). **A.** Single-layered cortex in *Spinularia spinularia* (original photo). Note the cortical palisade and the subcortical trichodragmata of raphides. **B.** Three-layered cortex in *Proteleia sollasi* (holotype BMNH 1887.5.2.62, original photo). Section stained with carmine. Note the superficial palisade of small tylostyles, the inner palisade of intermediary monactines and the innermost layer of tangentially arranged intermediary monactines. **C.** Three-layered cortex in *Polymastia arctica* (ZMBN 098068, photo from **Paper IV**: Figure 3D). Note the superficial palisade of small tylostyles, the middle layer with low concentration of spicules and the internal layer of criss-cross intermediary monactines. **D.** Three-layered cortex in *Sphaerotylus capitatus* (ZMBN 107485, original photo). Note the layers as in *P. arctica* and the surface hispidation reinforced with spherotylostyles. E. Three-layered cortex in *Quasillina brevis* (ZMBN 098084, photo from **Paper IV**: Figure 21D). Note the superficial bouquets of small monactines, the middle layer of criss-cross large monactines and the internal layer constituted by longitudinal tracts of large monactines. F. Three-layered cortex in *Ridleia oviformis* (holotype BMNH 1883.12.13.69, photo from **Paper I**: Figure 3B). Section stained with carmine. Note the layers as in *Q. brevis* and the subcortical bundles of intermediary monactines. G. Single-layered cortex in *Tylexocladus joubini* (lectotype MOM 04-0526a, photo from **Paper II**: Figure 32F). Note the palisade of small tylostyles reinforced with cladotylostyles. **H.** Skeleton in *Radiella sarsi* (ZMBN 107582, photo from **Paper IV**: Figure 29I). Note the basal cortex constituted by the tracts of principal monactines running parallel to the surface and the superficial palisade of small tylostyles.

1.2.4. Molecular data

Molecular data obtained from Polymastiidae before the present study was started in 2010 had been in fact very poor in comparison with the data on many other demosponge families and considering the number of polymastiid species. Using a short region of 18S rDNA Kelly-Borges et al. (1991) reconstructed the relationships between eight taxa of the former order Hadromerida including three polymastiids, *Polymastia fusca* and two other *Polymastia* spp. not identified to species level. These data are unfortunately not available in GenBank. Nichols (2005) included partial 28S rDNA sequences from three polymastiid species, *Polymastia invaginata*, *P. pachymastia* de Laubenfels, 1932 and *Spinularia spinularia*, and three operational taxonomic units (OTUs), *Pseudotrachya* sp. and two *Polymastia* spp., along with the sequences of the barcoding region of CO1 from the same OTUs in his analyses of the relationships between the demosponge orders. Kober & Nichols (2007) used a 18S rDNA sequence from *P. pachymastia* in the reconstruction of the relationships between Poecilosclerida Topsent, 1928 and former Hadromerida. Some data were obtained under studies of the microbial communities hosted by polymastiids, e.g. a short piece of 28S rDNA from *Polymastia corticata* (Meyer & Kuever 2008) and the barcoding CO1 region from *P. janeirensis* (Boury-Esnault, 1973) (Turque et al. 2008).

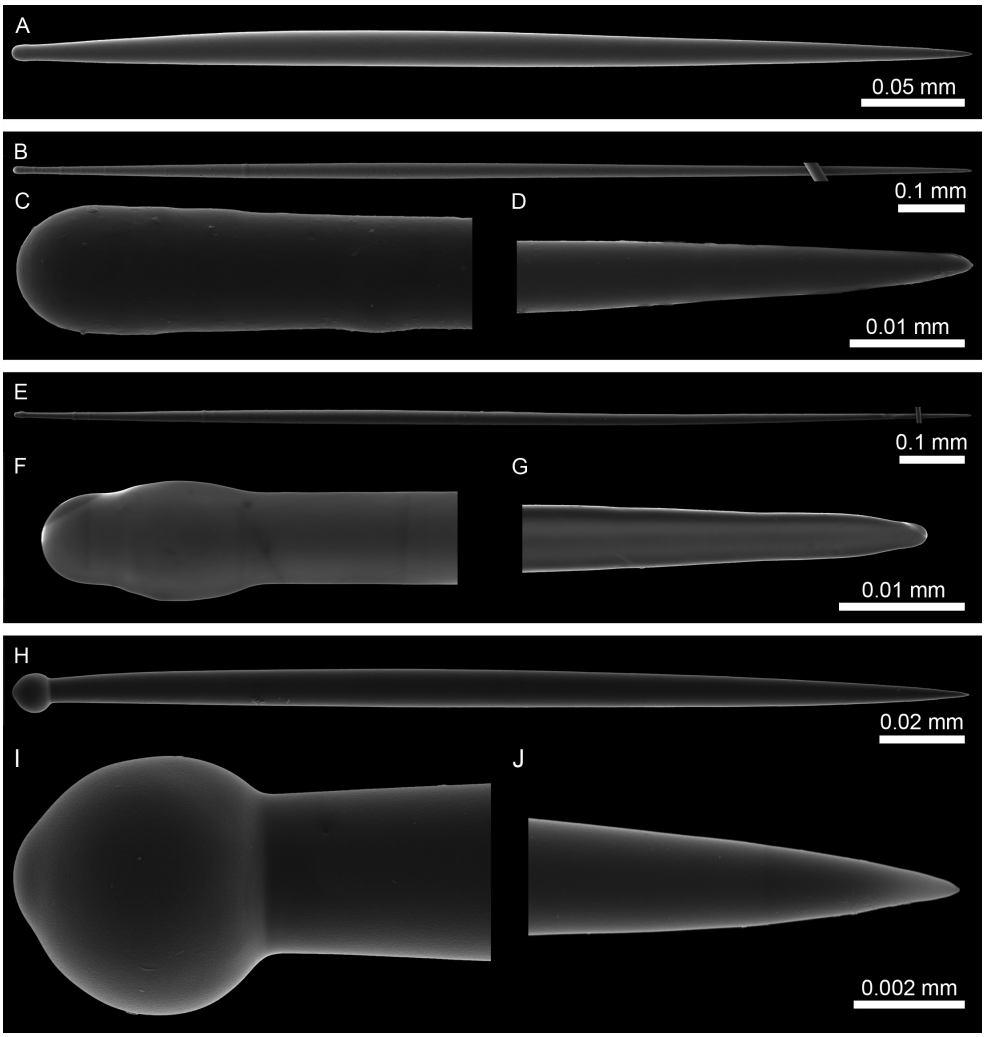

Figure 4. Ordinary monactines in Polymastiidae (SEM images). **A.** Strongyloxea in *Proteleia sollasi* (holotype BMNH 1887.5.2.62, photo from **Paper II**: Figure 5B). **B.** Style in *Sphaerotylus borealis*, general view. **C.** The same, detail of proximal extremity. **D.** The same, detail of distal extremity. **E. S**ubtylostyle in *Sphaerotylus borealis*, general view. **F.** The same, detail of proximal extremity. **G.** The same, detail of distal extremity (B–G: photos from **Paper II**: Figures 11A–F). **H.** Tylostyle in *Spinularia spinularia*, general view (photo from **Paper IV**: Figure 32B). **I.** The same, detail of proximal extremity. **J.** The same, detail of distal extremity.

During the last four years the amount of data on the polymastiids has considerably increased due to the intensive studies on the demosponge phylogeny. For the moment these data available in GenBank (apart from those obtained in the present study, Table 5) comprise nine 18S rDNA sequences from seven species and two OTUs (Kober & Nichols 2007; Redmond et al. 2013), 31 sequences of different 28S rDNA fragments

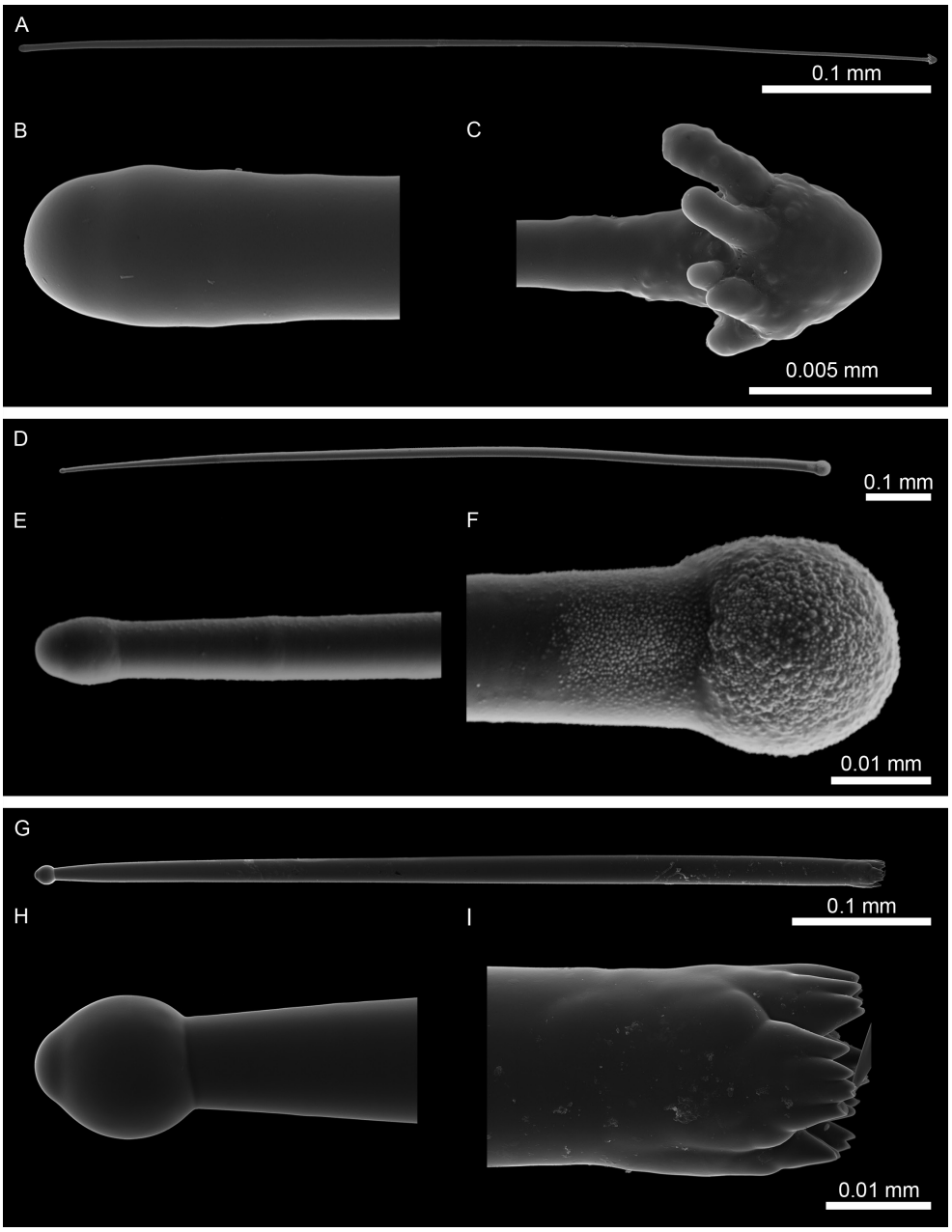

Figure 5. Ornamented exotyles in Polymastiidae (SEM images). **A.** Exotyle with grapnel-like ornamentation in *Proteleia sollasi*, general view. **B.** The same, proximal extremity. **C.** The same, distal ornamentation (A–C: photos from **Paper II**: Figures 5E, G, H). D. Spherotylostyle in *Sphaerotylus capitatus*, general view. **E.** The same, proximal tyle. **F.** The same, distal knob (D–F: photos from **Paper II**: Figures 14D–F). **G.** Cladotylostyle in *Tylexocladus joubini*, general view. **H.** The same, proximal tyle. **I.** The same, distal ornamentation (G–I: photos from **Paper II**: Figures 34I–K).

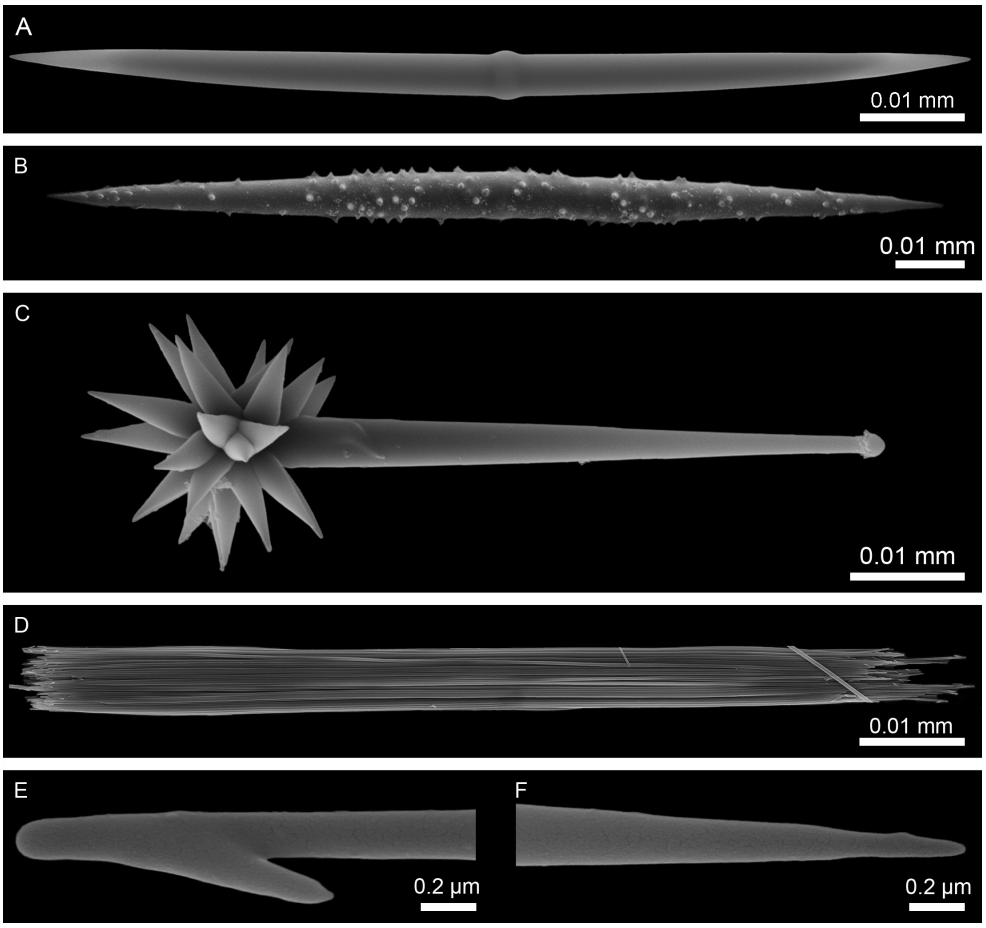

Figure 6. Extraordinary choanosomal microscleres in Polymastiidae (SEM images). **A.** Smooth centrotylote microxea in *Tylexocladus joubini* (photo from **Paper II**: Figure 34H). **B.** Acanthose microxea in *Acanthopolymastia pisiformis* (original photo). **C.** Astrotylostyle in *Astrotylus astrotylus* (photo from Plotkin & Janussen 2007: Figure 3D). **D.** Trichodragma of raphides in *Spinularia spinularia* (original photo). **E.** Proximal extremity of a raphide. **F.** Distal extremity of the same raphide (E–F: photos from **Paper IV**: Figures 32F–G).

from nine species and six OTUs (Nichols 2005; Meyer & Kuever 2008; Morrow et al. 2012; 2013; Thacker et al. 2013), three sequences of 5.8S rDNA and internal transcribed spacers, ITS 1–2, from one OTU (Nichols & Barnes 2005), 23 sequences of the CO1 barcoding region from seven species and six OTUs (Nichols 2005; Turque et al. 2008; Morrow et al. 2012; 2013; Alex et al. 2013; Vargas et al. 2015), sequences of five nuclear housekeeping genes from *Polymastia tenax* (Hill et al. 2013) and complete mitochondrial genomes from two individuals of *Polymastia littoralis* Stephens, 1915 (Del Cerro et al. 2016).

Table 5. Molecular data on Polymastiidae available in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/?term=Polymastiidae accessed 2016-06-18). Our data (**Paper III**) are highlighted with bold. Taxonomic names are given as in the original papers. See footnotes for changes proposed in Paper IV. See Paper I: Table 1 for GenBank accession numbers.

Species/OTU	18S rDNA	28S rDNA	ITS	CO1
Atergia corticata	Redmond et al. 2013	Morrow et al. 2013		
Polymastia andrica		**Our data**		**Our data**
Polymastia arctica		**Our data**		**Our data**
Polymastia bartletti		**Our data**		**Our data**
Polymastia cf. *bartletti*		**Our data**		**Our data**
Polymastia boletiformis	Redmond et al. 2013	Morrow et al. 2012; **our data**		**Our data**
Polymastia cf. *conigera*		**Our data**		**Our data**
Polymastia corticata		Meyer & Kuever 2008[1]; **our data**		**Our data**
Polymastia euplectella[2]		**Our data**		**Our data**
Polymastia grimaldii		**Our data**		**Our data**
Polymastia invaginata		Nichols 2005; **our data**		Vargas et al. 2015; **our data**
Polymastia isidis				Vargas et al. 2015
Polymastia janeirensis				Turque et al. 2008
Polymastia littoralis				Del Cerro et al. 2016[3]
Polymastia mamillaris		**Our data**		**Our data**
Polymastia pachymastia	Kober & Nichols 2007	Nichols 2005; Yang (unpub.)		
Polymastia penicillus	Redmond et al. 2013	Morrow et al. 2012; **our data**		Alex et al. 2013; **our data**

Table 5 (continued)

Species/OTU	18S rDNA	28S rDNA	ITS	CO1
Polymastia tenax[4]		Thacker et al. 2013		
Polymastia thielei		**Our data**		**Our data**
Polymastia uberrima		**Our data**		**Our data**
Polymastia sp. 1 AP-2013[5]		**Our data**		**Our data**
Polymastia sp. 1 CM-2013		Morrow (unpub.)[6]		Morrow et al. 2013[7]
Polymastia sp. 1 SN-2005[8]		Nichols 2005	Nichols & Barnes 2005	Nichols 2005
Polymastia sp. 2 AP-2013		**Our data**		**Our data**
Polymastia sp. 2 SN-2005		Nichols 2005		Nichols 2005
Polymastia sp. 3 AP-2014				**Our data**
Proteleia sollasi	Redmond et al. 2013			
Pseudotrachya sp.[8]		Nichols 2005		Nichols 2005
Quasillina brevis	Redmond et al. 2013	**Our data**		**Our data**
Radiella hemisphaerica[9]		**Our data**		**Our data**
Radiella sarsi[10]		**Our data**		**Our data**
Radiella cf. *sarsi*[11]		**Our data**		**Our data**
Radiella sp.[12]		**Our data**		**Our data**
Sphaerotylus antarcticus		**Our data**		Morrow et al. 2013; Vargas et al. 2015; **our data**
Sphaerotylus borealis		**Our data**		**Our data**
Sphaerotylus capitatus		**Our data**		**Our data**
Sphaerotylus sp. A[13]	Redmond et al. 2013	Morrow (unpub.); **our data**		**Our data**
Sphaerotylus sp. C[14]	Redmond et al. 2013	Morrow et al. 2012		Morrow et al. 2012

Table 5 (continued)

Species/OTU	18S rDNA	28S rDNA	ITS	CO1
Spinularia spinularia		Nichols 2005; **our data**		**Our data**
Tentorium papillatum		**Our data**		Vargas et al. 2015; **our data**
Tentorium semisuberites	Redmond et al. 2013	Morrow et al. 2012; **our data**		Morrow et al. 2012; **our data**
Tentorium cf. *semisuberites*		**Our data**		
Tentorium sp.				Vargas et al. 2015
Weberella bursa		**Our data**		**Our data**

[1]Meyer & Kuever (2008) published only the B10–C1 fragment of 28S rDNA from *Polymastia corticata*. We presented the B10–E19 region from this species (**Paper III**).

[2]*Polymastia euplectella* is regarded as a synonym of *P. nivea* in **Paper IV**.

[3]Del Cerro et al. (2016) published complete mitochondrial genome from *Polymastia littoralis*.

[4]Hill et al. (2013) published 7NHP from *Polymastia tenax*.

[5]*Polymastia* sp. 1 AP-2013 is described as a new species *P. svenseni* in **Paper IV**.

[6]Sequences of the B10–C1 fragment of 28S rDNA from five individuals identified as *Polymastia* sp. 1 CM-2013 and submitted to GenBank by Morrow in 2013 are still not employed in any published paper. Trial PhyML analysis of the alignment comprising these and our sequences have revealed that Morrow's sequences may belong to three or four different species. Sequence KF017187 is identical with our sequences from *Polymastia svenseni*, while sequence KF017190 is the sister to the pair *P. svenseni* + *Polymastia* sp. 2. Sequences KF017186 and KF017191 form a clade with our sequences from *P. bartletti* and *P.* cf. *bartletti*. Sequence KF017193 is the sister to the sequences from *P. nivea* (designated as *P. euplectella* in **Paper III**).

[7]CO1 sequence KC869420 from individual BELUM MC6488 identified as *Polymastia* sp. 1 CM-2013 (Morrow et al. 2013) is identical with the sequences of *P. penicillus* obtained by us (**Paper III**) and Alex et al. (2013).

[8]*Polymastia* sp. 1 SN-2005 and *Pseudotrachya* sp. fell remotely from other polymastiids in the CO1 and 28S rDNA phylogenies recovered by Nichols (2005) that may be an artefact resulting from inaccurate taxonomic identification of the samples (for details see Introduction: section 1.2.4).

[9]*Radiella hemisphaerica* is accepted as *Polymastia hemisphaerica* in **Paper IV**.

[10]*Radiella sarsi* is accepted as *Spinularia sarsi* in **Paper IV**.

[11]*Radiella* cf. *sarsi* is accepted as *Spinularia* cf. *sarsi* in **Paper IV**.

[12]*Radiella* sp. is described as a new species *Spinularia njordi* in **Paper IV**.

[13]*Sphaerotylus* sp. A (in Redmond et al. 2013) corresponding to *Sphaerotylus* sp. 2 (in our **Paper III**) is described as a new species *S. renoufi* in Plotkin et al. 2016 (**Paper II**).

[14]*Sphaerotylus* sp. C (in Morrow et al. 2012; Redmond et al. 2013) corresponds to *Sphaerotylus* sp. 1 in our **Paper III**.

The first solid phylogenetic study on demosponges involving polymastiid taxa was based on partial 28S rDNA and the barcoding region of CO1 (Nichols 2005). In both phylogenies Polymastiidae appeared to be non-monophyletic, with *Pseudotrachya* sp. and *Polymastia* sp. 1 falling remotely from four other polymastiids. However, the trees presented displayed several other inconsistencies, e.g. the sister relationship between *Geodia barretti*, an astrophorid, and *Lissodendoryx topsenti*, a poecilosclerid, that may question the credibility of taxonomic identification of the samples in this study. In all subsequent studies Polymastiidae appeared to be monophyletic (e.g. clade C2 in Morrow et al. 2012), although within this clade the genus *Polymastia* was non-monophyletic (Morrow et al. 2012; 2013; Redmond et al. 2013). Furthermore, phylogenies based on different genes supported contradicting hypotheses on the relationships between Polymastiidae and other families. In the 28S rDNA phylogeny Polymastiidae was the sister to clade C1 (Suberitidae + Halichondriidae), whereas in the CO1 tree Polymastiidae was the sister to the superclade C3 (Hemiasterellidae Lendenfeld, 1889 + Tethyidae Gray, 1848) + C4 (Clionaidae), although Bayesian supports for the relationships revealed were weak in both phylogenies (Morrow et al. 2012). The 18S rDNA phylogeny confirmed none of these hypotheses (Redmond et al. 2013), while the analysis of the combined dataset 18S rDNA + 28S rDNA + CO1 (Morrow et al. 2013) displayed a moderate support for the sister relationship between Polymastiidae and the superclade composed of five clades, C1 (Suberitidae + Halichondriidae), C3 (Hemiasterellidae + Tethyidae), C4 (Clionaidae + Placospongiidae Gray, 1867), C5 (Poecilosclerida) and C12 (Desmacellidae Ridley & Dendy, 1886). Based on these results Morrow & Cárdenas (2015) established for Polymastiidae a new order Polymastiida. However, the relationships between the polymastiid genera remained unresolved because the data on them are still poor.

1.2.5. Biogeography

For a long time there were no special studies focused on the diversity of polymastiid species and the data on their distribution were obtained under broader surveys of the regional sponge faunas. For obvious reasons the fauna of the European coasts and North Atlantic was explored most thoroughly, starting with the classical studies on the British sponges by Bowerbank (1864a; 1866; 1874) and Bowerbank & Norman (1882) followed by Schmidt (1870; 1880), who described species from both European and American Atlantic Coasts, Topsent (1892; 1898; 1900; 1904; 1913; 1928), who focused on the French and North African coasts, Azores and the Canadian Atlantic Coast, and many other authors. Substantial contribution to the knowledge of the deep-sea species

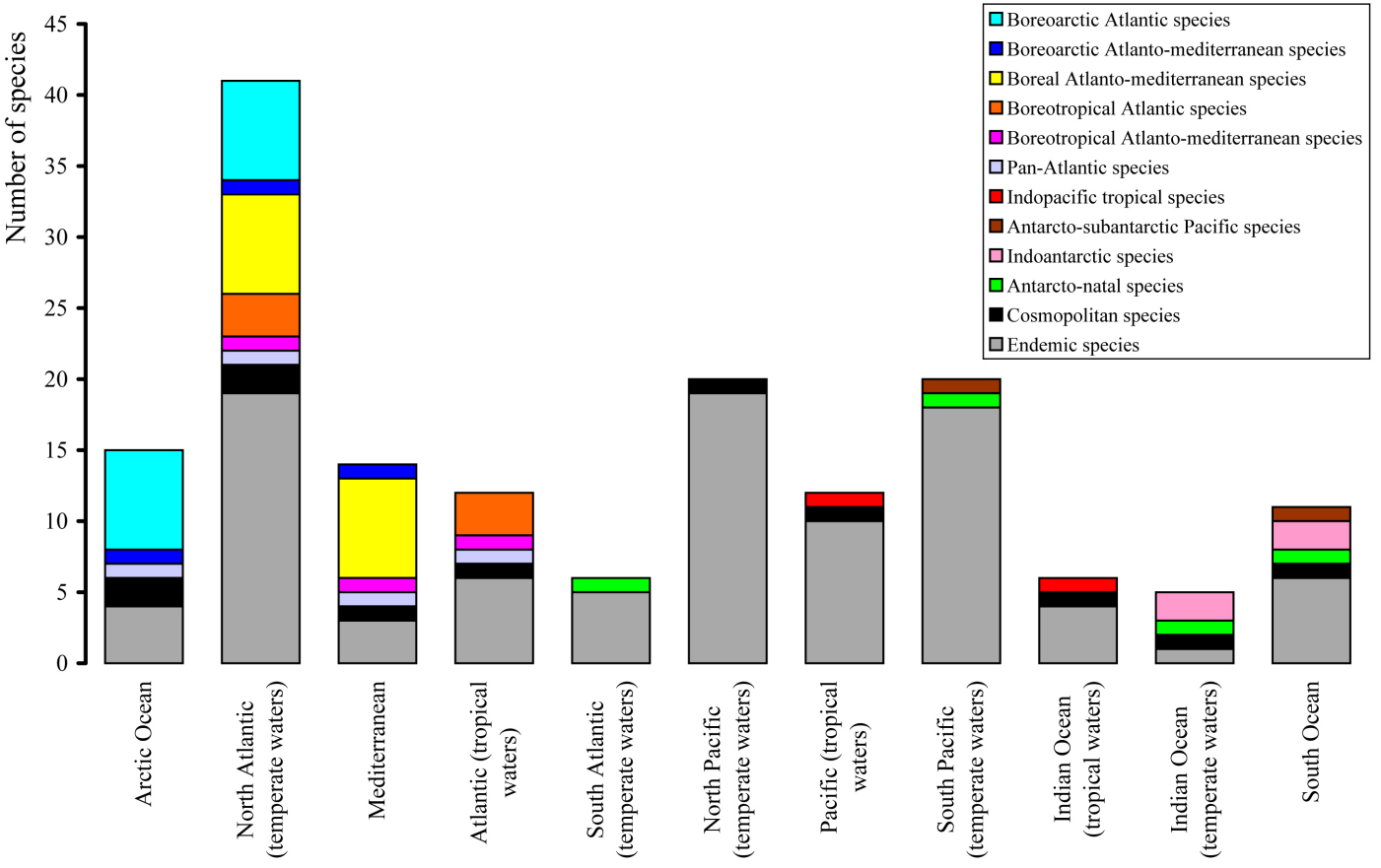

Figure 7. Geographical distribution of polymastiid species.

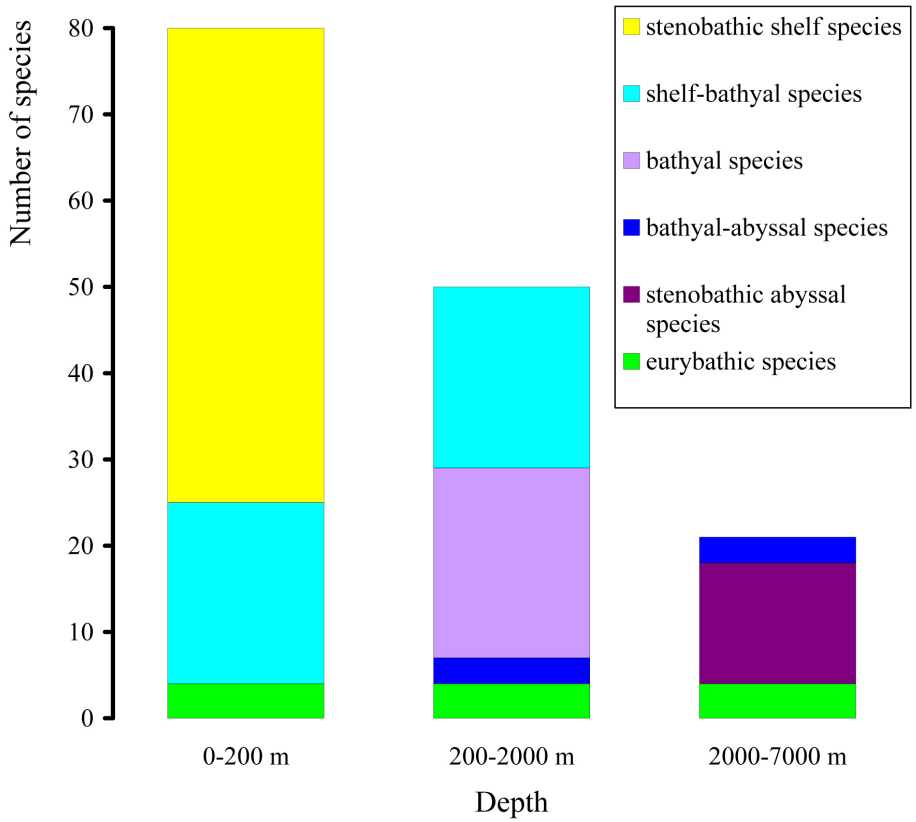

Figure 8. Vertical distribution of polymastiid species.

of the North Atlantic and other regions was done by Ridley & Dendy (1886; 1887). The data on the North Atlantic *Polymastia* spp. were summarized by Boury-Esnault (1987) and later critically revised, appended with the data on other polymastiid genera and compared with the Mediterranean species by Boury-Esnault et al. (1994). For the moment the polymastiid fauna of the North Atlantic including both open-sea and coastal areas comprises one third of all known polymastiid species (Figure 7). Almost a half of the North Atlantic polymastiids are endemic to this region, about 22% of the species are also recorded in the Mediterranean Sea and other 22% inhabit both the North Atlantic and the Arctic Ocean. Studies on the sponge fauna of the Nordic and Arctic Seas started by Hansen (1885), Fristedt (1885; 1887), Vosmaer (1885; 1887b) and Levinsen (1887) were continued by Lundbeck (1909), Hentschel (1916; 1929) and Burton (1930a). All this knowledge was summarized by Koltun (1959; 1966), and his books still remain the most exhaustive survey of the Arctic demosponges, although the data presented

there are definitely out-of-date and need a serious revision. Some revision was already performed by Plotkin (2004), who presented the most recent data on the diversity and distribution of polymastiids in the Arctic Seas. This region hosts about 12% of all known polymastiid species, of which most are of the North Atlantic origin and four species are supposed to be endemic (Figure 7). The knowledge of the South Atlantic polymastiids based on the sporadic data from early studies (Ridley & Dendy 1886; Stephens 1915; Uriz 1988) revised and appended by Samaai & Gibbons (2005) remains poor (Figure 7). The data on the polymastiids inhabiting the tropical areas of the Atlantic and Indopacific and the temperate waters of the Indian Ocean are out-of-date (Ridley & Dendy, 1886; 1887; Whitelegge 1897; Wilson 1904; 1925; Dendy 1916; 1922; Burton 1930b; 1934; 1959b; Dickinson 1945; Lévi 1964; 1967), except for the more recent survey of the New Caledonian deep-sea sponge fauna (Lévi 1993). The polymastiid fauna of the Southern Ocean was thoroughly described by Kirkpatrick (1907; 1908), Hentschel (1914) and Koltun (1964b), but the most recent review of the deep-sea Antarctic polymastiids (Plotkin & Janussen 2008) has demonstrated that the diversity of species in this region is apparently underestimated. For now about 9% of all polymastiid species are recorded from the Southern Ocean (Figure 7). Polymastiids of the temperate waters of the Pacific Ocean were for a long time explored sporadically except for the relatively solid data presented by Koltun (1966; 1970), who recorded nine species in the North Pacific. These data were substantially appended by Kelly-Borges & Bergquist (1997), who documented 14 species in the South Pacific, and Austin et al. (2014), who added five new polymastiid species to the North Pacific fauna. For the moment, 16 % of all polymastiid species are recorded in the North Pacific and other 16% in the South Pacific, most of them being endemics (Figure 7). It is evident that the polymastiid diversity of the Pacific Ocean is underestimated and further studies are required.

In all regions the distribution of most polymastiids is restricted to the continental shelf (more than 45% of all species, Figure 8). About 17% of the species inhabit both the shelf and the continental slope. Almost 32% of the species are known exclusively from the deep-sea, with 14 species recorded only in the abyssal. A long-lasting point of discussion in the sponge biogeography is the assumed cosmopolitan distribution of some species. This concept was quite common in the overconservative taxonomic and faunistic studies of the past (e.g. Schmidt 1870; Topsent 1900; Burton 1930a, b; Koltun 1966), but during the last decades the cosmopolitanism in sponges has been seriously challenged by use of data on their life histories and ecology, demonstrating the limited dispersal capacity of most sponge larvae (Boury-Esnault et al. 1993; Uriz

et al. 1998; Mariani et al., 2005; Uriz et al. 2008). Moreover, extensive application of molecular markers in taxonomy supplied with careful morphological re-examination of the type and comparative material has revealed that many sponges previously regarded as cosmopolitan species actually represent species complexes (e.g. Klautau et al. 1999; Lazoski et al. 2001; Miller et al. 2001; Wörheide et al. 2002; Boury-Esnault & Solé-Cava 2004; Blanquer & Uriz 2007; Pérez et al. 2011). For example, with the numerous records from the North and South Atlantic (e.g. Topsent 1900), Arctic and North Pacific (Koltun 1966), the polymastiid species *Polymastia mamillaris* (Müller, 1806) was for a long time regarded as a cosmopolitan. However, re-examination of the type material from Sweden compared with similar sponges from other regions revealed that the North Atlantic and Arctic records in fact represented three separate species: *P. mamillaris* restricted to the Swedish Coast, *P. penicillus* (Montagu, 1814) widely distributed around the British Isles, along the Southern European Coast and probably along the Canadian Coast, and *P. arctica* (Merejkowsky, 1878) inhabiting the Barents and White Seas (Morrow & Boury-Esnault 2000; Plotkin & Boury-Esnault 2004). The South Atlantic and North Pacific records of *P. mamillaris* definitely represent some other species, which require further studies (Plotkin 2004). For now two other polymastiid species, *Radiella sarsi* Ridley & Dendy, 1886 and *Tentorium semisuberites*, are still regarded as cosmopolitans (Figure 7). *R. sarsi* is reported from the deep-waters of the Arctic Ocean (Gorbunov 1946; Plotkin 2004), North Atlantic (Ridley & Dendy 1886; 1887; Topsent 1892; 1904; 1928), Pacific (Ridley & Dendy 1886; 1887) and Indian Ocean (Dendy 1922; Burton 1959b). Moreover, its relationships with *R. sol*, a morphologically similar deep-sea species of presumably Pan-Atlantic distribution (Schmidt 1870; Koltun 1966), remain unclear (Plotkin 2004; Plotkin & Janussen 2008). *T. semisuberites* is supposed to be eurybathic, with the numerous records from the Arctic (Koltun 1966; Plotkin 2004), North Atlantic (Topsent 1892; 1904; 1913; Lundbeck 1909; Barthel & Tendal, 1993) and Antarctic (Barthel et al. 1990; Plotkin & Janussen 2008; Göcke & Janussen 2013) and some records from the North Pacific (Austin et al. 2014) and Indian Ocean (Burton 1959b). Furthermore, the eurybathic distribution, although restricted to the certain regions, is also documented for three other species (Figure 8), *Polymastia invaginata* in the Southern Ocean (Koltun 1964b; Plotkin & Janussen 2008), *P. uberrima* (Schmidt, 1870) in the Arctic and North Atlantic (Lundbeck 1909; Arndt 1935; Koltun 1966; Plotkin 2004) and *Spinularia spinularia* in the North Atlantic (Stephens 1915b; Topsent 1928). All these cases require a careful comparison between the individuals from different regions and depths based on their morphology and molecular data.

2. Objectives of the thesis

Polymastiidae, widely recognized as one of the key demosponge families and one of the commonest groups of the marine benthos, still remains a “black box” with quite controversial taxonomy hardly managed by few dedicated experts and completely unintelligible for other marine biologists. Hence, the main aim of the present study was the revision of the Polymastiidae based on its phylogeny providing an essential contribution to the further development of the novel classification of the phylum Porifera and a practical tool for the identification of common species under biodiversity research and environmental monitoring. Four tasks were posed to achieve this aim:

- Reassessment of the polymastiid genera currently distinguished by the shape of exotyles applying multiple morphological characters (**Paper II**),
- Reconstruction of the polymastiid phylogeny based on morphology (**Paper I**),
- Reconstruction of the polymastiid phylogeny based on molecular markers (**Paper III**),
- Inventory of the polymastiid fauna in the Nordic and Siberian Seas based on integrative taxonomy (**Paper IV**).

3. Material and methods

3.1. Material

(**Papers I – IV**)

The study was based on the sponge collections of 18 museums (Table 6). Altogether about 1800 museum individuals were examined. Fresh material from the Nordic Seas was sampled by the author and Hans Tore Rapp (the first supervisor) by SCUBA diving, with dredging and trawling during the cruises onboard r/v "G.O. Sars", r/v "Håkon Mosby" and r/v "Hans Brattström" (by the University of Bergen and the Institute of Marine Research, Bergen) in 2009–2013 and the cruise of r/v "Helmer Hanssen" (by the University Centre in Svalbard) in 2011. Fresh material from other regions, including the Canadian Atlantic Coast and offshore areas, the Azores, the White Sea, the deep Weddell Sea, the Antarctic Peninsula and the Mozambiquean Coast was sampled and donated by our colleagues from different institutions (see Acknowledgements). Altogether 90 individuals were sampled and deposited mainly in the University Museum of Bergen. Material for both genetic and morphological studies, comprising 16 individuals from old collections and 71 freshly sampled individuals, was preserved in 96–100% alcohol. Other sponges, either preserved directly in alcohol, or primarily preserved in formalin and then transferred to 75% alcohol, or dried, were examined only with morphological methods.

3.2. Morphological laboratory methods

(**Papers I – IV**)

Sponges were photographed and their external morphology was examined under stereomicroscope. The architecture of the skeletons and aquiferous systems was studied on histological sections under light microscope. The sections were prepared following Vacelet (2006), Boury-Esnault & Bézac (2007) and Plotkin & Janussen (2008). Sponge fragments were dehydrated in 100% ethanol and acetone and embedded in Agar Low Viscosity epoxy resin (Agar Scientific Ltd, UK). The resulting embeddings were sectioned on a precise saw with a diamond wafering blade. The sections, 400–700 μm thick, were polished and mounted on slides with the epoxy resin. Some sections were stained with toluidine blue before mounting. In order to examine the spicules they were isolated from organic matter by cooking sponge fragments in nitric acid. The resulting

spicule suspensions were rinsed in several portions of distilled water and alcohol. Then the spicules were mounted on slides for light microscopy or on stubs for scanning electron microscopy (SEM). The samples on the stubs were coated with gold-palladium. Most SEM studies were performed at ZEISS Supra 55VP and JEOL 6400 microscopes (Laboratory for Electron Microscopy, University of Bergen). Taxonomic identification of sponge individuals followed Boury-Esnault (1987), Boury-Esnault & Rützler (1997), Boury-Esnault (2002) and Plotkin & Janussen (2008).

Table 6. List of museums whose collections were used in the present study.

Museum title and affiliation	Museum acronym
Canadian Museum of Nature, Ottawa	CMNI
Evolutionsmuseet, Uppsala Universitet	UPSZTY
Göteborgs Naturhistoriska Museum	GNM
Musée Océanographique de Monaco	MOM
Museum für Naturkunde, Berlin	ZMB
Muséum National d'Histoire Naturelle, Paris	MNHN
Museum of New Zealand, Te Papa Tongarewa, Wellington	NZNM
Natural History Museum, London	BMNH
Naturalis Biodiversity center, Leiden	RMNH
Naturhistorisk museum, Universitetet i Oslo	NHMUO
Senckenberg Forschungsinstitutt und Naturmuseum, Frankfurt am Main	SMF
Smithsonian National Museum of Natural History, Washington	USNM
Statens Naturhistoriske Museum, Københavns Universitet	ZMUC
Ulster Museum, National Museums of Northern Ireland, Belfast	BELUM
Universitetsmuseet i Bergen	ZMBN
Vitenskapsmuseet, Norges teknisk-naturvitenskapelige universitet, Trondheim	NTNU-VM
Zoological Institute of Russian Academy of Sciences, Saint-Petersburg	ZIN RAS

3.3. Phylogenetic analyses based on morphology

(**Paper I**)

The analyses were based on 25 binary morphological characters (**Paper I**: Table 1) of 21 polymastiid species (**Paper I**: Table 2 and Figures 1–2) and three suberitid species, of which *Suberites domuncula* (Olivi, 1792) was used as outgroup and two species of *Aaptos*, *A. aaptos* (Schmidt, 1864) and *Aaptos papillata*, demonstrating strong affinities

with the polymastiids were involved in the ingroup. The ingroup encompassed the type species of all polymastiid genera accepted for that time, except for *Trachyteleia* and *Radiella*, for which the available data were too poor. Other taxa of the ingroup were represented by seven non-type species, five of *Polymastia* and two of *Radiella*, and the type species of *Suberitechinus*, a genus regarded as a synonym of *Trachyteleia* in the generally accepted classification (Boury-Esnault 2002), but in fact displaying essential differences from the latter (**Paper II**). Three possible evolutionary scenarios based on three alternative interpretations of the body plan of *Quasillina brevis* (Bowerbank, 1866), the type of *Quasillina*, and *Ridleia oviformis*, the type of *Ridleia*, were reconstructed. First interpretation: *Ridleia* possesses aquiferous papillae, whereas *Quasillina* lacks them. Second interpretation: both genera lack papillae. Third interpretation: the body in both genera is a single hyper-developed papilla. The analyses were performed in PAUP* 4.0b10 (Swofford 2002) running heuristic search with the parsimony criterion. 50% majority rule consensus trees were computed and their consistency indices were calculated.

3.4. Molecular studies

(**Paper III**)

3.4.1. Taxonomic scope

Molecular analyses were performed on 24 unambiguously identified polymastiid species and ten OTUs, of which four were identified to species level with some uncertainty and six could not be referred to any known species and were therefore identified only to genus level (this synopsis: Table 5, **Paper III**: Table 1). These species and OTUs belonged to seven polymastiid genera, *Polymastia*, *Quasillina*, *Radiella*, *Sphaerotylus*, *Spinularia*, *Tentorium* and *Weberella*. Each genus was represented at least by the type species except for *Radiella*, the type species of which, *R. sol*, was unavailable and had an ambiguous status (see Discussion on *Radiella* in **Paper III**). Sequences from 19 species and nine OTUs were novel. Data on two species and one OTU were taken from GenBank and sequences from three species were both obtained by us and taken from GenBank. Two species were chosen as outgroups, the suberitid *Suberites ficus* (Johnston, 1842) and the tethyid *Tethya citrina* Sarà & Melone, 1965. Data on both species were taken from GenBank. This selection was based on the substantial morphological affinities between Suberitidae, Tethyidae and Polymastiidae and on the former affiliation of these three families with the order Hadromerida.

3.4.2. Phylogenetic markers, amplification and sequencing

Two phylogenetic markers were selected, the 5'-end barcoding region of CO1 (Folmer et al. 1994) successfully used for most invertebrates including many sponge families (e.g. Erpenbeck et al. 2007b; 2012a; Cárdenas et al. 2010; Pöppe et al. 2010) and a region of 28S rDNA coding rRNA from helix B10 to helix E19 (numeration of the helices according to De Rijk et al. (1999; 2000) and Wuyts et al. (2001)). This region, proposed by Morrow et al. (2012) and successfully used for a much larger set of polymastiid species than ever studied before, comprises three overlapping fragments, a highly variable fragment coding from helix B10 to helix C1, a moderately variable fragment coding from D1 to D19 and a more conservative fragment coding from D20 to E18–E19.

Details of the DNA extraction, amplification of the selected regions, estimation of the resulting PCR products and sequencing are described in **Paper III**: Material and Methods. CO1 barcoding regions were amplified with the primers dgLCO1490/dgHCO2198 (Meyer 2003) for most species and OTUs and with the primers jgLCO14901490/ jgHCO21982198 (Geller et al. 2013) for *Polymastia corticata*. The selected region of 28S rDNA was amplified with three pairs of primers designed by Morrow et al. (2012) and corresponding to three overlapping DNA fragments (see above), Por28S-15F/Por28S-878R for B10–C1, Por28S-830F/Por28S-1520R for D1–D19 and Por28S-1490F/Por28S-2170R for D20–E19. Sequence reads were performed with an automated ABI 3730XL DNA Analyser (Applied Biosystems) at the Department of Molecular Biology, University of Bergen.

The raw forward and reverse reads were analyzed with the SeqMan application of DNASTAR Lasergene 8.0. The resulting consensus sequences were checked by nucleotide BLAST search (Altschul et al. 1990) against GenBank sequences to verify their polymastiid origin. If strong contaminating signals or double signals on some sites were revealed in the reads, the extractions from the respective samples and the PCRs were repeated in order to exclude occasional cross-contamination and PCR errors. If these repetitive procedures failed to get rid of the contamination or double signals in the direct sequences, the PCR products were cloned, and 10–20 clones per product were sequenced by LGC Genomics GmbH in Berlin, Germany (see details on cloning in **Paper III**: Material and Methods). The resulting clones were checked for errors against the alignment of the approved direct sequences. The clones with unique nucleotides or gaps in the conservative sites were disregarded. The polymorphism in the remaining

clones was regarded as natural. Strict consensuses of the clones with the polymorphic sites encoded with IUPAC symbols were employed in the main phylogenetic analyses. Altogether 75 CO1 sequences and 236 sequences of the three 28S rDNA fragments including eight clone libraries were obtained and submitted to GenBank (**Paper III**: Table 1).

3.4.3. Alignments

All alignments were performed in SeaView 4.3.4 (Galtier et al. 1996; Gouy et al. 2010). The initial alignment of the 28S rDNA sequences was further refined manually under consideration of the RNA secondary structure (Erpenbeck et al. 2007a, e; 2008). A 90% consensus of all sequences was adjusted to a template adapting the secondary structures reconstructed from other sponge families (Figure 9). Search for unambiguously aligned sites was initially performed in GBlocks 0.91b (Castresana 2000). The resulting set of the sites was manually extended to exclude in total 43 sites, because some obviously homologous sites were neglected by the algorithm. For all computing procedures identical sequences were collapsed into one sequence. The resulting alignments, CO1 matrix (35 unique polymastiid sequences), 28S rDNA complete matrix and 28S rDNA matrix reduced by 43 sites (49 sequences), and the respective concatenated matrices, CO1 + complete 28S rDNA and CO1 + reduced 28S rDNA (47 sequences), were deposited at TreeBase and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S18487. These matrices were also used for the definition of the genetic synapomorphies and autoapomorphies of the species under the inventory of Polymastiidae in the Nordic and Siberian Seas (**Paper IV**).

3.4.4. Selection of evolutionary models and phylogenetic analyses

Search for the best fitting nucleotide substitution model for the CO1 dataset carried out in MrModeltest 2.0 (Nylander 2004) selected GTR+G+I. Three phylogenetic analyses were performed under this model with alternative partitioning of the CO1 data: no partitions, two partitions (codon positions 1 + 2 and codon position 3) and three partitions (codon position 1, codon position 2 and codon position 3). Additionally the CO1 matrix was analyzed under codon substitution model.

Search for the best fitting RNA specific substitution model for the 28S rDNA matrices was performed with the model testing application implemented in PHASE 3.0 (Allen & Whelan 2014), the modified version of PHASE 2.0 (Gowri-Shankar & Jow 2006).

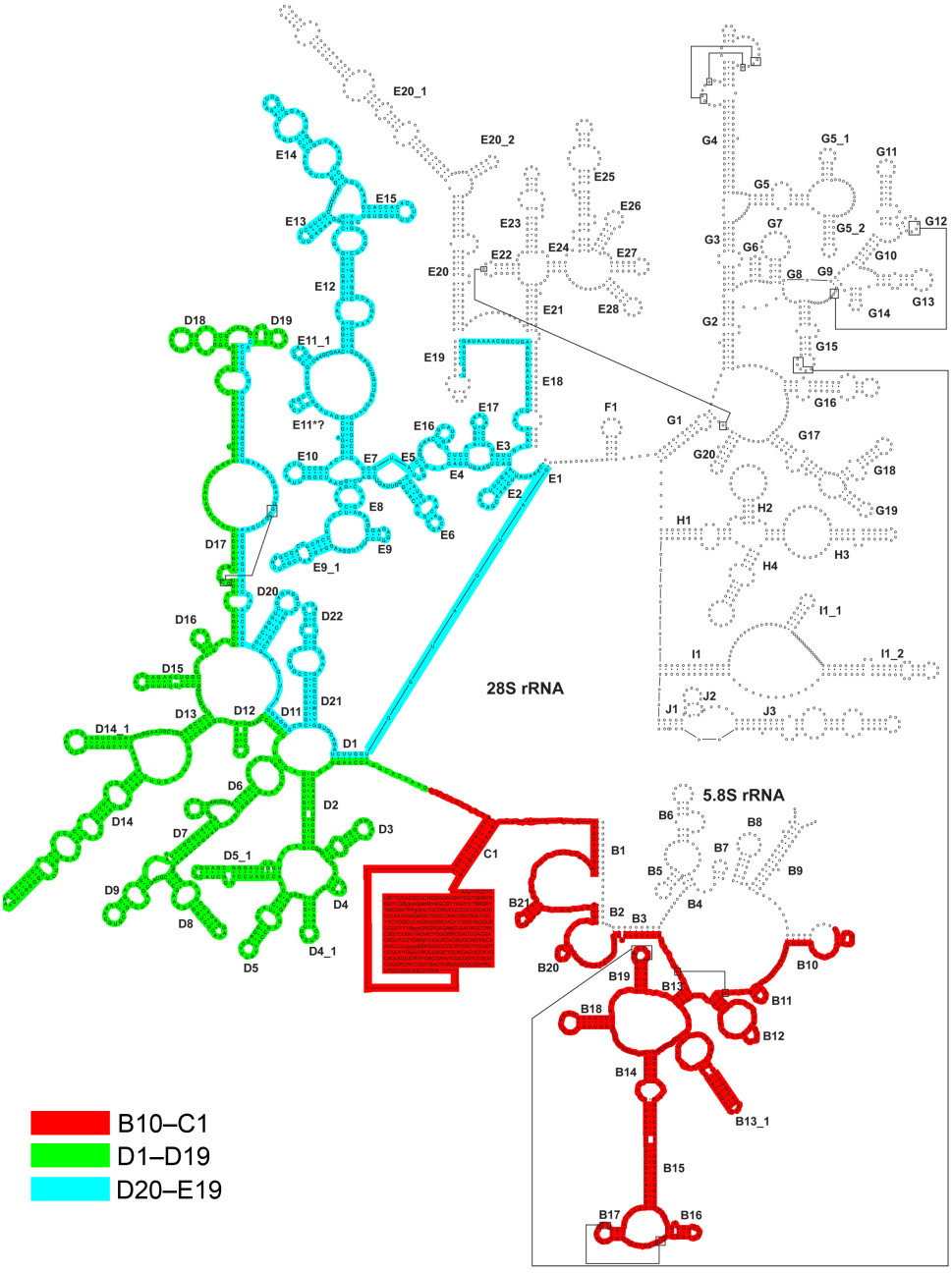

Figure 9. 90% consensus of 49 sequences from polymastiid species adapted to the 28S rRNA secondary structure reconstructed from other demosponge families (courtesy of D. Erpenbeck and O. Voigt (Ludwig-Maximilians-Universität München), unpublished data). The three fragments employed in the present study are highlighted with colour.

The mixed model comprising RNA16C+G for helix positions and REV+G (corresponding to GTR+G in other software) for loop positions was selected. In addition to the analyses under the best fitting model the 28S rDNA datasets were also analyzed under RNA16B+G+I / REV+G+I and under REV+G+I without partitioning of the data, in order to check how the model selection could affect the resulting phylogeny. Correspondingly, three analyses under the alternative models for 28S rDNA were run for the concatenated datasets CO1 + 28S rDNA. In these analyses the CO1 data were split in two partitions.

Phylogenetic analyses were performed in a Bayesian inference framework, with MrBayes 3.2 (Ronquist et al. 2011) for the CO1 matrix and with PHASE 3.0 (Allen & Whelan 2014) for the 28S rDNA matrices and the concatenated matrices CO1 + 28S rDNA, and in a Maximum Likelihood framework (ML) with RAxML 8.1.24 (Stamatakis 2014). Details of the settings for the analyses and the quality control of the results are described in **Paper III**: Material and Methods.

Bayesian analyses of the single-gene datasets revealed some incongruence between the CO1 and 28S rDNA phylogenies. To illustrate the conflicts a rooted galled network (Huson et al. 2009) based on the consensus trees was computed with Dendroscope 3 (Huson & Scornavacca 2012). To explore these conflicts an incongruence length difference test (ILD, Farris et al. 1994) on the concatenated dataset CO1 + reduced 28S rDNA was performed in PAUP* 4.0b10 (Swofford 2002). Furthermore, the conflicting topological hypotheses were tested with Bayes factor comparisons of the model likelihoods (Kass & Raftery 1995). To obtain more accurate likelihoods stepping-stone samplings, with the monophyly of the congruent clades constrained as recommended by Bergsten et al. (2013), were performed in MrBayes 3.2.

Intragenomic polymorphism revealed in the D1–D19 fragment of 28S rDNA in three species of *Polymastia*, *P. andrica* de Laubenfels, 1949, *P. arctica* and *P. grimaldii*, was regarded as natural. A dataset comprising all versions of this fragment in the three species was analysed with Minimum-spanning network algorithm (Bandelt et al. 1999) implemented in PopArt 1.7 (http://popart.otago.ac.nz) and in a ML framework with PhyML (Guindon et al. 2010).

Consensus trees resulting from the Bayesian analyses along with the ML-tree illustrating the intragenomic polymorphism were deposited at TreeBase and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S18487.

3.4.5. Tracing of the evolution of morphological characters

The consensus tree resulting from the Bayesian analysis of the concatenated dataset CO1 + reduced 28S rDNA was chosen for tracing of the morphological evolution. The branches corresponding to different individuals of the same species or OTU were collapsed, resulting in 32 branches. A matrix with 21 morphological characters of the respective 30 polymastiid taxa and two outgroup taxa was built by the modification of the dataset for evolutionary scenario 3 from **Paper I** (see **Paper III**: Online Resources 1–2). The ancestral state reconstruction with parsimony criterion for each character was performed in Mesquite 3.04 (Maddison & Maddison 2015), while the consistency indices were computed in PAUP* 4.0b10 (Swofford 2002).

4. Results

4.1. Polymastiidae with ornamented exotyles: a taxonomic revision

(**Paper II**)

In this paper we presented detailed descriptions of all known species of *Proteleia*, *Sphaerotylus*, *Trachyteleia* and *Tylexocladus*. Furthermore, we established a new genus *Koltunia* and three new species of *Sphaerotylus* and proposed the transfer of two species from *Polymastia*, one to *Proteleia* and the other to *Sphaerotylus*, based on the presence of ornamented exotyles in these species. *Koltunia* was established for the deep-sea Antarctic species *Proteleia burtoni* Koltun, 1964. It displays some similarities with *Polymastia invaginata*, *Sphaerotylus antarcticus* and *S. borealis*, but considering the overall combination of its morphological traits and the unique shape of its exotyles, *K. burtoni* could not be allocated to any previously known sponge genus. The original allocation of this species to *Proteleia* was based only on the presumable similarity between the grapnel-like distal ornaments on the exotyles in *K. burtoni* and the type species of *Proteleia*, *P. sollasi* Dendy & Ridley, 1886. However, our study revealed that in *P. sollasi* the exotyles are relatively small (350–555 μm in length and 5–6.5 μm in diameter), with irregular distal ornaments varying from umbrelliform to grapnel-like and bearing weakly developed claws (**Paper II**: Figures 5F–J), whereas in *K. burtoni* the exotyles are much larger (1900–4300 μm in length and 24–40 μm in diameter), all with the grapnel-like distal ornaments composed of several prominent, symmetrically arranged claws (**Paper II**: Figures 2E–H). Other features distinguishing *K. burtoni* from *P. sollasi* include the shaggy body surface, the single-layered cortex and the presence of only three categories of spicules.

The proposal to transfer *Polymastia tapetum* to *Proteleia* was based on the presence of thin and short exotyles with umbrelliform distal ornaments in this species (**Paper II**: Figure 6G–I) and the similarities between *P. tapetum* and *P. sollasi* in external morphology. Meanwhile, these species differ by the architecture of cortex, which is two-layered in *P. tapetum* (**Paper II**: Figure 6B) and three-layered, including an additional spicule palisade, in *P. sollasi* (**Paper II**: Figure 4C). Previously this difference was an argument for keeping these species in different genera (Kelly-Borges & Bergquist 1997), but considering the variability of the cortical skeletons within many polymastiid genera, e.g. the presence of extra spicule palisades in the cortex of several *Polymastia* spp., this argument was disputed.

One more species with small exotyles bearing umbrelliform or grapnel-like distal ornaments, *P. umbraculum*, was originally allocated to *Polymastia* (Kelly-Borges & Bergquist 1997). However, at present this species cannot with proper confidence be placed in any known polymastiid genus, since it displays the characteristic traits of four genera, *Atergia*, *Proteleia*, *Pseudotrachya* and *Weberella*. Because of this unusual combination of traits and the impossibility to define any solid autapomorphy of *P. umbraculum*, we refrained from establishing of a new genus for this species and proposed to regard it as incertae sedis awaiting evidence from molecular data.

In another species of *Polymastia*, *P. isidis* Thiele, 1905, exotyles with spherical distal ornaments were revealed (**Paper II**: Figures 20G–L). By this feature, along with the three-layered cortex and some external traits, this species strongly resembles the type species of *Sphaerotylus*, *S. capitatus* (Vosmaer, 1885). Consequently, we proposed to transfer *P. isidis* to *Sphaerotylus*. The ornamented exotyles discovered in the type material of *P. isidis* from Chile were in fact not reported by the species' author (Thiele 1905). Neither were the exotyles reported in the numerous records of this species from other regions, Palmer Archipelago and Falkland Islands (Burton 1932), South Shetland Islands (Desqueyroux 1975), Kerguelen (Boury-Esnault & Van Beveren 1982), Namibian coast (Uriz 1988) and eastern Weddell Sea (Barthel et al. 1990). Hence, these records may represent another species, probably of *Polymastia*.

Reconsidering *Sphaerotylus* we also documented great morphological similarities between *S. antarcticus* and *S. borealis* and their considerable distinctions from the type species *S. capitatus*. These distinctions include the umbrelliform or fungiform distal ornaments on the exotyles, the larger size of the principle spicules and exotyles and some traits in the external morphology and cortical skeleton. Despite these distinctions we retained *S. antarcticus* and *S. borealis* in *Sphaerotylus* until better classification could be proposed based on molecular phylogenies.

Meanwhile, we established three new species of *Sphaerotylus*, S. renoufi from the British Isles, S. strobilis from South Africa and S. tjalfei from West Greenland. S. strobilis distinguished by the unique strobile-shaped exotyle ornaments (**Paper II**: Figures 25J and M) resembles *S. capitatus* by the three-layered cortex and some external features. S. tjalfei resembles *S. capitatus* by the spherical exotyle ornaments (**Paper II**: Figures 27F–H), but differs from the latter by the shaggy body surface and the two-layered cortex. S. renoufi was allocated to *Sphaerotylus* with some doubt, because it differs from *S. capitatus* by the hispid body surface, the two-layered cortex and the unique lobate exotyle ornaments (**Paper II**: Figures 22I and K).

The CO1 and 28S rDNA phylogenies confirmed the validity of S. renoufi and its allocation to *Sphaerotylus* (**Paper III**). In both phylogenies this species was the sister to the pair *S. capitatus* + *Sphaerotylus* sp. (an aberrant sponge with reduced exotyle ornaments), whereas *S. antarcticus* and *S. borealis* fell in other clades (**Paper III**: Figures 1–2), that was congruent with the morphological data. At the same time the latter two species did not group together, that was in contradiction with their morphological similarities, while the clades, where they fell, were weakly supported. Unfortunately, no molecular data on other new or previously known *Sphaerotylus* spp. were obtained.

We disputed the synonymization of *Suberitechinus* with *Trachyteleia* proposed by Boury-Esnault (2002). The type species of these nominal genera, *S. hispidus* (Bowerbank, 1864) and *T. stephensi* Topsent, 1928 respectively, possess exotyles, which differ from the principal choanosomal monactines mainly by larger size. In *T. stephensi* the exotyles are 650–770 μm in length, with tiny spines on the distal parts (**Paper II**: Figures 30G–H), that is regarded as the distinguishing feature of *Trachyteleia* (Boury-Esnault 2002). Conversely, in *S. hispidus* the exotyles are much longer (up to 4000 μm) and smooth. *S. hispidus* differs from *T. stephensi* also by the presence of polytylote spicules among the principal monactines. *T. stephensi* has not been reported since Topsent (1928) described this species from Azores, and the available type material is limited to histological sections and spicule preparations. This hinders further comparison between *T. stephensi* and *S. hispidus* by external morphology. We proposed to regard both *Trachyteleia* and *Suberitechinus* as valid genera until molecular data and more comprehensive morphological data become available.

Tylexocladus differs from other genera by the denticulate distal ornaments on the exotyles and comprises two well-defined species, *T. joubini* from Azores and *T. hispidus* Lévi, 1993 from New Caledonia. The latter species is distinguished from the former by the heterogeneous cortex with exotyles in three categories. We found a great variability of characters in *T. joubini*. Some individuals possess centrotylote microxeas in the choanosome, whereas the others lack these spicules. In some individuals the exotyles are uniformly distributed over the cortex, while in the others the exotyles are concentrated at the body edge. The shape of the denticulate distal extremities of the exotyles also varies greatly. This variability may testify that *T. joubini* is in fact a complex of two or more species, but more studies are required to check this assumption.

4.2. Phylogenies of Polymastiidae based on morphology

(**Paper I**)

All three reconstructed evolutionary scenarios demonstrated consistency of six characters: the presence/absence of radial growth pattern, basal surface, specialized basal cortex, exhalant papillae, oscula on the body surface and middle cortical layer of aquiferous cavities. Other characters displayed higher or lesser level of homoplasy at least in one of the scenarios (**Paper I**: Table 3). Furthermore, all three scenarios revealed the non-monophyly of Polymastiidae as well as the non-monophyly of *Aaptos* and *Polymastia*. Five phylogenetic patterns were supported in all consensus trees (**Paper I**: Figure 4):

1) The majority of polymastiid species and the suberitid *Aaptos papillata* formed a superclade distinguished by two synapomorphies, the acquisition of exhalant papillae and the loss of oscula on the body surface. Another suberitid *Aaptos aaptos* and one polymastiid, *Pseudotrachya hystrix* (Topsent, 1892), fell remotely from this superclade.

2) Within this superclade at least 14 polymastiid species (all polymastiids excluding *Polymastia boletiformis*, *P. uberrima*, *Pseudotrachya hystrix*, *Tentorium semisuberites* and *Weberella bursa* (Müller, 1806) in all scenarios and also *Quasillina brevis* and *Ridleia oviformis* in scenarios 1 and 2) formed a strongly supported group designated as the main polymastiid clade in **Paper I** and as Clade 1 herein. The synapomorphies of Clade 1 comprised the loss of differentiated lateral surface and the shift from globular to thickly encrusting or radial growth pattern. Meanwhile, these synapomorphies were also shared by *Pseudotrachya hystrix* falling outside the polymastiid superclade.

3) *P. boletiformis* and *W. bursa* were sisters sharing three synapomorphies, the acquisition of a middle cortical layer of aquiferous cavities, the shift from the radial to reticulate architecture of the main choanosomal skeleton and the reduction in the number of spicule size categories from three to two. However, the number of spicule size categories appeared to be a very homoplasious character with several reversals in all reconstructed trees. The pair *P. boletiformis* + *W. bursa* formed an uncertain trichotomy with Clade 1 and *P. uberrima*.

4) Clade 1 diverged in two smaller clades, one (here Clade 1.1) comprising *Polymastia grimaldii*, *Radiella hemisphaerica* and *R. sarsi* and the other (here Clade 1.2) encompassing the rest of polymastiids including the type species of *Polymastia*,

P. mamillaris. Clade 1.1 was distinguished by three synapomorphies, the shift to radial growth pattern, the acquisition of a basal surface and the specialization of the basal cortex. Clade 1.2 was characterized by thickly encrusting growth pattern, but this feature was also shared by *Pseudotrachya hystrix* falling remotely from other polymastiids.

5) Within Clade 1.2 *Polymastia euplectella* Rezvoj, 1927 was the sister to the group of ten species in scenarios 1 and 2, which was appended with *Quasillina brevis* and *Ridleia oviformis* in scenario 3. This group was characterized by the hispid body surface lacking ostia. Within this group most trees revealed an unresolved trichotomy between *Polymastia mamillaris* and two small clades, *Acanthopolymastia acanthoxa* (Koltun, 1964) + *Astrotylus astrotylus* Plotkin & Janussen, 2007 + *Atergia corticata* Stephens, 1915 + *Polymastia invaginata* + *Spinularia spinularia* + *Tylexocladus joubini* (here Clade 1.2.1) and *Proteleia sollasi* + *Sphaerotylus borealis* + *Suberitechinus hispidus* (here Clade 1.2.2). Clade 1.2.1 was characterized by the loss of an inner cortical layer of criss-cross monactines and the reduction in the number of size categories of ordinary monactines. But these features also appeared in some other clades. Within Clade 1.2.1 five species distinguished by the presence of unordinary microscleres in the choanosome (*Acanthopolymastia acanthoxa*, *Astrotylus astrotylus*, *Atergia corticata*, *Spinularia spinularia* and *Tylexocladus joubini*) grouped together (Clade 1.2.1.1). But such microscleres were also typical of *Pseudotrachya hystrix* falling remotely from other polymastiids. All species of Clade 1.2.2 were characterized by the presence of exotyles. But the exotyles were also present in *T. joubini* from Clade 1.2.1. In few trees of scenarios 1 and 2 Clades 1.2.1 and 1.2.2 were not revealed.

Since the three reconstructed scenarios were based on three alternative interpretations of the body plan of *Quasillina brevis* and *Ridleia oviformis* (see section 3.3 in Material and Methods above) the differences between the phylogenies mainly concerned the position of these species. In scenario 1 (*Ridleia* possesses a papilla, *Quasillina* lacks papilla) *R. oviformis* grouped with the polymastiid superclade, while *Q. brevis* fell remotely. In scenario 2 (both *Ridleia* and *Quasillina* lack papillae), these species were sisters and fell outside the polymastiid superclade. In scenario 3 (the body in both genera is a single hyper-developed papilla) *Q. brevis* and *R. oviformis* were sisters and this pair joint Clade 1.2.1 as the sister to Clade 1.2.1.1. We favoured scenario 2 as the most parsimonious and consistent. This scenario assumed that the most recent common ancestor of *Aaptos aaptos*, *Pseudotrachya hystrix* and the superclade of 18 polymastiid species acquired the regular main choanosomal

skeleton, which was never lost afterwards, remaining radial in most taxa and having transformed to reticulate in *Polymastia boletiformis* and *Weberella bursa*. However, these assumptions required a careful verification with molecular phylogenies.

4.3. Molecular phylogenies of Polymastiidae

(**Paper III**)

Analyses of the 28S rDNA under three alternative substitution models resulted in the same phylogenies with negligible differences in the supporting values (Appendix 1). Consequently, in **Paper III** we presented only the phylogeny based on the best fitting model RNA16C+G / REV+G. The phylogenies reconstructed from the complete 28S rDNA matrix and the matrix reduced by 43 ambiguously aligned sites were also congruent, except for the unresolved relationships between *Spinularia spinularia* and *Radiella* sp. in the tree based on the reduced dataset. Similarly, the analyses of the CO1 dataset under the nucleotide substitution model with three alternative data partitionings provided congruent phylogenies (Appendix 2: Supplementary Figure 2 and Table 2). Meanwhile, the phylogeny reconstructed under the codon model was slightly different (Appendix 2: Supplementary Figure 3 and Table 2), although these differences concerned only the clades weakly supported by the analyses under the nucleotide model. The codon model is useful for the analyses of the datasets comprising long protein-coding sequences, while it was obviously inappropriate for the short barcoding region we analyzed. Hence, the results obtained under this model were disregarded in **Paper III**. Analyses of the concatenated datasets 28S rDNA + CO1 under alternative substitution models resulted in the same phylogenies (Appendix 3).

Both the 28S rDNA and CO1 phylogenies supported the monophyly of all polymastiids studied against the outgroups and the non-monophyly of four genera, *Polymastia*, *Radiella*, *Sphaerotylus* and *Tentorium* (**Paper III**: Figures 1–3). *Polymastia* spp. were scattered over different clades, *Radiella hemisphaerica* fell distantly from other *Radiella* spp., *Sphaerotylus borealis* lay remotely from its congeners and *Tentorium papillatum* fell on a long branch as the sister group to a clade of the remaining polymastiids. Moreover, in the 28S rDNA tree the type species of *Tentorium*, *T. semisuberites*, and *T.* cf. *semisuberites* did not group together, although the support for their non-monophyly was very weak (**Paper III**: Figure 1). Unfortunately, no CO1 data from *T.* cf. *semisuberites* were obtained. Three clades of species were recovered in all phylogenies:

Clade I comprised *Radiella hemisphaerica* and six *Polymastia* spp. including the type species *P. mamillaris*. It diverged in two sister subclades, each of three species, and *P. uberrima*, which fell alone. In one subclade *P. thielei* Koltun, 1964 and *R. hemisphaerica* were sisters against *P. mamillaris*. The relationships within the other subclade comprising *P. andrica*, *P. arctica* and *P. grimaldii* were unresolved in the 28S rDNA trees because of the intraspecific and intragenomic polymorphism and resolved with a weak support for the sister relationships between *P. andrica* and *P. arctica* in the CO1 phylogenies. 28S rDNA phylogenies strongly supported the sister relationships between Clade I and the pair *Polymastia* sp. 1 + *Polymastia* sp. 2. CO1 phylogenies under the nucleotide model revealed the sister relationship between Clade I and the trio *Polymastia* sp. 1 + *Polymastia* sp. 2 + *Polymastia* sp. 3, although with a weak support. In the phylogeny under the codon model this relationship was not supported. Unfortunately, no 28S rDNA were obtained for *Polymastia* sp. 3.

Clade II comprised three species of *Sphaerotylus*, *S. capitatus* (the type species), *Sphaerotylus* sp. 1 and S. renoufi (designated as *Sphaerotylus* sp. 2 in **Paper III** and described as a new species in **Paper II**). The first two species had identical CO1 sequences and were sisters in the 28S rDNA phylogeny.

Clade III included *Spinularia spinularia* (the type species of *Spinularia*) and three species of *Radiella*, *R. sarsi*, *R.* cf. *sarsi* and *Radiella* sp. All these species except for *R.* cf. *sarsi* had identical CO1 and formed a subclade, with *S. spinularia* and *Radiella* sp. being sisters, in the 28S rDNA phylogeny. *S. spinularia* was presented by two groups of individuals differing in 28S rDNA, while all studied individuals of *Radiella* sp. had identical 28S rDNA. Some sites in this gene, in which the two groups of *S. spinularia* differed from each other and from *Radiella* sp., were regarded as ambiguously aligned in the alignment as a whole and consequently excluded in the reduced matrix that resulted in a polytomy between these branches. However, within Clade III these excluded sites could be aligned unambiguously and provided a sufficient signal to resolve the polytomy. In the CO1 phylogenies Clade III was the sister to *Tentorium semisuberites*, but this relationship was not supported in the 28S rDNA phylogeny.

Furthermore, both the 28S rDNA and CO1 phylogenies strongly supported the pair *Polymastia boletiformis* + *Quasillina brevis* and revealed the grouping *Sphaerotylus borealis* + *Polymastia* cf. *conigera* Bowerbank, 1874 + *Weberella bursa*, although the support for it in the single gene analyses was weak. Within this grouping the latter two species were sisters with a strong support in the CO1 phylogeny (**Paper III**: Figure 2),

but a much weaker support in the 28S rDNA phylogeny (**Paper III**: Figure 1).

ILD test of the concatenated dataset CO1 + 28S rDNA rejected the hypothesis of congruent data. Six conflicts between the single gene phylogenies were revealed (**Paper III**: Figure 4). The earnest conflict concerned the position of the pair *Polymastia boletiformis* + *Quasillina brevis*. In the CO1 phylogenies this pair was the sister to *Polymastia invaginata*, while in the 28S rDNA phylogenies it was the sister to the grouping Clade I + *Polymastia* sp. 1 + *Polymastia* sp. 2. Bayesian support for the indicated relationships was strong in each consensus tree, while Bayes factor tests revealed no support for the alternative hypothesis in either of the two topologies. Two other conflicts were evidently due to the low resolution in the CO1 phylogenies, which failed to resolve the relationships of *Polymastia corticata* with other taxa and were inconsistent on the position of *Sphaerotylus antarcticus*. In the trees reconstructed under the nucleotide model the latter species was the sister to Clade II, while in the phylogeny based on the codon model it was the sister to Clade I, with very weak support in both cases. Conversely, the 28S rDNA phylogenies strongly supported the sister relationship between *P. corticata* and Clade II as well as between *S. antarcticus* and the pair *P. corticata* + Clade II (**Paper III**: Figure 1). Three conflicts between the CO1 and 28S rDNA phylogenies revealed within small terminal subclades, the trio *Polymastia andrica* + *P. arctica* + *P. grimaldii* in Clade I, the pair *P. boletiformis* + *Quasillina brevis* and the group of three individuals of *P. invaginata* (**Paper III**: Figure 4) were caused by the gene polymorphism reviewed below.

4.4. Intraspecific and intragenomic polymorphism in Polymastiidae

(**Paper III**)

In *Polymastia andrica*, *P. arctica* and *P. grimaldii* a 28S rDNA polymorphism was revealed in four sites of the B10–C1 fragment (positions 578–580 and 583 in the complete matrix) and in seven sites of the D1–D19 fragment (positions 941–943, 947–948 and 1294–1295). The variation in B10–C1 was estimated on the direct sequences. The sequences of this fragment from three *P. andrica* were identical, while *P. arctica* displayed a polymorphism – individual ZMBN 098063 differed from *P. andrica* just by one ambiguity, individual ZMBN 098068 by three nucleotides and two individuals, ZMBN 098060 and ZMBN 098062, by four nucleotides. *P. grimaldii* ZMBN 098064 differed from the latter two individuals of *P. arctica* just by one ambiguity. The variation

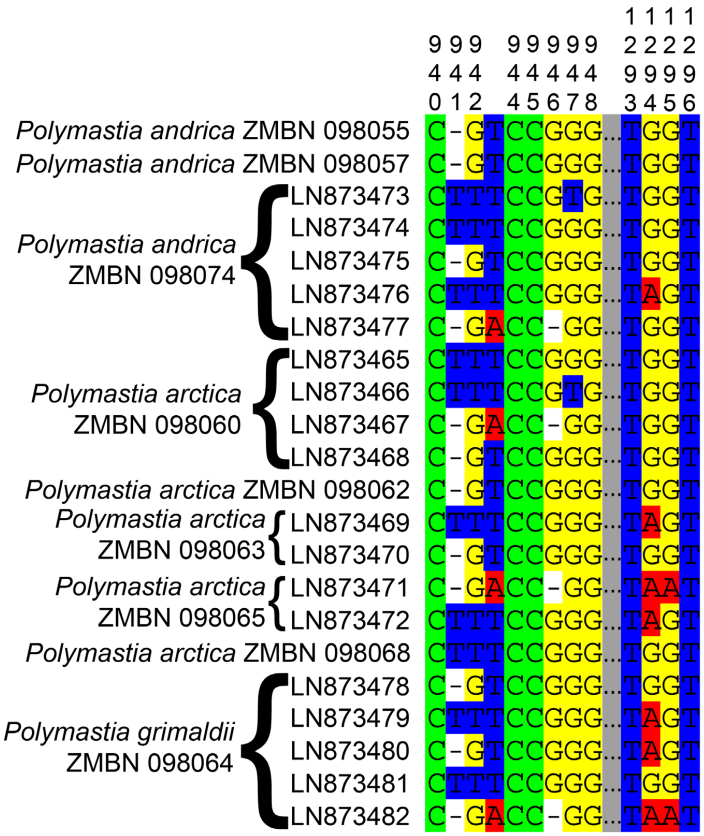

Figure 10. Fragment of the 28S rDNA alignment demonstrating intragenomic polymorphism in three species of *Polymastia* (direct sequences and clones shown).

in D1–D19 was estimated on four direct sequences (two from *P. andrica* and two from *P. arctica*) and 18 clones from five individuals (one of *P. andrica*, three of *P. arctica* and one of *P. grimaldii*). An intragenomic polymorphism was discovered in this fragment. Seven versions of D1–D19 were spread among the individuals of different species (**Paper III**: Figure 5; this synopsis: Figure 10). Meanwhile, the CO1 data from *P. andrica*, *P. arctica* and *P. grimaldii* were consistent, i.e. the sequences from the individuals of the same species were identical.

Intraspecific polymorphism of CO1 was revealed in *Polymastia boletiformis*. One individual of *P. boletiformis*, ZMBN 098047, differed from the sister species *Q. brevis* just by one nucleotide in this gene, while the other, ZMBN 098089, differed from *Q. brevis* by six nucleotides. These results primarily obtained by direct sequencing were also

confirmed by cloning of the PCR products. At the same time the 28S rDNA sequences obtained from seven *P. boletiformis*, including the two individuals with different CO1, were identical. Mismatch between the CO1 and 28S rDNA data was also revealed in *Polymastia invaginata*. Two individuals of this species, ZMBN 098093 and ZMBN 098094, had identical 28S rDNA, whereas individual ZMBN 098046 differed from them by two nucleotides. Conversely, CO1 of ZMBN 098046 and ZMBN 098093 were identical, while ZMBN 098094 differed from them by 19 nucleotides.

4.5. Homoplasy of the morphological characters in Polymastiidae

(**Paper III**)

All phylogenies based on morphology assumed the consistency of six characters: the presence/absence of radial growth pattern, basal surface, specialized basal cortex, exhalant papillae, oscula on the body surface and middle cortical layer of aquiferous cavities (see section 4.2 above and **Paper I**). Furthermore, the favoured scenario 2 assumed the consistency of the presence/absence of the regular main choanosomal skeleton and the radial vs. reticulate architecture of this skeleton. However, the tracing of the morphological characters along the molecular phylogenetic tree confirmed full consistency of only two characters. Acquisition of exhalant papillae and the loss of oscula on the body surface were assumed to be the synapomorphies of the polymastiid clade in the molecular phylogenies, while all other morphological characters appeared to be more or less homoplasious. The shift to radial growth pattern and the acquisition of basal surface and specialized basal cortex occurred independently in Clade III (in *Radiella sarsi* and *Radiella* cf. *sarsi*) and in two subclades of Clade I (in *Radiella hemisphaerica* and *Polymastia grimaldii*). Transformation of the radial architecture of the choanosomal skeleton to reticulate, along with acquisition of aquiferous cavities in the cortex took place in taxa belonging to three remote groupings: in *Weberella bursa* (*W. bursa* + *Polymastia* cf. *conigera* + *Sphaerotylus antarcticus*), in *Polymastia corticata* (the sister to Clade II in the 28S rDNA phylogeny and the taxon with unresolved relationships in the CO1 phylogeny) and in *Polymastia boletiformis* (*P. boletiformis* + *Q. brevis*). Furthermore, the molecular phylogenies indicated a secondary loss of the regular main choanosomal skeleton in *Q. brevis* (**Paper III**) as supported in morphology-based scenario 3, but rejected in scenario 2 (**Paper I**). Of other key events in the morphological evolution of Polymastiidae the acquisition of

ornamented exotyles occurred in all species of *Sphaerotylus* scattered in three remote branches, Clade II (three species), *S. antarcticus* and *S. borealis*, while the acquisition of a marginal spicule fringe took place in two remote clades, Clade III (inherited by all species) and Clade I (only in *Polymastia grimaldii* and *Radiella hemisphaerica* falling in the sister subclades).

4.6. Polymastiidae of the Nordic and Siberian Seas revisited with integrative taxonomy

(**Paper IV**)

4.6.1. Scope of the study

This faunistic inventory covered a large geographical area including the coastal waters from the Southern Scandinavia to the easternmost point of Russia and the deep-waters from the Norwegian and Iceland Seas in the south-west to the Chukchi Sea and adjacent regions of the Arctic Ocean in the north-east. We also compared the Nordic and Siberian polymastiids with the species from the British Isles, Canadian Atlantic Coast and some other regions. Revising the species we employed newly obtained morphological data as well as molecular data resulting from **Paper III**. Based on the molecular phylogenies, we accepted the abandonment of *Radiella*. Two species formerly placed in this genus, *R. sarsi* and *Radiella* sp. (described as *Spinularia njordi* sp. nov. in **Paper IV**), were transferred to *Spinularia* and one species, *R. hemisphaerica* was allocated to *Polymastia*. Other genera were retained as in the previous classification (Boury-Esnault 2002). Altogether 20 species and one OTU from six polymastiid genera were recorded in the area of the study. For each species we presented a detailed morphological description, defined the synapomorphies and autapomorphies in 28S rDNA and CO1 and reported the occurrence based on own and literature data. Furthermore, we provided a key for identification of the Nordic and Siberian polymastiids (**Paper IV**: Appendix). Finally, we discussed the distribution patterns of the polymastiids and compared them with the biogeographical data on other sponge families.

4.6.2. Species new to science

Two new polymastiid species, *Polymastia svenseni* sp. nov. and *Spinularia njordi* sp. nov., were erected predominantly based on their genetic apomorphies and

molecular phylogenies. A large population of *Polymastia svenseni* sp. nov. (designated as *Polymastia* sp. 1 in **Paper III**) was discovered in the coastal waters near Stavanger. In the 28S rDNA phylogenies *P. svenseni* was the sister to *Polymastia* sp. from the deep-sea area west of Bergen (designated as *Polymastia* sp. 2 in **Paper III**) sharing with the latter two synapomorphies and differing by two autapomorphies in this gene. In the CO1 phylogenies *P. svenseni*, *Polymastia* sp. and an unidentified *Polymastia* from the Canadian Atlantic Coast (designated as *Polymastia* sp. 3 in **Paper III** and not covered by **Paper IV**) formed a clade distinguished by nine synapomorphies from all other polymastiids. Inside this clade the Norwegian *Polymastia* sp. and the Canadian *Polymastia* sp. were sisters sharing three additional synapomorphies. The Norwegian *Polymastia* sp. was distinguished by two autapomorphies in CO1. Meanwhile, no morphological autapomorphies distinguishing *P. svenseni* and the Norwegian *Polymastia* sp. from other polymastiids could be defined. Moreover, the morphological affinities of these species were inconsistent with the molecular phylogenies. By its smooth surface and the presence of only two categories of spicules *P. svenseni* resembles *P. boletiformis*. On the contrary, by its radial main choanosomal skeleton and three-layered cortex *P. svenseni* resembles the type species of *Polymastia*, *P. mamillaris*, and also *P. andrica*, *P. arctica* and *P. grimaldii*. *Polymastia* sp. strongly resembles *P. andrica* by its external features, skeleton architecture and the presence of four categories of spicules including the non-ornamented exotyles reinforcing the cortex and the surface hispidation. *Polymastia* sp. may potentially be a species new to science, but the formal erection of this species was postponed until more material in addition to the single individual becomes available.

Spinularia njordi sp. nov. (designated as *Radiella* sp. in **Paper III**) was first discovered on the seamounts of Loki's Castle / Schultz Massive at the border between the Norwegian and Greenland Seas. Later additional material on this species came from Storegga, north-west of the Møre coast, Middle Norway. *S. njordi* is distinguished from all other polymastiids by a unique autapomorphy in 28S rDNA and from the congeners also by six other autapomorphies in this gene. Morphologically *S. njordi* resembles the type species of *Spinularia*, *S. spinularia*, by the encrusting growth pattern, the consequent absence of the basal cortex and the relatively small marginal fringe composed of the spicules of the same category as those forming the main choanosomal tracts. These features distinguish *S. njordi* and *S. spinularia* from other *Spinularia* spp. At the same time *S. njordi* differs from *S. spinularia* by the shaggy surface, the presence of an additional cortical layer made of intermediary monactines and the absence of trichodragmata with raphides in the choanosome. These features rather resemble *S. sarsi*. The presence of

trichodragmata with raphides was previously regarded as the apomorphy of *Spinularia*, while the presence of the specialized basal cortex was considered as the apomorphy of *Radiella* (Boury-Esnault 2002). However, the molecular phylogenies revealed the homoplasy of these characters. This assumption is also confirmed by the morphology of *S. australis* Lévi, 1993 from New Caledonia, which possesses both raphides and a specialized basal cortex.

4.6.3. Species new to local faunas

We expanded the list of the Scandinavian and Nordic sponges with three polymastiid species, *Polymastia andrica*, *P. bartletti* de Laubenfels, 1942 and *P. penicillus*. Before our study *Polymastia andrica* was only known from the type locality in the Gulf of St. Lawrence, Canadian Atlantic (de Laubenfels 1949). We recorded this species from Western and Northern Norway and from Svalbard based on the morphological comparison of the material from the type locality with similar sponges from other regions and on the genetic data from the Canadian and Nordic individuals. *P. andrica* is morphologically very similar to *P. mamillaris* and *P. arctica*, but differs from these two by the possession of an additional spicule category, the non-ornamented exotyles. Genetically *P. andrica* is related to *P. arctica* and morphologically distinct from *P. grimaldii*. These three species are clearly distinguished in CO1, while their 28S rDNA is very polymorphic with some identical gene versions found in the individuals from different species (see section 4.4 above).

Polymastia bartletti was previously only known from the type locality in the Foxe Bay (Canadian Atlantic) (de Laubenfels 1942). This species is morphologically very similar to the NE Atlantic *P. nivea* (Hansen, 1885) (regarded as a senior synonym of *P. euplectella* in **Paper IV**), but the genetic difference between these two is large (27 base pairs (bps) in CO1 and 60 bps in 28S rDNA, see Papers III and IV). An individual discovered by us in the coastal waters of Western Sweden could be identified either as *P. bartletti* or as *P. euplectella* based on morphology, while in the molecular phylogenies it appeared to be the sister to the Canadian *P. bartletti* sharing with the latter nine synapomorphies in CO1 and two synapomorphies in 28S rDNA and differing just by two bps in CO1 and four bps in 28S rDNA. Provisionally, we identified this Swedish sponge as *P.* cf. *bartletti*. Further studies on larger material are required to check whether the Canadian *P. bartletti* and the Swedish *P.* cf. *bartletti* are indeed conspecific or represent different species.

Polymastia penicillus is widely distributed around the British Isles and along the South European Coast (Boury-Esnault 1987). Previously it was often confused with *P. mamillaris*, until Morrow & Boury-Esnault (2000) clearly defined the differences between these species in the architecture of cortex and fine details of spicule shape. We identified as *P. penicillus* two individuals from the Swedish Western Coast based on the morphological comparison with the type material. Our identification was also confirmed by the identity of the partial 28S rDNA obtained from the Swedish sponges and an Irish *P. penicillus*. This was the first record of *P. penicillus* from the Scandinavian Coast.

4.6.4. Challenging cosmopolitanism

Among the polymastiid species previously recorded from the Nordic and Siberian Seas two species, *Spinularia sarsi* and *Tentorium semisuberites*, were supposed to have a cosmopolitan distribution. We challenged this concept based on molecular phylogenies. *T. semisuberites* originally described from Greenland (Schmidt 1870) was later reported from the various localities in the North Atlantic, North Pacific, Indian Ocean and Antarctic (for references see Introduction: section 1.2.5 above). We obtained molecular data from three morphologically very similar individuals of this species, from Svalbard, Western Norway and the Antarctic Weddell Sea. In the 28S rDNA phylogenies the Antarctic *T.* cf. *semisuberites* did not group together with *T. semisuberites* from the northern hemisphere (**Paper III**) differing from them by 42 bps. Consequently, we regarded the Antarctic individual as another species. Unfortunately, no CO1 was obtained from it. The Norwegian and Svalbard individuals differed by four bps in 28S rDNA and two bps in CO1. Further studies are required to check whether all *T. semisuberites* from the northern hemisphere are conspecific or represent different species.

The type localities of *S. sarsi* included such remote regions as Azores and Australia (Ridley & Dendy 1886). Later this species was also reported from the Arctic Ocean, Nordic Seas and Indian Ocean (for references see Introduction: section 1.2.5 above). We got molecular data from three morphologically indistinguishable individuals of *S. sarsi*, two from the Norwegian Sea and one from the Mozambiquean Coast. In the molecular phylogenies the Norwegian *S. sarsi* (with no genetic differences between the two individuals) and the Mozambiquean *S.* cf. *sarsi* were not sisters differing by eight bps in CO1 and 13 bps in 28S rDNA. We assumed that the Mozambiquean sponge represented another species. At the same time all individuals

from the North Atlantic and Arctic, morphologically similar to the type material from Azores, were provisionally regarded as *S. sarsi*. However, more molecular data on sponges from different regions are required to check whether they all are indeed conspecific.

For one more polymastiid species of the Nordic sponge fauna, *Spinularia spinularia*, the allegedly wide distribution was disputed. This species originally described from the British Isles (Bowerbank 1866), was later reported from Sweden, Norway and Greenland (see **Paper IV** for references). The assumed distribution of *S. spinularia* was extended after Stephens (1915) synonymized *Rhaphidorus setosus* Topsent, 1898 from Azores with this species. Examination of the type material of both *S. spinularia* and *R. setosus* and comparative material from Norway and Sweden revealed that the Azorean individual could be distinguished from the British and Scandinavian sponges by the shape of raphides, bearing umbrelliform or subspherical ornaments. Based on this difference we proposed to resurrect the Azorean species as *Spinularia setosa*. However, this proposal needs to be verified with the molecular data.

4.6.5. Patterns of sponge distribution in the Nordic Seas and Arctic Ocean

Among the polymastiids inhabiting the Nordic and Siberian Seas ten species (*Polymastia andrica*, *P. grimaldii*, *P. hemisphaerica*, *P. thielei*, *P. uberrima*, *Quasillina brevis*, *Sphaerotylus capitatus*, *Spinularia sarsi*, *Tentorium semisuberites* and *W. bursa*) were regarded as amphi-Atlantic boreoarctic, with distributions ranging from the Canadian Atlantic Coast and north-eastwards over the Nordic Seas and along the coasts of Greenland, Iceland, Scandinavia and Russia up to the Arctic Ocean. In the south-western parts of the area these species were mainly recorded at the depths below 100–200 m, while in the north-east most of them were registered both in the deep- and shallow-waters, except for *Polymastia* hemisphaerica and *Spinularia sarsi* recorded only in the deep-sea, deeper than 150 m and 300 m correspondingly, in all regions. The prevalence of the amphi-Atlantic boreoarctic species in the Nordic and Arctic faunas was earlier demonstrated for several other demosponge families, e.g. for Geodiidae Gray, 1867 (Cárdenas et al. 2013) and Theneidae Carter, 1883 (Cárdenas & Rapp 2012), and for hexactinellids, e.g. for Rossellidae Schulze, 1885 (Tabachnick & Menshenina 2007).

Four polymastiid species, *Polymastia arctica*, *P. nivea*, *Sphaerotylus borealis* and *Spinularia spinularia*, were regarded as NE Atlantic high-boreoarctic. *S. borealis* was

recorded from Iceland in the south-west to the eastern Kara Sea in the north-east. The records of *P. arctica* and *P. nivea* were limited to the Russian Coast of the Barents and White Sea and the Norwegian Coast, with the first species never recorded to the south-west from Northern Norway and the second species found up to Southern Norway. The allegedly wide distribution of *S. spinularia* was disputed (see section 4.6.4 above) and the confirmed records of this species were limited to the area between Ireland, East Greenland and Northern Norway. Atlantic high-boreoarctic species were also recorded among other demosponge families, e.g. Geodiidae (Cárdenas et al. 2013), Tetillidae Sollas, 1886 (Koltun 1966) and Theneidae (Cárdenas & Rapp 2012). We concluded that the Arctic sponge fauna was predominantly composed of the species dispersed from the Atlantic. A few early records of the sponge species distribution limited to the Arctic Ocean, e. g. *Cladorhiza arctica* Koltun, 1959, *Hemimycale rhodus* (Hentschel, 1929), and *Pseudosuberites sadko* Koltun, 1966, need verification on the additional material.

Two polymastiid species, *Polymastia boletiformis* and *P. penicillus*, being quite common in the European coastal waters, were regarded as the southern boreal component in the Scandinavian sponge fauna. We documented the northernmost occurrence of these species, Møre and Romsdal, Middle Norway for *P. boletiformis* and the British Isles and the Swedish Western Coast for *P. penicillus*. The dispersal of the southern boreal species to the Scandinavian Coast was also recorded for other sponge families, e.g. for calcareans of the family Clathrinidae Minchin, 1900 (Rapp 2006) and demosponges of the families Pachastrellidae Carter, 1875 and Theneidae (Cárdenas & Rapp 2012).

5. Discussion

5.1. Incongruence between the molecular and morphological phylogenies

The molecular phylogenies have challenged the applicability of morphological characters, most of which appeared to be highly homoplasious, for the natural classification of Polymastiidae. Homoplasy is a common problem in morphological taxonomy of the largest demosponge subclass Heteroscleromorpha (e.g. Cárdenas et al. 2011; Morrow et al. 2013). None of the evolutionary scenarios based on morphological evidence (**Paper I**) have been confirmed with the molecular data (**Paper III**). The only rational point resulting from these scenarios is the non-monophyly of *Polymastia*, but the groupings of *Polymastia* spp. proposed in **Paper I** are not consistent with the clades recovered in **Paper III**. Meanwhile, the assumption that the body in *Quasillina* and *Ridleia* is a hyper-developed papilla, as suggested by the morphology-based scenario 3, seems quite likely. Based on this assumption and the molecular phylogenies, where *Quasillina* grouped with papilla-bearing polymastiids, we can regard the presence of exhalant papillae and the absence of oscula on the body surface as consistent characters, at least for the set of taxa in **Paper III**, although no molecular data on *Ridleia* are available so far.

5.2. Is the Polymastiidae monophyletic?

Although the molecular phylogenies reconstructed by us for 34 taxa have supported the monophyly of the family Polymastiidae and this hypothesis coincides with the results from most other studies (e.g. Morrow et al. 2012; 2013; Redmond et al. 2013; based on much smaller sets of taxa though), the alternative hypothesis assuming the non-monophyly of this family still cannot be rejected. For the moment there are no molecular data (at least no credible ones) on *Pseudotrachya* spp. and *Polymastia umbraculum*, the taxa lacking papillae, but currently regarded as polymastiids. The phylogenies recovered by Nichols (2005), where Polymastiidae was not monophyletic, with *Pseudotrachya* sp. and *Polymastia* sp. falling remotely from other taxa, were probably artefacts resulting from inaccurate taxonomic identification (see section 1.2.4 above). Furthermore, there are no molecular data on *Aaptos papillata*, which possesses papillae, cortical and choanosomal skeleton as in polymastiids, but is at present placed in Suberitidae. Hence, two questions on the

evolutionary history of Polymastiidae remain unanswered: 1) whether the papillae were acquired by the polymastiids or by the common ancestor of Polymastiidae and some other families, and 2) whether the secondary loss of the papillae occurred during the polymastiid evolution.

5.3. Non-monophyly of traditional polymastiid genera and background for a new classification

All four polymastiid genera represented by more than one species in the molecular phylogenies, i.e. *Polymastia*, *Radiella*, *Sphaerotylus* and *Tentorium*, appeared to be non-monophyletic (**Paper III**). The non-monophyly of *Polymastia* and *Sphaerotylus* was already assumed based on the morphological data (Papers I and II). Meanwhile, the molecular phylogenies have recovered three strongly supported clades, each including the type species of the certain genus: Clade I with the type species of *Polymastia*, *P. mamillaris*, five other species allocated to *Polymastia* in earlier classifications and one species transferred from *Radiella*, Clade II with the type species of *Sphaerotylus*, *S. capitatus*, one new species and one OTU, and Clade III with the type species of *Spinularia*, *S. spinularia*, one new species and one species transferred from *Radiella*, which is probably a complex of two species. We also assume that our new species *Polymastia svenseni*, along with its unidentified sibling, the Norwegian *Polymastia* sp., are related to Clade I, that is evident from the 28S rDNA phylogeny, although causes some doubt considering the CO1 phylogeny. The clades revealed may be used as reference points in future classification of the Polymastiidae. However, no morphological synapomorphies can at present be defined for these clades. Moreover, about half of the species studied do not fall into any of the recovered clades. Thus, for the time being, no satisfactory classification of Polymastiidae can be proposed. Based on our phylogenetic reconstructions (**Paper III**) and taxonomic revisions (Papers II and IV) we can only present a provisional list of the polymastiid genera, provide them with emended diagnoses and indicate their species content with the monophyletic groups, where present, and molecular data available (see Appendix 4). Three main emendations from the currently accepted classification (Boury-Esnault 2002; Van Soest et al. 2016) are proposed: addition of *Koltunia* (erected in **Paper II**), abandonment of *Radiella* (proposed in Papers III–IV) and resurrection of *Suberitechinus* (proposed in **Paper II**). Hence, this list comprises 16 genera.

5.4. Inconsistence between the nuclear and mitochondrial gene phylogenies

Inconsistence between the phylogenies recovered from different molecular datasets is a well-known phenomenon thoroughly discussed at the example of the early metazoan evolution and the origin of sponges (Philippe et al. 2011; Wörheide et al. 2012; Dohrmann & Wörheide 2013; Nosenko et al. 2013; see details in Introduction: section 1.1.4 above). Nuclear and mitochondrial genes as well as coding and non-coding genes have unequal evolutionary rates and different genealogical histories. This may explain the most prominent conflict between our CO1 and 28S rDNA phylogenies concerning the relationships of the pair *Polymastia boletiformis* + *Quasillina brevis* with other clades. The multi-gene datasets undoubtedly may overcome such conflicts between the single-gene signals.

On the other hand, the position of *P. boletiformis* + *Q. brevis* in the CO1 trees could be affected by very low resolution leading to Clade I. Likewise, unresolved relationships of *Polymastia corticata* along with weakly supported grouping of *Sphaerotylus antarcticus* with Clade II in the CO1 trees were obviously due to low resolution and hence to a weak phylogenetic signal provided by our CO1 data. To reconstruct the CO1 phylogeny of Polymastiidae we used so called "Folmer's" barcoding region successfully applied to recover the phylogenies of two large sponge families, Geodiidae (Cárdenas et al. 2010) and Halichondriidae (Erpenbeck et al. 2012a). However, in the polymastiids the variation of this region was evidently too low and may therefore have caused inconsistencies between the CO1 and 28S rDNA phylogenies and also hindered the separation of the species in Clades II and III based on CO1 alone, while these species were otherwise successfully separated by the 28S rDNA data. A similar problem with the "Folmer's" region was reported for some other sponge families, e.g. Lubomirskiidae (Schröder et al. 2003), Clionaidae (Ferrario et al. 2010) and Irciniidae Gray, 1867 (Pöppe et al. 2010). To overcome this problem sequencing of an additional downstream region of the CO1 gene providing more variability was recommended (Erpenbeck et al. 2006, Sponge Barcoding Project at http://www.palaeontologie.geo.uni-muenchen.de/SBP/).

5.5. Intraspecific and intragenomic polymorphism: possible reasons

Intraspecific polymorphism observed in *Polymastia boletiformis* and *P. invaginata* represents another example of inconsistence between the nuclear and mitochondrial gene data, when the individuals of the same species may exhibit identical 28S rDNA, but fairly different CO1 and vice versa. This example may probably be regarded as an artefact caused by the insufficient phylogenetic signal in the sequenced CO1-region as suggested above. But some other explanation is definitely required for the case of *P. andrica*, *P. arctica* and *P. grimaldii*, which are clearly distinguished in morphology and in CO1, but exhibit an intragenomic polymorphism in 28S rDNA, with some individuals of different species possessing the same versions of this gene and vice versa the individuals of the same species having different versions. We assume that this example may indicate incomplete lineage sorting in closely related sponge species and their populations. For instance, each lineage may carry one unique version of CO1, but several versions of 28S rDNA, if its ancestor was polymorphic by this gene, and vice versa. When further divergence of the lineages takes place, some gene versions inherited from the polymorphic ancestor may be lost owing to genetic drift or selection (Rogers & Gibbs 2014). Another explanation of the revealed intragenomic polymorphism may be a gene flow through hybridization between the sibling species, but this assumption requires more thorough studies.

5.6. Problems in sponge biogeography at the example of Polymastiidae

Two big and well-known problems in biogeography of sponges are alleged cosmopolitanism (Boury-Esnault et al. 1993; Klautau et al. 1999) and disjunct distribution of some species (Hooper 1994). The cosmopolitanism or, broadly speaking, any strangely wide distribution often results from the lack of data on type material to be compared with the individuals from non-type localities or from the overlooking of fine morphological details distinguishing the sponges from different regions, e.g. the details of spicule shape visible only under SEM could not be observed in early studies. Among the polymastiids an alleged cosmopolitan *Polymastia mamillaris* (e.g. see the list of its records by Topsent 1900) appeared to be a complex of at least four species revealed after a careful morphological comparison between the type and other material, *P. mamillaris* limited to the Swedish Western Coast (Morrow & Boury-Esnault 2000) and Southern Norway (**Paper IV**), *P. penicillus* widely distributed along the European coasts up to the British Isles (Morrow & Boury-Esnault 2000) and Sweden (**Paper IV**) in the north, *P. arctica*

limited to the Northern Norway and NW Russia (Plotkin & Boury-Esnault 2004; **Paper IV**) and *P. andrica* originally erected by de Laubenfels (1949) for the Canadian sponges earlier identified as *P. mamillaris* by Whiteaves (1874) and Lambe (1896), and reported from the Norwegian Coast and Svalbard by us (**Paper IV**). The validity of these four species is now confirmed with molecular data (**Paper III**). Furthermore, based on the fine distinctions in spicule shape, we proposed to resurrect a deep-water Azorean species *Spinularia setosa* from a synonym of the British-Scandinavian *Spinularia spinularia* (**Paper IV**), although this needs verification with genetic data. At the same time we "lumped" the Nordic *Quasillina richardi* Topsent, 1913 with the British *Q. brevis* because the assumed difference between them (small spicules being bent in the former) appeared to be unstable, with no correlation to geography. Molecular data on *Quasillina* spp. from different regions are required to follow up on this issue.

Another reason leading to the concept of cosmopolitan or wide distribution may be the existence of morphologically indistinguishable (cryptic) species, which can be revealed only with molecular tools (Klautau et al. 1999; Wörheide et al. 2002; Blanquer & Uriz 2007). Applying these tools we revealed that each of two presumably cosmopolitan polymastiids, *Spinularia sarsi* and *Tentorium semisuberites*, represents at least two species, one in the northern and the other in the southern hemisphere (**Paper III**), but still the numerous records of *S. sarsi* and *T. semisuberites* in the Northern Atlantic and Arctic call for further studies. Furthermore, we revealed a long genetic distance between the two morphologically indistinguishable Atlantic species, *Polymastia bartletti* and *P. nivea* (**Paper III**).

The other problem in biogeography, the disjunct distribution, may result from inaccurate taxonomic identification as in the case of the cosmopolitanism, but more often it just pinpoints the "blank" areas where the fauna remains unexplored. In our study the disjunct distribution was reported for *P. bartletti*, with its only records along the Canadian Atlantic Coast and at the Swedish Western Coast (**Paper IV**). Data on similar sponges from the area in-between are required to conclude whether the small genetic difference between the Canadian and Swedish individuals indicate two separate species or just an intraspecific polymorphism. The most discussable cases of the disjunct distribution are represented by the bipolar species. Strong morphological similarities, with practically no distinctions, between two polymastiids, *Sphaerotylus antarcticus* from the Southern Ocean and *S. borealis* from the Nordic and Arctic Seas, led to an assumption about a single species with bipolar distribution (Koltun 1976), but based on molecular phylogenies where these species fell in remote clades, this assumption was rejected (**Paper III**).

6. Conclusions

- Our study has built a solid background for further research on the demosponge phylogeny providing a much larger set of molecular data on Polymastiidae, one of the key demosponge families, than ever obtained before. Both 28S rDNA and CO1 sequences are obtained from 25 polymastiid species (including three new species erected by us) and five OTUs, of which the data on 23 species and all the five OTUs are fairly new (Table 5).

- We have presented the first comprehensive phylogenies of the Polymastiidae. All reconstructed phylogenies have showed the polyphyly of several polymastiid genera, suggesting that the widely accepted classification of the family needs a thorough revision. It is concluded that the phylogenies based on morphology are in a strong conflict with the molecular phylogenies and, accordingly, the majority of assumed morphological synapomorphies appear to be highly homoplasious. In the molecular phylogenies we have recovered several strongly supported clades, which may be used as the reference points for building a new classification. In order to determine the morphological synapomorphies of these clades a re-interpretation of the currently used characters and a selection of additional characters are required.

- Nevertheless, we have demonstrated the usefulness of multiple morphological characters for the distinction of some species. Based on morphological data we have revised four problematic polymastiid genera, erected three new species and one new genus (*Koltunia*) for the previously known species, resurrected one genus (*Suberitechinus*) from synonym and proposed several re-allocations.

- Applying integrative taxonomy (based on both morphological and molecular data) we have explored the polymastiid fauna of the Nordic and Siberian Seas, proposed the abandonment of one genus (*Radiella*), erected two new species and resurrected one species from synonym. We have also questioned the cosmopolitanism of two species and discussed the patterns of sponge distribution in the North Atlantic and Arctic. Finally we have presented a key for identification of the Nordic and Siberian polymastiids.

7. Future perspectives

The molecular data obtained by us will be hopefully included in the further studies on the demosponge phylogeny and shed more light on the relationships of the monofamilian order Polymastiida with other orders. As proposed above, the data on *Pseudotrachya* spp., *Polymastia umbraculum* and *Aaptos papillata* will be required in order to verify the monophyly of Polymastiidae and to reconstruct its early evolution. Establishment and development of a new classification for the polymastiids should preferably be based on phylogenies employing much larger set of taxa than covered in our study in order to verify the support for the clades revealed by us and to recover more clades. Data on any additional species will be useful, but we recommend concentrating the efforts on the taxa with the most uncommon morphological traits, e.g. the insufficiently studied deep-sea genera with extraordinary spicules (*Acanthopolymastia*, *Astrotylus*, *Trachyteleia* and *Tylexocladus*) and an unordinary skeleton architecture (*Ridleia*). Furthermore, it seems reasonable to focus on the various species of *Polymastia*, the largest and the worst defined polymastiid genus. Comparing the diversity of polymastiids in different regions with the amount of molecular data available from them, we propose focusing future efforts on the Pacific Ocean. This region hosts a large number of endemic polymastiids with unique features, but, unfortunately, the molecular data obtained from the Pacific species are much poorer in comparison with other regions.

Regarding the perspectives of the molecular markers applied for reconstruction of the polymastiid phylogeny, we assume that our comprehensive sequence dataset of the B10–E19 region of 28S rDNA represents a useful framework for future studies. Furthermore, the promising data on 18S rDNA obtained from six species and two OTUs by Redmond et al. (2013) inspire sequencing of this marker from more polymastiid taxa. Concerning the utility of the barcoding region of CO1, we would recommend complementing the data on this marker with the sequences of an additional downstream region as proposed by Erpenbeck et al. (2006) in order to overcome the inconsistencies in the CO1 phylogenies, which we have faced. Standardizing of the DNA information on the polymastiid species by combining the three molecular markers, the B10–E19 region of 28S rDNA, complete 18S rDNA and the extended barcoding region of CO1, will facilitate the comparison between the phylogenies reconstructed under different studies on this and other sponge families and increase the credibility of the results. Furthermore, the phylogenies based on the

standard molecular markers definitely should be verified on the multi-gene datasets including nuclear protein-coding genes, e.g. ALG11 proposed by Belinky et al. (2012) may be a good starting point.

Since the separation of some polymastiid species based on morphological characters is difficult or impossible, there is a great need for a practical molecular tool that should be universal and easy for use, not demanding much of sequencing efforts. We suppose that B10–C1, one of the three fragments of 28S rDNA employed in our study, could be a good candidate for such a tool, providing much higher variability than the traditional "Folmer's" region of CO1 and being applicable for most species. However, applying this fragment we must be aware of intraspecific and intragenomic polymorphism, which in some cases may hinder identification, e.g. the case of *Polymastia andrica*, *P. arctica* and *P. grimaldii*. There is no other way to get over this hindrance than to apply integrative taxonomy employing data on two or more molecular markers and morphological characters. Gene polymorphism revealed in some polymastiids definitely needs more comprehensive exploration than we managed to perform. Accumulation of clone libraries of the 28S rDNA fragments, which we studied, and overlapping fragments, obtaining for each species the data on individuals from different regions and sequencing of ITS will shed more light on this phenomenon.

Regarding the perspectives in the biogeography of Polymastiidae, there is still a great need for additional records of species from different localities, even within the region thoroughly studied by us, i.e. the Nordic and Siberian Seas, in order to make judgements on the patterns of distribution and dispersal more credible. Rich sponge collections still remaining completely or partially unstudied, e.g. the collections of most Norwegian and Swedish natural history museums, may be a good source of new records, while fresh material is continually coming from the expeditions arranged each year by different institutions in Norway and other countries. It is preferable that each new record is documented with both morphological and molecular data, at least the sequence of the B10–C1 fragment of 28S rDNA. The new techniques make the DNA extraction with the subsequent PCR of the desired taxonomic marker possible even from age-old sponge samples (Erpenbeck et al. 2016). Hence, the importance of museum collections is still great, while the potential opportunity to get molecular data from most type specimens will secure an effective quality control of taxonomic identification.

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Source of data

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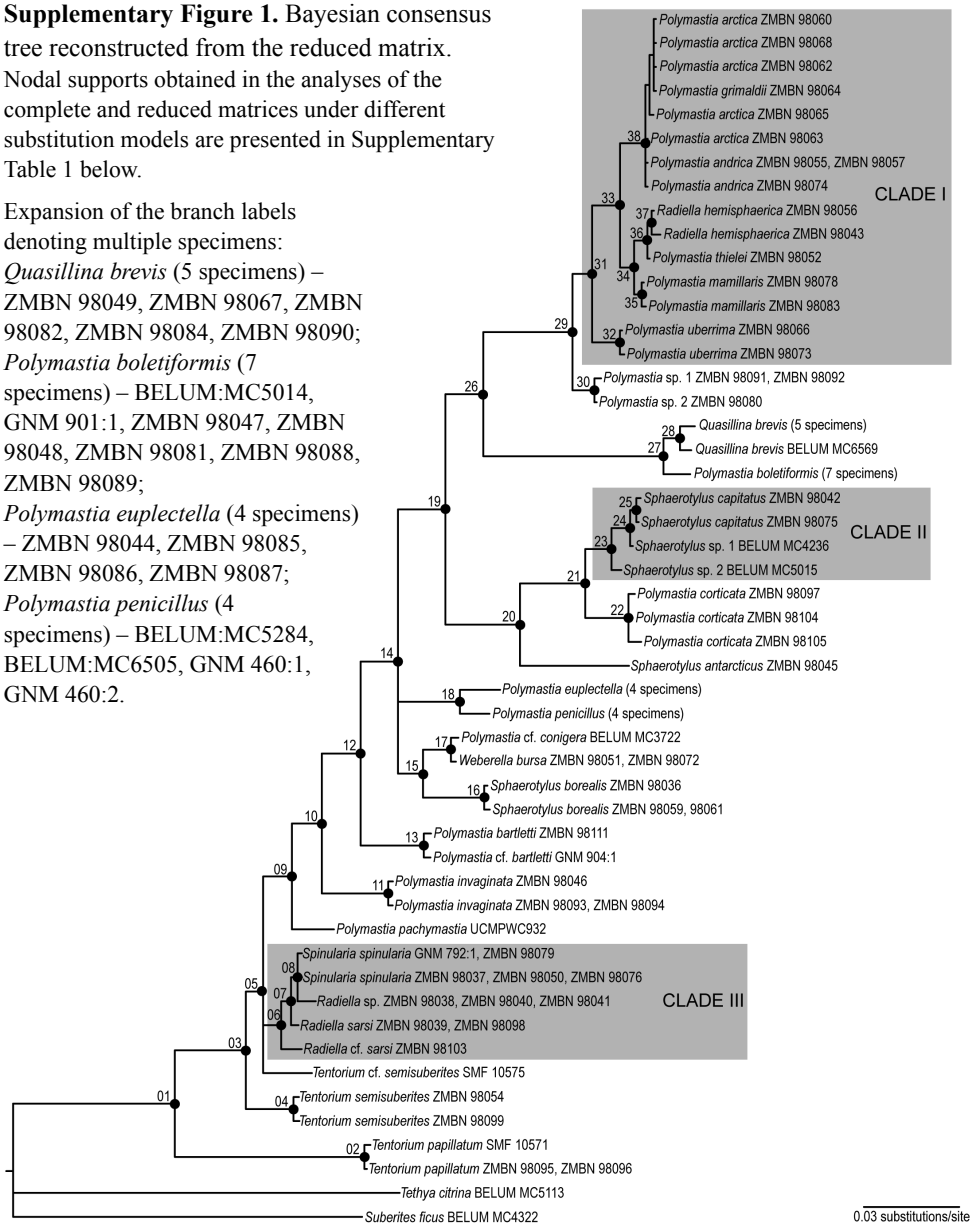
Appendices

Appendix 1

Results of the phylogenetic analyses of the 28S rDNA matrices (data on Polymastiidae) obtained under different substitution models.

Supplementary Figure 1. Bayesian consensus tree reconstructed from the reduced matrix. Nodal supports obtained in the analyses of the complete and reduced matrices under different substitution models are presented in Supplementary Table 1 below.

Expansion of the branch labels denoting multiple specimens: *Quasillina brevis* (5 specimens) – ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090; *Polymastia boletiformis* (7 specimens) – BELUM:MC5014, GNM 901:1, ZMBN 98047, ZMBN 98048, ZMBN 98081, ZMBN 98088, ZMBN 98089; *Polymastia euplectella* (4 specimens) – ZMBN 98044, ZMBN 98085, ZMBN 98086, ZMBN 98087; *Polymastia penicillus* (4 specimens) – BELUM:MC5284, BELUM:MC6505, GNM 460:1, GNM 460:2.



Appendix 1 (continued)

Supplementary Table 1. Support values for the nodes shown in the consensus tree (Supplementary Figure 1).

Models named as in PHASE 2.0 (www.bioinf.manchester.ac.uk/resources/phase/index.html).
Nodes corresponding to the main clades are highlighted with grey.
BPP – Bayesian posterior probabilities (for each PHASE analysis two values corresponding to two runs are given).
ML-BS – Maximum likelihood bootstrap supports in percent.

Matrix	**Complete**						**43 ambiguously aligned sites excluded**					
Model	**RNA16B+G +I, REV+G+I**		**RNA 16C +G, REV +G**	**RNA 16A +G, REV +G**	**REV+G+I (no partitioning)**		**RNA16B+G +I, REV+G+I**		**RNA 16C +G, REV +G**	**RNA 16A +G, REV +G**	**REV+G+I (no partitioning)**	
Support values	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**
Million iterations	**30**		**15 15**		**15**		**20**		**10 30**		**15**	
Node 01	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 02	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 03	0.99	69	1 1	84	1	63	1	77	1 1	87	1	70
Node 04	1	96	1 1	99	1	92	1	95	1 1	98	1	90
Node 05	0.60	-	0.80 0.82	62	0.68	-	0.62	-	0.82 0.83	64	0.73	-
Node 06 (Clade III)	1	78	1 1	86	1	79	1	84	1 1	89	1	81
Node 07	1	77	0.99 0.99	73	1	79	1	83	0.99 1	77	1	79
Node 08	0.93	81	0.95 0.96	74	0.96	79	0.88	79	0.93 0.94	73	0.96	75
Node 09	1	76	1 1	86	1	75	1	77	1 1	86	1	77
Node 10	1	85	1 1	95	1	90	1	81	1 1	91	1	84
Node 11	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 12	1	86	1 1	89	1	89	1	92	1 1	93	1	92
Node 13	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 14	1	85	1 1	86	1	80	1	81	1 1	84	1	72
Node 15	0.95	-	0.93 0.91	-	0.84	-	0.88	-	0.95 0.83	-	0.58	-
Node 16	1	99	1 1	100	1	100	1	99	1 1	99	1	100
Node 17	0.95	-	0.93 0.91	-	0.83	-	0.96	-	0.98 0.87	-	0.61	-
Node 18	1	100	1 1	100	1	100	1	100	1 1	100	1	100

Appendix 1 (continued)

Supplementary Table 1 (continued)

Matrix	Complete						43 ambiguously aligned sites excluded					
Model	RNA16B+G +I, REV+G+I		RNA 16C +G, REV +G	RNA 16A +G, REV +G	REV+G+I (no partitioning)		RNA16B+G +I, REV+G+I		RNA 16C +G, REV +G	RNA 16A +G, REV +G	REV+G+I (no partitioning)	
Support values	BPP	ML-BS	BPP	ML-BS	BPP	ML-BS	BPP	ML-BS	BPP	ML-BS	BPP	ML-BS
Million iterations	30		15 15		15		20		10 30		15	
Node 19	0.99	73	1 1	67	1	66	0.99	68	1 1	60	1	59
Node 20	1	97	1 1	99	1	98	1	98	1 1	99	1	99
Node 21	1	100	1 1	100	1	100	1	100	1 1	99	1	100
Node 22	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 23 (Clade II)	1	96	1 1	91	1	95	1	95	1 1	89	1	92
Node 24	1	97	1 1	96	1	97	1	97	1 1	95	1	95
Node 25	0.94	56	0.87 0.87	53	0.97	60	1	78	0.99 0.99	77	1	78
Node 26	1	67	1 1	72	0.99	61	1	64	0.99 0.99	69	0.98	59
Node 27	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 28	0.94	99	0.99 0.99	99	0.95	99	0.89	99	0.97 0.97	100	0.93	99
Node 29	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 30	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 31 (Clade I)	0.94	75	0.95 0.95	72	0.97	73	0.94	77	0.93 0.94	73	0.97	77
Node 32	1	100	1 1	100	1	100	1	100	1 1	100	1	100
Node 33	1	95	1 1	94	1	94	1	95	1 1	94	1	93
Node 34	0.98	62	0.99 0.98	76	0.99	82	0.97	61	0.98 0.98	73	0.98	77
Node 35	1	99	1 1	98	1	99	0.99	97	0.99 1	97	0.99	96
Node 36	1	99	1 1	99	1	100	1	98	1 1	98	1	100
Node 37	0.97	86	0.98 0.98	86	0.96	66	0.96	85	0.97 0.98	81	0.96	67
Node 38	1	99	1 1	100	1	100	1	99	1 1	99	1	100

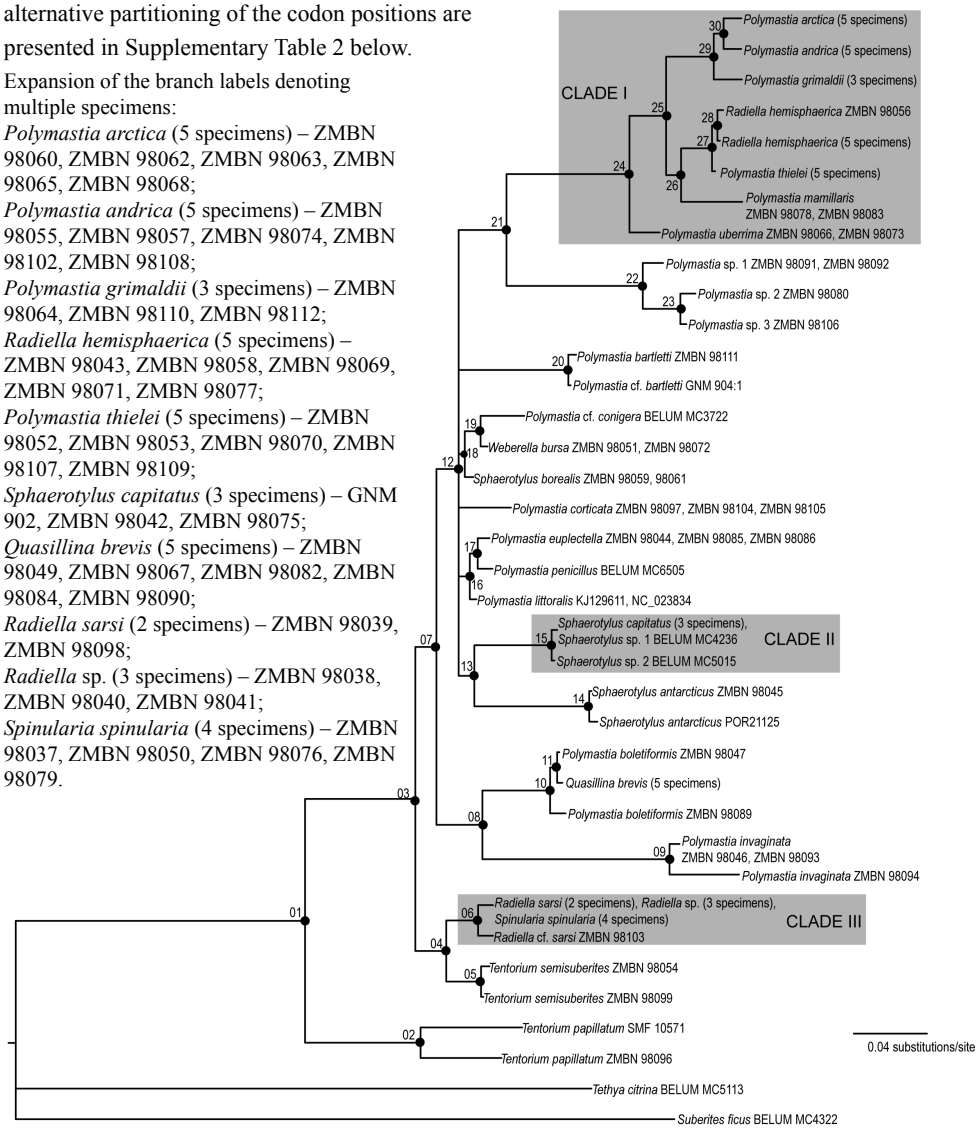
Appendix 2

Results of the phylogenetic analyses of the 28S rDNA matrices (data on Polymastiidae) obtained under different substitution models.

Supplementary Figure 2. Bayesian consensus tree reconstructed under GTR+G+I.

Nodal supports obtained in the analyses with alternative partitioning of the codon positions are presented in Supplementary Table 2 below.

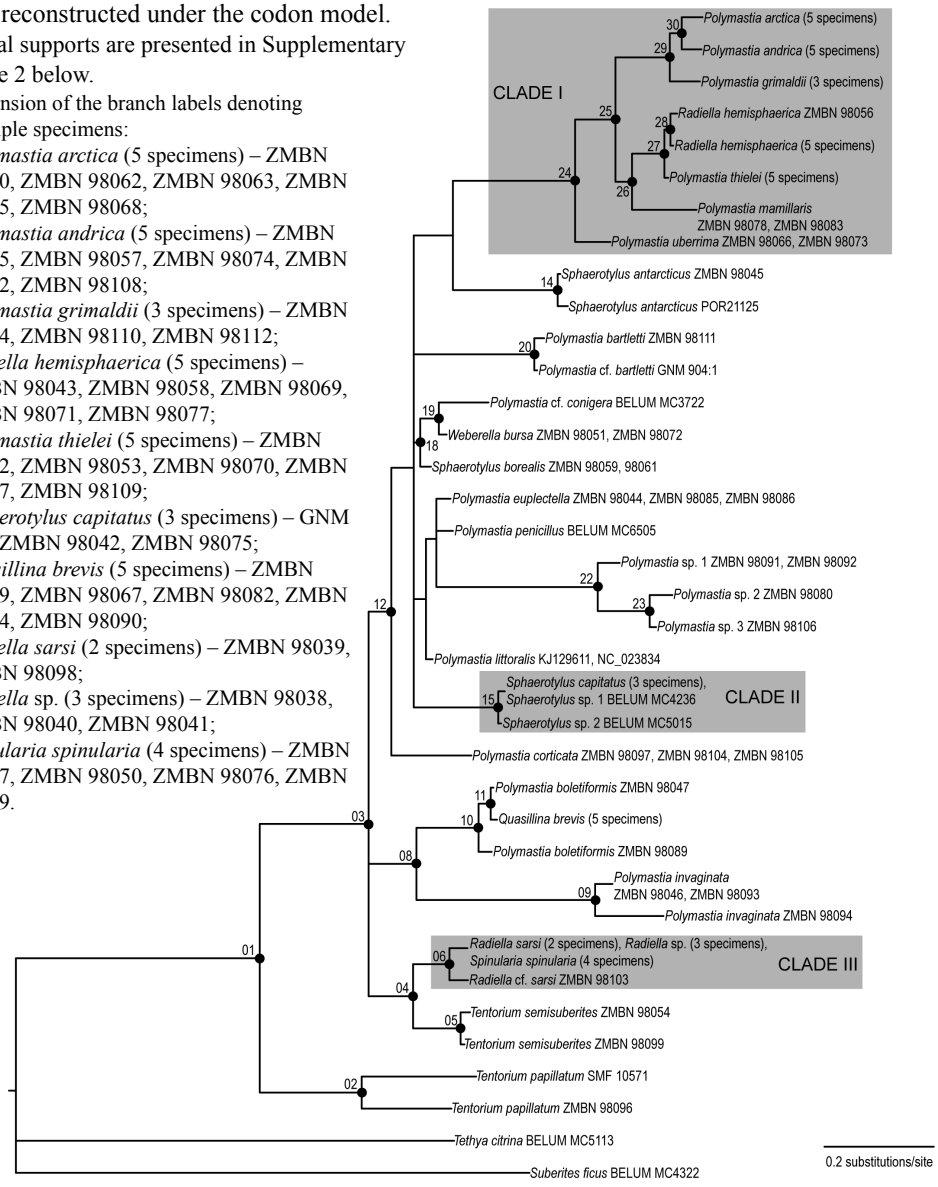
Expansion of the branch labels denoting multiple specimens:
Polymastia arctica (5 specimens) – ZMBN 98060, ZMBN 98062, ZMBN 98063, ZMBN 98065, ZMBN 98068;
Polymastia andrica (5 specimens) – ZMBN 98055, ZMBN 98057, ZMBN 98074, ZMBN 98102, ZMBN 98108;
Polymastia grimaldii (3 specimens) – ZMBN 98064, ZMBN 98110, ZMBN 98112;
Radiella hemisphaerica (5 specimens) – ZMBN 98043, ZMBN 98058, ZMBN 98069, ZMBN 98071, ZMBN 98077;
Polymastia thielei (5 specimens) – ZMBN 98052, ZMBN 98053, ZMBN 98070, ZMBN 98107, ZMBN 98109;
Sphaerotylus capitatus (3 specimens) – GNM 902, ZMBN 98042, ZMBN 98075;
Quasillina brevis (5 specimens) – ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090;
Radiella sarsi (2 specimens) – ZMBN 98039, ZMBN 98098;
Radiella sp. (3 specimens) – ZMBN 98038, ZMBN 98040, ZMBN 98041;
Spinularia spinularia (4 specimens) – ZMBN 98037, ZMBN 98050, ZMBN 98076, ZMBN 98079.



Appendix 2 (continued)

Supplementary Figure 3. Bayesian consensus tree reconstructed under the codon model. Nodal supports are presented in Supplementary Table 2 below.

Expansion of the branch labels denoting multiple specimens:
Polymastia arctica (5 specimens) – ZMBN 98060, ZMBN 98062, ZMBN 98063, ZMBN 98065, ZMBN 98068;
Polymastia andrica (5 specimens) – ZMBN 98055, ZMBN 98057, ZMBN 98074, ZMBN 98102, ZMBN 98108;
Polymastia grimaldii (3 specimens) – ZMBN 98064, ZMBN 98110, ZMBN 98112;
Radiella hemisphaerica (5 specimens) – ZMBN 98043, ZMBN 98058, ZMBN 98069, ZMBN 98071, ZMBN 98077;
Polymastia thielei (5 specimens) – ZMBN 98052, ZMBN 98053, ZMBN 98070, ZMBN 98107, ZMBN 98109;
Sphaerotylus capitatus (3 specimens) – GNM 902, ZMBN 98042, ZMBN 98075;
Quasillina brevis (5 specimens) – ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090;
Radiella sarsi (2 specimens) – ZMBN 98039, ZMBN 98098;
Radiella sp. (3 specimens) – ZMBN 98038, ZMBN 98040, ZMBN 98041;
Spinularia spinularia (4 specimens) – ZMBN 98037, ZMBN 98050, ZMBN 98076, ZMBN 98079.



Appendix 2 (continued)

Supplementary Table 2. Support values for the nodes shown in the consensus trees (Supplementary Figures 2–3).

Nodes corresponding to the main clades are highlighted with grey.
BPP – Bayesian posterior probabilities (for each PHASE analysis two values corresponding to two runs are given).
ML-BS – Maximum likelihood bootstrap supports in percent.

Model	**GTR+G+I**						**Codon**
Partitions	**No partitions**		**Two: codon pos. 1+2, codon pos. 3**		**Three: codon pos. 1, codon pos. 2, codon pos. 3**		**No partitions**
Analysis	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**
Million iterations	**15**		**10**		**25**		**43**
Node 01	1	58	1	74	1	77	1
Node 02	1	95	1	98	1	99	1
Node 03	0.97	-	0.95	-	0.95	-	1
Node 04	1	89	1	87	0.99	86	1
Node 05	1	96	1	99	1	98	1
Node 06 (Clade III)	1	98	1	99	1	99	1
Node 07	0.59	-	0.76	-	0.70	-	-
Node 08	0.98	51	0.99	61	0.96	61	0.99
Node 09	1	80	1	84	1	86	1
Node 10	1	98	1	99	1	99	1
Node 11	0.86	99	0.70	99	0.72	99	0.95
Node 12	0.98	-	0.94	-	0.97	-	0.92
Node 13	0.79	-	0.71	53	0.75	57	-
Node 14	1	100	1	100	1	100	1
Node 15 (Clade II)	1	100	1	100	1	100	1
Node 16	0.56	-	0.63	-	0.64	-	-
Node 17	0.63	-	0.68	-	0.69	-	-
Node 18	0.89	-	0.87	-	0.88	-	0.69
Node 19	1	88	1	88	1	86	1
Node 20	1	100	1	100	1	100	1
Node 21	0.74	-	0.70	-	0.78	-	-
Node 22	1	91	1	83	1	86	1
Node 23	1	97	1	91	1	94	1
Node 24 (Clade I)	1	99	1	100	1	100	1
Node 25	0.99	90	0.99	93	0.99	93	1
Node 26	0.78	72	0.83	81	0.81	81	0.56
Node 27	1	85	1	88	1	88	1
Node 28	0.70	59	0.80	63	0.70	61	0.76
Node 29	1	100	1	100	1	100	1
Node 30	0.87	77	0.82	68	0.81	68	0.86

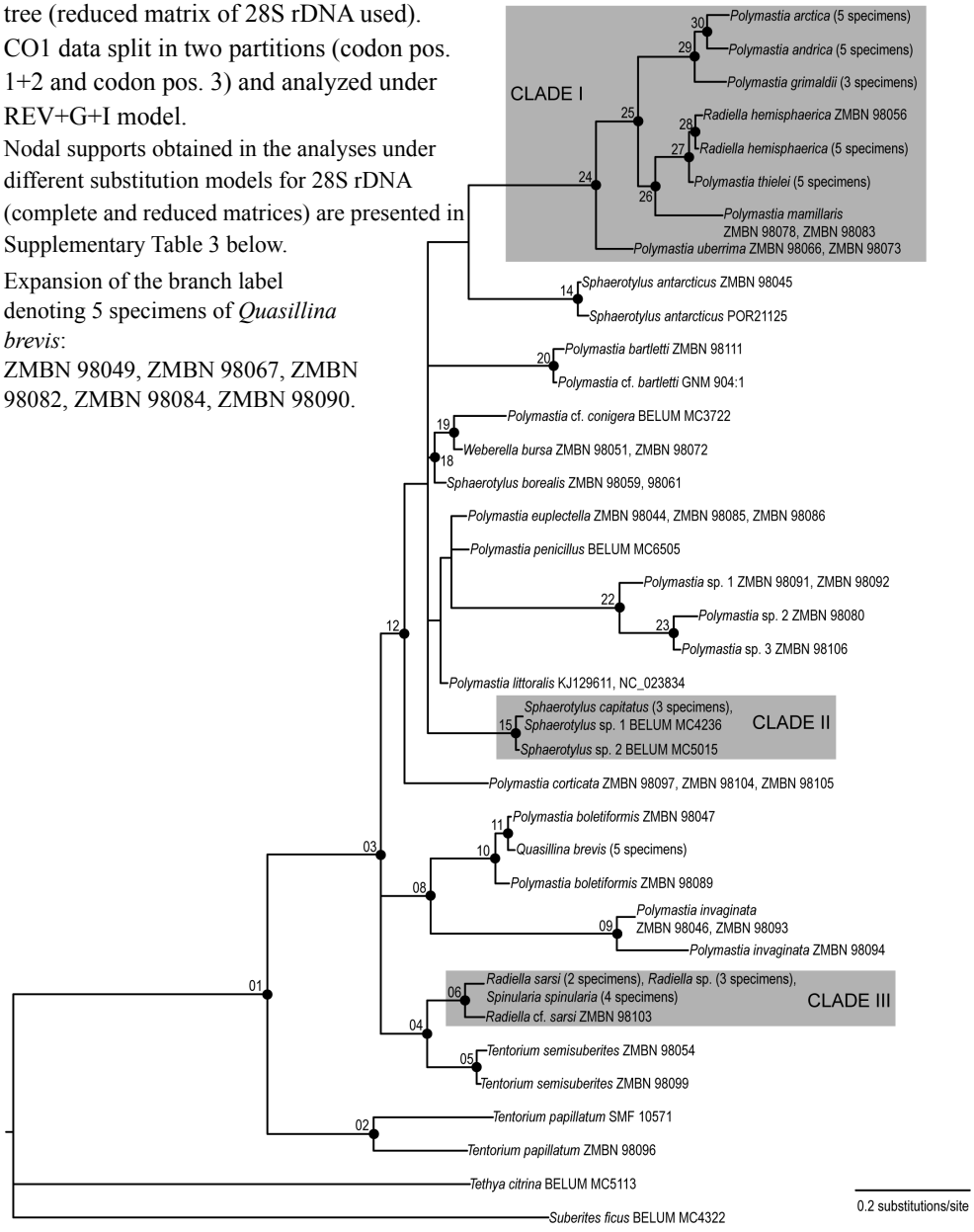
Appendix 3

Results of the phylogenetic analyses of of the concatenated matrices 28S rDNA + CO1 (data on Polymastiidae) obtained under different substitution models.

Supplementary Figure 4. Bayesian consensus tree (reduced matrix of 28S rDNA used). CO1 data split in two partitions (codon pos. 1+2 and codon pos. 3) and analyzed under REV+G+I model.

Nodal supports obtained in the analyses under different substitution models for 28S rDNA (complete and reduced matrices) are presented in Supplementary Table 3 below.

Expansion of the branch label denoting 5 specimens of *Quasillina brevis*: ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090.



Appendix 3 (continued)

Supplementary Table 3. Support values for the nodes shown in the consensus tree (Supplementary Figure 4).

Models named as in PHASE 2.0 (www.bioinf.manchester.ac.uk/resources/phase/index.html).
CO1 data split in two partitions (codon pos. 1+2 and codon pos. 3) and analyzed under REV+G+I model.
Nodes corresponding to the main clades are highlighted with grey.
BPP – Bayesian posterior probabilities (for each PHASE analysis two values corresponding to two runs are given).
ML-BS – Maximum likelihood bootstrap supports in percent.

Matrix	**Complete**				**43 ambiguously aligned sites excluded**			
Model for 28S rRNA	**RNA16B+G+I REV+G+I**		**RNA 16C+G, REV+G**	**RNA 16A+G, REV+G**	**RNA16B+G+I REV+G+I**		**RNA 16C+G, REV+G**	**RNA 16A+G, REV+G**
Support values	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**	**BPP**	**ML-BS**
Million iterations	**50**		**40 40**		**50**		**40 40**	
Node 01	1	100	1 1	100	1	100	1 1	100
Node 02	1	100	1 1	100	1	100	1 1	100
Node 03	1	92	1 1	99	1	94	1 1	100
Node 04	0.92	75	0.88 0.89	74	0.92	72	0.88 0.89	72
Node 05	1	100	1 1	100	1	100	1 1	100
Node 06 (Clade III)	1	99	1 1	100	1	100	1 1	100
Node 07	1	98	1 1	97	1	99	1 1	98
Node 08	0.99	91	0.99 0.99	90	0.98	87	0.98 0.98	88
Node 09	1	90	1 1	97	1	89	1 1	96
Node 10	1	100	1 1	100	1	100	1 1	100
Node 11	0.79	71	0.81 0.81	68	0.81	69	0.83 0.82	67
Node 12	1	90	1 1	89	1	93	1 1	92
Node 13	1	100	1 1	100	1	100	1 1	100
Node 14	1	72	1 1	65	1	68	1 1	57
Node 15	1	100	1 1	100	1	100	1 1	100
Node 16	1	66	1 1	69	0.93	56	0.95 0.96	61
Node 17	1	98	1 1	98	1	98	1 1	98

Appendix 3 (continued)

Supplementary Table 1 (continued)

Matrix	Complete				43 ambiguously aligned sites excluded			
Model for 28S rRNA	RNA16B+G+I REV+G+I		RNA 16C+G, REV+G	RNA 16A+G, REV+G	RNA16B+G+I REV+G+I		RNA 16C+G, REV+G	RNA 16A+G, REV+G
Support values	BPP	ML-BS	BPP	ML-BS	BPP	ML-BS	BPP	ML-BS
Million iterations	50		40 40		50		40 40	
Node 18	1	62	1 1	-	1	60	1 0.99	-
Node 19	1	95	1 1	98	1	96	1 1	99
Node 20	1	100	1 1	100	1	100	1 1	100
Node 21	1	100	1 1	100	1	100	1 1	100
Node 22 (Clade II)	1	100	1 1	100	1	100	1 1	100
Node 23	1	98	1 1	98	1	99	1 1	99
Node 24	0.91	56	0.84 0.84	54	1	73	1 0.99	72
Node 25	1	56	0.99 0.99	53	1	54	0.99 0.99	-
Node 26	1	100	1 1	100	1	100	1 1	100
Node 27	1	100	1 1	99	1	100	1 1	99
Node 28	1	100	1 1	100	1	100	1 1	100
Node 29	1	100	1 1	100	1	100	1 1	100
Node 30 (Clade I)	1	100	1 1	100	1	100	1 1	100
Node 31	1	100	1 1	100	1	100	1 1	100
Node 32	1	100	1 1	99	1	99	1 1	99
Node 33	1	87	1 1	90	1	85	1 1	92
Node 34	1	100	1 1	100	1	100	1 1	100
Node 35	1	98	1 1	97	1	98	1 1	97
Node 36	1	90	1 1	89	1	88	1 1	88
Node 37	1	100	1 1	100	1	100	1 1	100
Node 38	0.86	59	0.85 0.86	64	0.81	57	0.79 0.81	60
Node 39	1	100	1 1	100	1	100	1 1	100
Node 40	1	75	1 1	78	1	89	1 1	92

Appendix 4

Polymastiidae Gray, 1867

Diagnosis (following Paper IV):

Demosponges of encrusting, massive, globular, hemispherical, discoid, columnar or pedunculate body shape. Oscula are often located at the summits of papillae or, sometimes, directly on the surface of the main body. Assortment of spicules comprises at least two size categories of smooth monactines. Tracts of principal monactines radiating from the sponge base or forming a reticulation constitute the main choanosomal skeleton or the innermost layer of the cortex. Auxiliary choanosomal skeleton comprises smaller spicules, free-scattered or grouped in little bundles, which may be smooth monactines, smooth or acanthose oxeas, raphides in trichodragmata or astrotylostyles. A complex specialized cortical skeleton is developed to a greater or lesser degree, composed of at least a palisade of smooth tylostyles, subtylostyles, or oxeas and/or exotyles. A fringe of extra long monactines may be present at the edge of the body where it is in contact with the substrate.

***Acanthopolymastia* Kelly-Borges & Bergquist, 1997**

Type species: Atergia acanthoxa Koltun, 1964.

Scope: three species.

Molecular data: not available.

Diagnosis (emended from Boury-Esnault 2002):

Polymastiidae of thickly encrusting or discoid body shape, with a single weakly developed exhalant papilla. Main choanosomal skeleton composed of tracts of principal monactines radiating from the sponge base, with the longest monactines often projecting above the surface at the body edge. Auxiliary choanosomal skeleton comprises free-scattered acanthose microxeas. Cortical skeleton constituted by a superficial palisade of small smooth tylostyles and an internal dense mass of irregularly distributed acanthose microxeas.

***Astrotylus* Plotkin & Janussen, 2007**

Type species: Astrotylus astrotylus Plotkin & Janussen, 2007.

Scope: monotypic genus.

Molecular data: not available.

Diagnosis (emended from Plotkin & Janussen 2007):

Polymastiidae of thickly encrusting body shape, with a hispid surface and a single rather weakly developed exhalant papilla. Main choanosomal skeleton composed of tracts of principal tylostyles radiating from the sponge base. Auxiliary choanosomal skeleton comprises free-scattered numerous astrotylostyles and occasional small smooth tylostyles. Cortical skeleton constituted by the tracts of principal tylostyles ascending from the choanosome and forming bouquets reinforced with an irregular palisade of small tylostyles. The bouquets project above the surface.

***Atergia* Stephens, 1915**

Type species: Atergia corticata Stephens, 1915.

Scope: three species.

Molecular data: 18S rDNA (Redmond et al. 2013) and 28S rDNA (Morrow et al. 2013) from the type species. In the reconstructed phylogenies (Morrow et al. 2013; Redmond et al. 2013) the type species joins the polymastiid clade, but does not group with any other taxon.

Diagnosis (emended from Boury-Esnault 2002):

Polymastiidae of spherical to hemispherical body shape, with a single rather weakly developed exhalant papilla. Main choanosomal skeleton composed of tracts of principal tylostyles radiating from the sponge base, with some tracts projecting above the surface making it more or less hispid overall and forming a fringe at the body edge. Auxiliary choanosomal skeleton comprises free-scattered small tylostyles and smooth centrotylote microxeas. Cortical skeleton constituted by a palisade of small tylostyles.

Koltunia* Plotkin, Gerasimova, Morrow & Rapp, 2016 *interim unpublished

Type species: Proteleia burtoni Koltun, 1964.

Scope: monotypic genus.

Molecular data: not available.

Diagnosis (following Paper II: Plotkin et al. 2016):

Polymastiidae of thickly encrusting body shape, with a shaggy surface. Papillae unknown (no intact sponges were studied though). Main choanosomal skeleton composed of longitudinal tracts of principal monactines. Ascending tracts form cortical bouquets reinforced with small tylostyles and a surface hispidation reinforced with exotyles. Exotyles longer than 1.9 mm, with

grapnel-like ornaments of several long claws on distal extremities. Auxiliary choanosomal skeleton comprises free-scattered small tylostyles.

***Polymastia* Bowerbank, 1864**

Type species: Spongia mamillaris Müller, 1806

Scope: polyphyletic genus with 73 species.

Molecular data: CO1 from the type species, 15 other species and six OTUs (Turque et al. 2008; Nichols 2005; Alex et al. 2013; Morrow et al. 2013; Vargas et al. 2015; this study), 18S rDNA from four species (Kober & Nichols 2007; Redmond et al. 2013), 28S rDNA from the type species, 15 other species and one OTU (Nichols 2005; Meyer & Kuever 2008; Morrow et al. 2012; Thacker et al. 2013; this study), complete mitochondrial genome from one species (Del Cerro et al. 2016), five nuclear housekeeping proteins from one species (Hill et al. 2013). In the reconstructed phylogenies (this study) the type species together with *P. andrica*, *P. arctica*, *P. grimaldii*, *P. hemisphaerica*, *P. thielei* and *P. uberrima* forms a monophyletic group.

Diagnosis (following Paper IV):

Polymastiidae of encrusting, massive, globular, hemispherical or discoid body shape, always bearing exhalant papillae. Main choanosomal skeleton composed of tracts of principal monactines radiating from the sponge base or forming a reticulation. Auxiliary choanosomal skeleton comprises smaller monactines, free-scattered or grouped in little bundles. Cortical skeleton constituted at least by a superficial palisade of small smooth tylostyles or subtylostyles and an internal layer of larger monactines lying obliquely to the surface and may include middle layers. A fringe of extra long monactines may be present at the edge of the body.

***Proteleia* Dendy & Ridley, 1886**

Type species: Proteleia sollasi Dendy & Ridley, 1886.

Scope: two species.

Molecular data: 18S rDNA (Redmond et al. 2013) from the type species. In the reconstructed phylogenies (Redmond et al. 2013) the type species joins the polymastiid clade, but does not group with any other taxon.

Diagnosis (following Paper II: Plotkin et al. 2016):

Polymastiidae of thickly encrusting body shape, with velvety surface and exhalant papillae. Main choanosomal skeleton made of longitudinal tracts of principal monactines (usually fusiform styles). Auxiliary choanosomal skeleton comprises free-scattered small and

intermediary monactines. Cortex constituted by a superficial palisade of small tylostyles and an inner layer of tangentially arranged intermediary monactines, and reinforced by exotyles. In some species an additional palisade of intermediary monactines may be present between the superficial palisade and the inner tangential layer. Exotyles thin, shorter than 1 mm, with prominent distal ornamentations, which are umbrelliform, fungiform or grapnel-shaped, with short protuberances on the edges.

***Pseudotrachya* Hallmann, 1914**

Type species: Trachya hystrix Topsent, 1892.

Scope: two species.

Molecular data: CO1 and 28S rDNA available only from the type species (Nichols 2005) need verification. In the reconstructed phylogenies (Nichols 2005) the type species falls outside the polymastiid clade, that may be an artifact resulting from inaccurate taxonomic identification.

Diagnosis (emended from Boury-Esnault 2002):

Polymastiidae of encrusting body shape, without papillae. Main choanosomal skeleton composed of tracts of principal monactines radiating from the sponge base and projecting above the surface making it very hispid. Auxiliary choanosomal skeleton comprises free-scattered small oxeas. Cortical skeleton constituted by a palisade of the small oxeas.

***Quasillina* Norman, 1869**

Type species: Polymastia brevis Bowerbank, 1866.

Scope: three species (we regard *Q. richardi* as a synonym of *Q. brevis* – see Paper IV).

Molecular data: CO1 (this study), 18S rDNA (Redmond et al. 2013) and 28S rDNA (this study) from the type species, which is the sister to *Polymastia boletiformis* in the reconstructed phylogenies (Redmond et al. 2013; this study).

Diagnosis (following Paper IV):

Polymastiidae of pedunculate or columnar body shape, with a smooth surface and a single osculum located either directly at the summit of the main body or at the summit of a short papilla. Choanosomal skeleton is a mass of small monactines. Cortex comprises a superficial palisade of small monactines, a middle layer of criss-cross large or intermediary monactines and an inner layer of longitudinal tracts of large monactines lying parallel to the surface.

***Ridleia* Dendy, 1888**

Type species: Ridleia oviformis Dendy, 1888.

Scope: two species.

Molecular data: not available.

Diagnosis (emended from Boury-Esnault 2002):

Polymastiidae of pedunculate body shape, with a single exhalant papilla at the top. Choanosomal skeleton restricted to a subcortical layer of tangentially arranged small tylostyles. Cortex comprises a superficial palisade of small tylostyles, a middle layer of criss-cross intermediary monactines and an inner layer of longitudinal tracts of large monactines lying parallel to the surface.

***Sphaerotylus* Topsent, 1898**

Type species: Polymastia capitata Vosmaer, 1885.

Scope: polyphyletic genus with 12 species.

Molecular data: CO1 (Morrow et al. 2012; 2013; Vargas et al. 2015; this study) and 28S rDNA (Morrow et al. 2012; this study) from the type species, three other species and one OTU, 18S rDNA from one non-type species and one OTU (Redmond et al. 2013). The type species together with *S. renoufi* and *Sphaerotylus* sp. forms a monophyletic group in the CO1 and 28S rDNA phylogenies (this study).

Diagnosis (emended from Paper II: Plotkin et al. 2016):

Polymastiidae of thickly encrusting, spherical, hemispherical, dome-like or button-like shape. Some species with a single exhalant papilla, others with up to several tens of papillae. Main choanosomal skeleton made of radial or longitudinal tracts of principal monactines, often polytylote. Auxiliary choanosomal skeleton comprises free-scattered, small and intermediary monactines, occasionally exotyles. Cortical skeleton constituted by a superficial cortical palisade of either exotyles with sparse small monactines or small monactines reinforced with exotyles. An inner layer of criss-cross intermediary monactines may be also present. Distal extremities of the exotyles rough, spined, granulated, tuberculated or wrinkled, often with knobs varying from spherical to hemispherical, fungiform, umbrelliform or lobate.

***Spinularia* Gray, 1867**

Type species: Spinularia tetheoides Gray, 1867 (objective junior synonym of *Tethea spinularia* Bowerbank, 1866).

Scope: presumably monophyletic genus with at least four species in addition to the type: *S. australis* (original allocation based on morphology), *S. njordi* (our new species, allocation based on molecular data), *S. sarsi* (transferred from the former *Radiella* based on molecular data) and *S. setosa* (resurrected from a synonym of *S. spinularia* based on morphology). *S. sarsi* very probably represents a complex of at least two species, from the northern and southern hemisphere. The type species of the abandoned *Radiella*, *R. sol*, is evidently congeneric to *Polymastia hemisphaerica* or *S. sarsi*. Re-examination of *R. sol* and six other species of the former *Radiella* not covered by our study is required to determine their generic allocation.

Molecular data: CO1 and 28S rDNA from the type species, two other species and one OTU (Nichols 2005 (dubious data on the type species); this study). In the reconstructed phylogenies (this study) the type species together with *S. njordi*, *S. sarsi* and *S.* cf. *sarsi* forms a monophyletic group.

Diagnosis (following Paper IV):

Polymastiidae of discoid, hemispherical, lenticular or thickly encrusting body shape, with a shaggy or minutely hispid surface and one to fifteen weakly developed exhalant papillae. Main choanosomal skeleton composed of longitudinal or radial tracts of principal monactines crossing the cortex. Auxiliary choanosomal skeleton comprises free-scattered small and/or intermediary (sub)tylostyles and may also include raphids in trichodragmata. Cortical skeleton may, in addition to the superficial palisade of small tylostyles, include extra spicule layers. Basal cortex, if present, reinforced with the peripheral tracts of principal monactines lying parallel to the surface. A spicule fringe is always present at the body edge.

***Suberitechinus* de Laubenfels, 1949**

Type species: Suberitechinus hispida (Bowerbank, 1864).

Scope: monotypic genus.

Molecular data: not available.

Diagnosis (proposed herein):

Polymastiidae of subhemispherical or massive body shape, with a hispid surface. Oscula at the summits of papillae located in a shallow depression at the body top. Main choanosomal skeleton composed of radial tracts of principal monactines (often polytylote) crossing the

cortex. Auxiliary choanosomal skeleton comprises infrequent, free-scattered small tylostyles and intermediary monactines. Cortical skeleton constituted by a superficial palisade of small tylostyles and an internal layer of loosely lying criss-cross intermediary monactines. The cortex is reinforced with exotyles, which differ from the principal monactines only by larger size.

Tentorium **Vosmaer, 1887**

Type species: Thecophora semisuberites Schmidt, 1870.

Scope: polyphyletic genus with three species.

Molecular data: CO1 (Morrow et al. 2012; Vargas et al. 2015; this study) and 28S rDNA (Morrow et al. 2012; this study) from the type species, *T. papillatum* and one OTU, 18S rDNA from the type species (Redmond et al. 2013). In the reconstructed phylogenies (Redmond et al. 2013; this study) *Tentorium* spp. join the polymastiid clade, but group neither together, nor with any other taxon. Based on the 28S rDNA phylogeny (this study) the type species evidently represents a complex of two species, *T. semisuberites sensu stricto* from the northern hemisphere and a morphologically similar species from the southern hemisphere (to be erected).

Diagnosis (following Paper IV):

Polymastiidae of columnar or globular body shape, always with papillae. Main choanosomal skeleton constituted by longitudinal or radial tracts of principal monactines. Skeleton of the upper cortex comprises a palisade of small monactines. Skeleton of the lateral cortex may be either the same palisade or a dense layer of criss-cross principal or intermediary monactines.

Trachyteleia **Topsent, 1928**

Type species: Trachyteleia stephensi Topsent, 1928.

Scope: monotypic genus.

Molecular data: not available.

Diagnosis (following Paper II: Plotkin et al. 2016):

Polymastiidae of thickly encrusting body shape. Papillae unknown (no intact sponges were studied though). Main choanosomal skeleton made of radial tracts of principal tylostyles. Auxiliary choanosomal skeleton comprises free-scattered intermediary tylostyles. Cortex composed of a palisade of small tylostyles and an inner layer of criss-cross intermediary tylostyles, and reinforced by exotyles, which differ from principal tylostyles only by larger size and distal extremities with fine spines.

***Tylexocladus* Topsent, 1898**

Type species: Tylexocladus joubini Topsent, 1898.

Scope: two species. Based on morphological data the type species may represent a complex of two sympatric species (further studies required).

Molecular data: not available.

Diagnosis (following Paper II: Plotkin et al. 2016):

Polymastiidae of thickly encrusting, spherical or hemispherical body shape, usually with a single exhalant papilla. Main choanosomal skeleton composed of radial tracts of principal monactines. Auxiliary choanosomal skeleton comprises free-scattered small monactines and may also include smooth centrotylote microxeas. All species with a superficial cortical palisade made either of small monactines reinforced by exotyles or exclusively of exotyles. Some species also with an inner cortical layer of criss-cross monactines. Exotyles with denticulate distal ornaments and often with proximal tyles (cladotylostyles).

***Weberella* Vosmaer, 1885**

Type species: Alcyonium bursa Müller, 1806.

Scope: four species.

Molecular data: CO1 and 28S rDNA (this study) from the type species. In the reconstructed phylogenies it is the sister to *Polymastia* cf. *conigera* and this pair is the sister to *Sphaerotylus borealis*, although with a weak support.

Diagnosis (following Paper IV):

Polymastiidae of massive or globular body shape, with a smooth surface always bearing exhalant papillae. Spicule assortment restricted to two size categories of smooth monactines. Main choanosomal skeleton is a reticulation formed by tracts of principal monactines. Auxiliary choanosomal skeleton comprises free-scattered small monactines. Cortical skeleton composed of a superficial palisade of small tylostyles or subtylostyles and an internal layer of criss-cross principal monactines separated by a middle layer with aquiferous cavities.

Paper II

II

Plotkin, A., Morrow, C., Gerasimova, E., & Rapp, H.T. (2016): Polymastiidae (Demospongiae: Hadromerida) with ornamented exotyles: a review of morphological affinities and description of a new genus and three new species. Journal of the Marine Biological Association of the United Kingdom, on-line early view, available at http://dx.doi.org/10.1017/S0025315416000655

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Polymastiidae (Demospongiae: Hadromerida) with ornamented exotyles: a review of morphological affinities and description of a new genus and three new species

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All polymastiid sponges displaying ornamented exotyles are reviewed and their morphological affinities are reconsidered. The study embraces all known species of Proteleia, Sphaerotylus, Trachyteleia *and* Tylexocladus *as well as several species of* Polymastia. *A new genus,* Koltunia, *is established for the Antarctic species* Proteleia burtoni *based on the unique shape of distal ornamentations of its giant exotyles and on the absence of a spicule palisade in its cortex, a rare feature among the polymastiids. Three new species of* Sphaerotylus *are described* – S. renoufi *from the British Isles,* S. strobilis *from South Africa and* S. tjalfei *from West Greenland. Transfer of one New Zealand species from* Polymastia *to* Proteleia *and of one Chilean species from* Polymastia *to* Sphaerotylus *is proposed. The present study provides a background for future integrative phylogenetic analyses based on comprehensive molecular and morphological datasets which should reveal the natural relationships between the polymastiid taxa.*

Keywords: sponges, Demospongiae, Polymastiidae, morphological affinities, new species

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INTRODUCTION

Sponges of the family Polymastiidae Gray, 1867 have a simple spicule assortment which is usually limited to several size categories of smooth monactines (Boury-Esnault, 2002). However, in addition to these common spicules, some species also possess distally ornamented monactines. This additional category of spicules was first recorded in polymastiids by Sollas (1882) who noticed the rounded swellings on the distal tips of projecting monactines in his new species *Radiella schoenus* from the Norwegian coast. Three years later Vosmaer (1885) recorded similar spicules in his new species *Polymastia capitata* from the Arctic. Dendy & Ridley (1886) noted the similarity between *R. schoenus* and *P. capitata* relegating the latter to synonymy with the former. They also established a new genus, *Proteleia*, for their new species, *P. sollasi* from South Africa, which was distinguished by the grapnel-like distal ornamentations of its protruding spicules.

In 1898 Topsent erected two more polymastiid genera displaying ornamented monactines, *Tylexocladus* for his new species, *T. joubini* from Azores, which was notable for the denticulate distal ornamentations on its cortical spicules, and *Sphaerotylus* for Vosmaer's *P. capitata*, which was characterized by the spherical swellings on its projecting spicules. To identify these spicules with usual tyles on the proximal extremities and ornaments on the distal extremetities protruding above the sponge surface Topsent used the term exotyle introduced by him 2 years earlier (Topsent, 1896) for the similar spicules in *Gomphostegia loricata* (now *Mycale* (*Rhaphidotheca*) *loricata*, see Van Soest *et al.*, 2015) from the family Mycalidae.

For the time being nine species of *Sphaerotylus* from various locations in polar and temperate waters of both hemispheres, two species of *Proteleia* from the southern hemisphere and two species of *Tylexocladus*, one from the North Atlantic and the other from the South Pacific are recognized as valid (Van Soest *et al.*, 2015). Exotyles have also been recorded in *Trachyteleia stephensi* Topsent, 1928 and in two New Zealand species of *Polymastia* Bowerbank, 1864, *P. tapetum* Kelly-Borges & Bergquist, 1997 and *P. umbraculum* Kelly-Borges & Bergquist, 1997. Affinities between all these taxa have been discussed (Kelly-Borges & Bergquist, 1997; Boury-Esnault, 2002), but they have never been properly revised, and there is still no agreement on the differences at the generic level.

In this paper we review all known species and varieties of *Proteleia, Sphaerotylus, Trachyteleia* and *Tylexocladus* along

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with those species of *Polymastia* which display ornamented exotyles. We establish a new genus, *Koltunia* gen. nov. for the Antarctic species *Proteleia burtoni* Koltun, 1964, describe three new species of *Sphaerotylus* – from South Africa, Ireland and West Greenland and propose the transfer of two South Pacific species of *Polymastia*, one to *Sphaerotylus*, the other to *Proteleia*. Finally, we reconsider the affinities of the species studied based on multiple morphological characters.

MATERIALS AND METHODS

This study was based on the type specimens and other material stored in Ulster Museum, Belfast (BELUM), Natural History Museum, London (BMNH), Göteborg Natural History Museum (GNM), Muséum National d'Histoire Naturelle, Paris (MNHN), Musée Océanographique de Monaco (MOM), Museum of New Zealand, Te Papa Tongarewa, Wellington (NZNM), National Museum of Natural History, Leiden (RMNH), Smithsonian National Museum of Natural History, Washington (USNM), Zoological Institute of Russian Academy of Sciences, Saint-Petersburg (ZIN RAS), Museum für Naturkunde, Berlin (ZMB), University Museum of Bergen (ZMBN) and Natural History Museum of Denmark, University of Copenhagen (ZMUC). Additional fresh material was collected from the Norwegian coast during cruises by the University of Bergen. The architecture of the sponge skeletons was examined under light microscope on histological sections prepared on a precise saw with a diamond wafering blade after embedding sponge fragments in epoxy resin as described by Boury-Esnault *et al.* (2002), Vacelet (2006) and Boury-Esnault & Bézac (2007). Spicules were examined under light microscope and SEM after their isolation from organic matter in nitric acid following standard procedures. The number of specimens used for spicule measurements is given in the corresponding section of the description of each species. The number of spicules of each category measured in one specimen is indicated as N. Measurements are presented as minimum–mean–maximum, unless otherwise indicated.

SYSTEMATICS

Systematic index

Class DEMOSPONGIAE Sollas, 1885
Suborder HETEROSCLEROMORPHA Cárdenas, Perez & Boury-Esnault, 2012
Order POLYMASTIIDA Morrow & Cárdenas, 2015
Family POLYMASTIIDAE Gray, 1867
Genus *Koltunia* gen. nov.
K. burtoni (Koltun, 1964) comb. nov.
Genus *Proteleia* Dendy & Ridley, 1886
P. sollasi Dendy & Ridley, 1886
P. tapetum (Kelly-Borges & Bergquist, 1997) comb. nov.
Genus *Sphaerotylus* Topsent, 1898
S. antarcticus Kirkpatrick, 1907
S. antarcticus drygalskii Hentschel, 1914
S. borealis (Swarczewsky, 1906)
S. capitatus (Vosmaer, 1885)
S. exospinosus Lévi, 1993
S. exotylotus Koltun, 1970
S. isidis (Thiele, 1905) comb. nov.
S. raphidophora Austin, Ott, Reiswig, Romagosa & McDaniel, 2014
S. renoufi sp. nov.
S. sceptrum Koltun, 1970
S. strobilis sp. nov.
S. tjalfei sp. nov.
S. vanhoeffeni Hentschel, 1914
S. verenae Austin, Ott, Reiswig, Romagosa & McDaniel, 2014
Genus *Trachyteleia* Topsent, 1928
T. stephensi Topsent, 1928
Genus *Tylexocladus* Topsent, 1898
T. hispidus Lévi, 1993
T. joubini Topsent, 1898
Incertae sedis
Polymastia umbraculum Kelly-Borges & Bergquist, 1997

Description of taxa

Family POLYMASTIIDAE Gray, 1867

DIAGNOSIS
Sponges of massive, encrusting, globular, discoid or pedunculate growth form. Surface slightly velvety to very hispid. Choanosomal skeleton composed of radial megasclere tracts. A complex specialized cortical skeleton is developed to a greater or lesser degree, composed of at least a palisade of tylostyles, or oxeas and/or exotyles. Spicules comprise two or more size categories and include tylostyles, subtylostyles, strongyloxeas, styles or oxeas. Free spicules are always present in the choanosome; they may be intermediary or small tylostyles as well as various microscleres including smooth centrotylote microxeas, acanthose microxeas, raphides in trichodragmata and astrotylostyles. A fringe of long spicules is often present bordering the edge of the body where it is in contact with the substratum (from Plotkin & Janussen, 2008).

Genus *Koltunia* gen. nov.

TYPE SPECIES
Proteleia burtoni Koltun, 1964 (designation herein).

DIAGNOSIS
Thickly encrusting sponges with shaggy surface. Main choanosomal skeleton composed of longitudinal tracts of large styles and subtylostyles. These tracts ascend forming cortical bouquets and a thick surface hispidation. Auxiliary choanosomal skeleton comprises free-scattered small tylostyles. Cortex and surface hispidation reinforced by small tylostyles and giant exotyles (several mm in length). Distal extremities of the exotyles with several long claws resembling grapnels.

ETYMOLOGY
Named after the late Dr Vladimir M. Koltun, the greatest Russian sponge expert of the 20th century who described the type species of this genus.

REMARKS
This new genus is established due to the unique ornamentations of its exotyles in combination with a single-layered cortex and two size categories of monactines. The single layered-cortex is recorded in some species of several

polymastiid genera, but usually it is composed of a palisade of either small tylostyles (e.g. in *Polymastia invaginata* Kirkpatrick, 1907, *Sphaerotylus raphidophora* Austin, Ott, Reiswig, Romagosa & McDaniel, 2014, *Spinularia spinularia* (Bowerbank, 1866) and *Tentorium semisuberites* (Schmidt, 1870)) or exotyles (e.g. in *Sphaerotylus exotylotus* Koltun, 1970 and *S. vanhoeffeni* Hentschel, 1914) while in *Koltunia* the cortex is made of the bouquets of principal spicules with small tylostyles and exotyles embedded in between. The absence of intermediary size monactine category is typical of *Weberella* Vosmaer, 1885. Apart from this feature, there are no other similarities between *Weberella* and *Koltunia*.

Koltunia burtoni (Koltun, 1964) comb. nov.
(Figures 1 & 2)

Original description: *Proteleia burtoni* Koltun, 1964, p. 28, text- figure 4.

SYNONYMS AND CITATIONS

Proteleia burtoni (Koltun, 1976, p. 168; Kelly-Borges & Bergquist, 1997, p. 374; Boury-Esnault, 2002, p. 204).

TYPE MATERIAL

Holotype: ZIN RAS 10605 (specimen in alcohol and slides 6299, 11864, Figure 1A), BMNH 1986.7.9.6 (fragment of holotype in alcohol, Figure 1B), North of Balleny Islands, Southern Ocean, 64°03′S 161°59.2′E, 3000 m, RV 'Ob', station 57, 29.03.1956, coll. Ushakov and Belyaev.

DESCRIPTION

External morphology

Holotype – considerably damaged, ~ 1.9 × 1.3 × 0.5 cm in size, with shaggy dark-grey surface, without visible papillae (Figure 1A).

Skeleton

Main choanosomal skeleton composed of longitudinal tracts of principal spicules (Figure 1C). These tracts cross the cortex, where they expand into bouquets forming a 380–790 μm thick layer, and penetrate the surface, giving it a hirsute appearance (Figure 1D). Cortical bouquets reinforced by small spicules and giant exotyles. Auxiliary choanosomal skeleton comprises free-scattered small spicules.

Spicules

(N = 7 for exotyles, N = 10 for other categories)

- Principal spicules – straight or gently curved, slender or slightly fusiform styles to subtylostyles (Figure 2A–C). Length 1700–2488–3201 μm, diameter of tyle 14.2–16.6–18.5 μm, proximal diameter of shaft 13.5–14.9–17.9 μm, maximum diameter of shaft 23.8–26.5–29.3 μm. Koltun (1964) also recorded much longer principal spicules, up to 6000 μm. However, on the slides examined the spicules longer than 3200 μm were broken and therefore their length could not be estimated.
- Small spicules – straight, slender or slightly fusiform tylostyles (Figure 2D). Length 165–310–418 μm, diameter of tyle 5.9–6.5–7.1 μm, proximal diameter of shaft 3.3–4.0–5.0 μm, maximum diameter of shaft 6.0–8.0–10.0 μm. Koltun (1964) recorded small tylostyles from 150 to 550 μm in length.
- Exotyles flexuous and slender. Length 1900–3005–4300 μm, maximum diameter of shaft 24.0–33.2–40.0 μm. Exotyles may reach greater size, but the longest spicules were broken. Proximal extremities of the exotyles rounded, occasionally with weakly developed tyles (Figure 2E). Distal extremities ornamented with two to five curved or bent claws directed towards the proximal ends resembling the clads of anatriaenes in spirophorid and astrophorid sponges (grapnel-shaped). Each claw 37.9–59.2–80.0 μm long, divided into three to six processes at the tip. The claws may be symmetrically arranged (Figure 2F) or concentrated on one side of the shaft (Figure 2G, H).

OCCURRENCE

(Figure 3)

Southern Ocean: continental sectors 4 (off Sabrina Coast – Koltun, 1976) and 5 (off Balleny Islands – Koltun, 1964) (sectors numbered according to Sarà *et al.*, 1992), 2267–3000 m.

REMARKS

Koltun (1964) placed his new species in *Proteleia* based on the grapnel-like distal ornamentations on the exotyles that were considered to be the main distinguishing feature of this genus (Dendy & Ridley, 1886). Subsequent authors followed Koltun (Kelly-Borges & Bergquist, 1997; Boury-Esnault, 2002). However, the exotyles of the type species of *Proteleia, P. sollasi*, are in fact filiform spicules less than 600 μm long, with small distal ornamentations varying from irregularly grapnel-shaped to umbrelliform. These exotyles are sparsely scattered over the surface. Conversely, in *K. burtoni* the exotyles are thick and reach several millimetres in length. They are densely scattered over the sponge surface. Their distal ornamentations are large claws resembling the clads of anatriaenes, which is a unique feature among the polymastiids. Moreover, neither the external morphology, nor the cortical architecture, or the spicule assortment of *K. burtoni* bears any similarities with *P. sollasi*. The shaggy surface and large principal spicules of *K. burtoni* rather resemble those of *Sphaerotylus borealis* (Swarczewsky, 1906), *S. antarcticus* Kirkpatrick, 1907 and *Polymastia invaginata* than the velvety surface and smaller spicules of *Proteleia sollasi.* A single-layered cortex of *K. burtoni* is similar to that of *P. invaginata*, although the cortex of the latter species comprises an ordinary palisade of small tylostyles overlapped by bouquets of principal spicules (Plotkin & Janussen, 2008), whereas in *K. burtoni* there is no palisade and single small tylostyles are embedded between the bouquets of large spicules. Conversely, the cortex of *Proteleia sollasi* comprises three layers, a superficial palisade of small tylostyles, an inner tangential layer of intermediary spicules and a palisade of intermediary spicules in between.

Genus *Proteleia* Dendy & Ridley, 1886

TYPE SPECIES

Proteleia sollasi Dendy & Ridley, 1886 (by monotypy).

DIAGNOSIS

Thickly encrusting sponges with velvety surface and papillae. Main choanosomal skeleton made of longitudinal tracts of

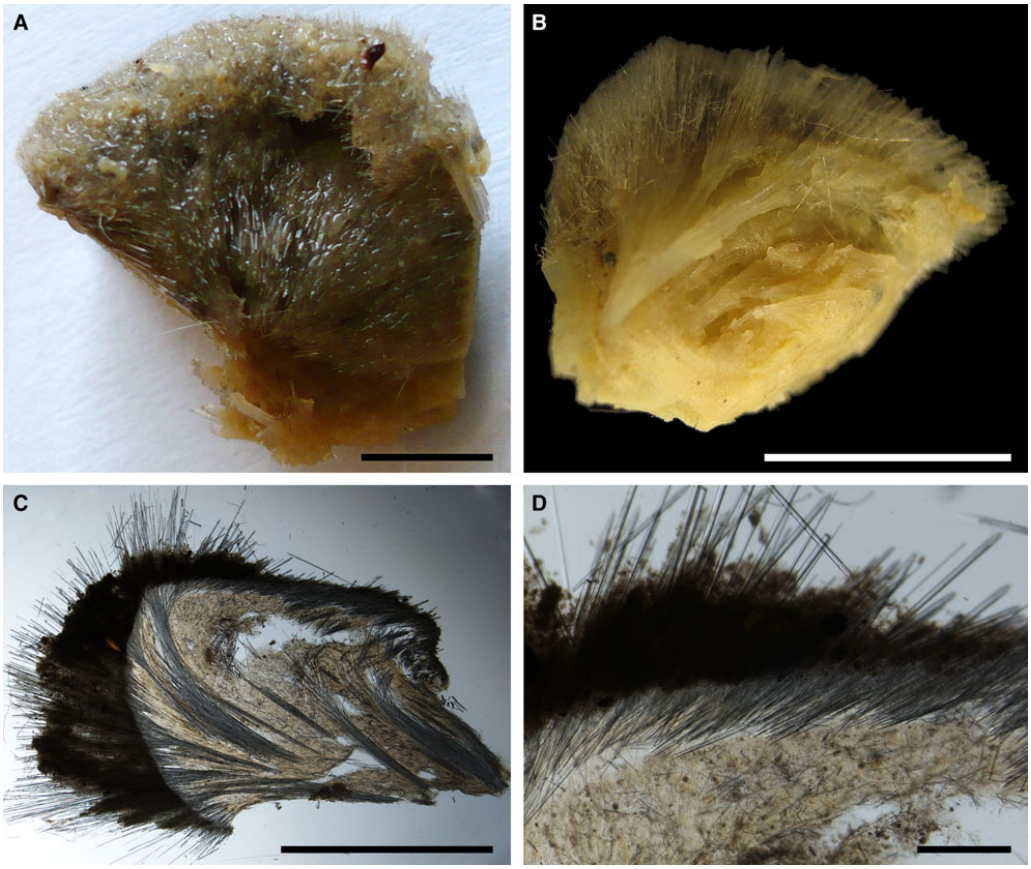

Fig. 1. *Koltunia burtoni*: (A) holotype ZIN RAS 10605, habitus; (B) fragment of the holotype BMNH 1986.7.9.6, habitus; (C) longitudinal section through the body of the holotype, general view; (D) the same section, detail of cortex. Scale bars: A–C, 5 mm; D, 0.5 mm.

principal spicules. Auxiliary choanosomal skeleton comprises free-scattered small and intermediary spicules. Cortex constituted by a superficial palisade of small spicules and an inner layer of tangentially arranged intermediary spicules, and reinforced by exotyles. In some species an additional palisade of intermediary spicules may be present between the superficial palisade and the inner tangential layer. Principal spicules are usually fusiform styles. Small and intermediary spicules are mainly tylostyles. Exotyles thin, shorter than 1 mm, with prominent distal ornamentations which may be umbrelliform, fungiform or grapnel-shaped with short protuberances on the edges.

Proteleia sollasi Dendy & Ridley, 1886
(Figures 4 & 5)

Original description: *Proteleia sollasi* Dendy & Ridley, 1886, p. 152, pl. 5.

SYNONYMS AND CITATIONS

Proteleia sollasi (Ridley & Dendy, 1886, p. 488; 1887 p. 214, pl. XLII figures 6–8, pl. XLIV figure 2; Von Lendenfeld, 1903, p. 29; Kelly-Borges & Bergquist, 1997, p. 374, figure 5D–E; Boury-Esnault, 2002, p. 204, figure 3).

TYPE MATERIAL

Holotype: BMNH 1887.5.2.62 (specimen in alcohol and eight slides), BMNH 1891.10.3.95 (one slide prepared from holotype), BMNH 1891.10.3.96 (one slide prepared from holotype), Simon's Bay near the Cape of Good Hope, South Africa, SE Atlantic, 18–36 m (10–20 fathoms), expedition on RV 'Challenger' in 1873–1876.

DESCRIPTION

External morphology

Holotype cushion-shaped, detached from substratum, ~ 5 × 3 × 0.3 cm in size (Figure 4A). Surface velvety, covered by small amounts of debris and shell pieces, with 27 cylindrical or conical papillae up to 0.8 cm long and 0.4 cm in diameter at base. Both surface and papillae pale yellow in colour. Oscula not visible. Some papillae sectioned transversally demonstrating a central canal surrounded by numerous peripheral canals.

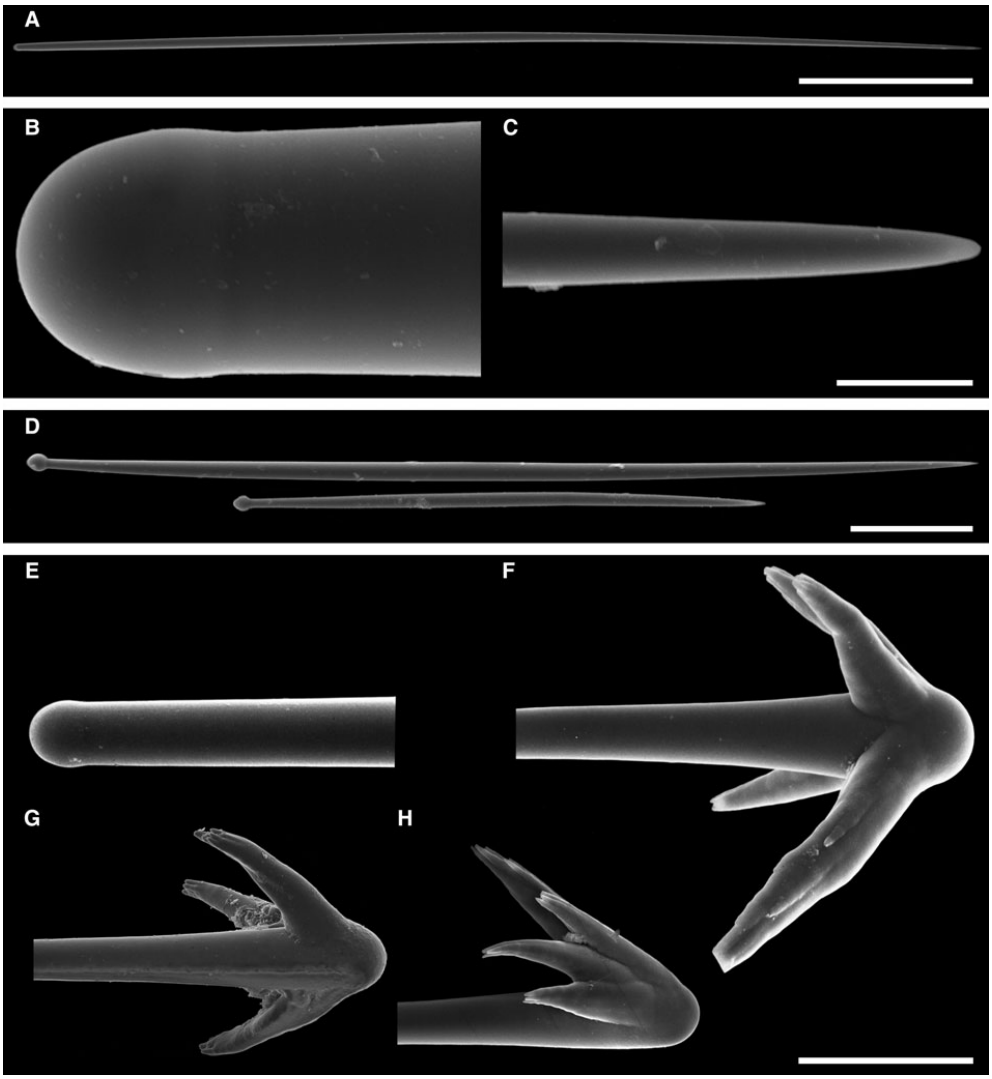

Fig. 2. *Koltunia burtoni*, spicules: (A) principal subtylostyle, general view; (B) proximal tip of the subtylostyle depicted in A, detailed view; (C) distal tip of the subtylostyle depicted in A, detailed view; (D) small tylostyles; (E) proximal tip of an exotyle, detailed view; (F) the same exotyle, distal ornamentation, detailed view; (G) and (H) distal ornamentations of other exotyles, detailed view. Scale bars: A, 0.5 mm; B and C, 0.01 mm; D–H, 0.05 mm.

Skeleton

Main choanosomal skeleton composed of longitudinal tracts (~ 250 μm thick) of principal spicules which enter the cortex (Figure 4B). Auxiliary choanosomal skeleton comprises singly scattered intermediary and small spicules. Cortex consists of a superifical palisade (~ 150 μm thick) of small spicules, an inner tangential layer (300–500 μm thick) of intermediary spicules and a palisade (~ 350 μm thick) of intermediary spicules in between, the two palisades intermingling (Figure 4C). The superficial palisade reinforced by sparse exotyles. All three cortical layers stretch along the walls of papillae, but the boundary between the inner palisade and the tangential layer is not well defined (Figure 4D–F). Central exhalant canal surrounded by ascending choanosomal tracts (Figure 4F). Bulkheads between peripheral canals reinforced by intermediary spicules.

Spicules

(N = 8 for exotyles, N = 10 for other categories)

- Principal spicules – straight strongyloxeas or fusiform subtylostyles with weakly developed tyles (Figure 5A, B). Length 473–974–1200 μm, proximal diameter of shaft 6.7–8.0–9.2 μm, maximum diameter of shaft 15.0–28.0–37.6 μm.
- Intermediary spicules – gently curved, fusiform subtylostyles (Figure 5C). Length 191–206–240 μm, diameter of

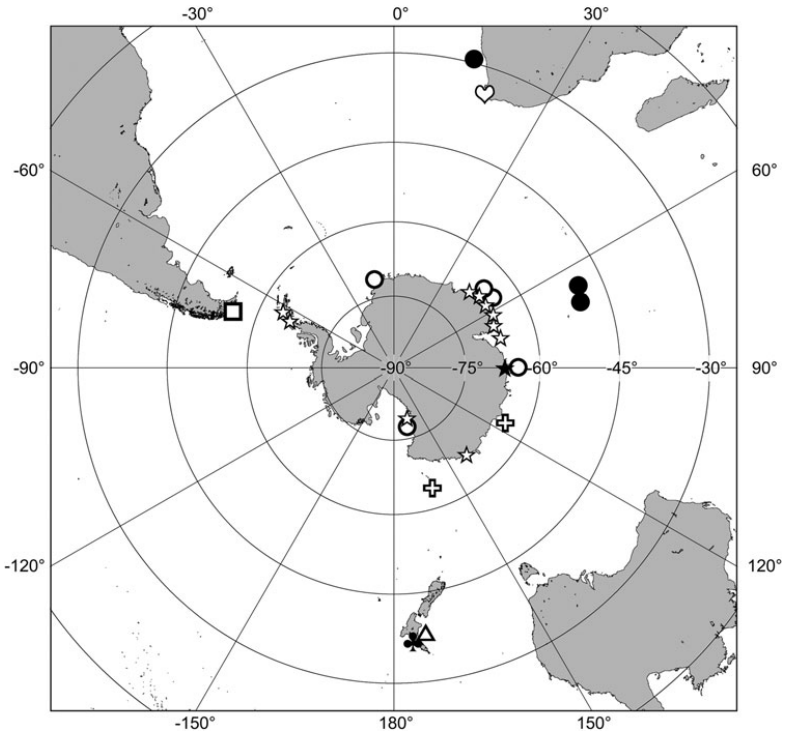

Fig. 3. Distribution of Polymastiidae with ornamented exotyles in the southern hemisphere: white crosses, *Koltunia burtoni*; white heart, *Proteleia sollasi*; white triangle, *Proteleia tapetum*; white stars, *Sphaerotylus antarcticus*; black star, *Sphaerotylus antarcticus drygalskii*; white square, *Sphaerotylus isidis*; white circles, *Sphaerotylus vanhoeffeni*, identification approved; black circles, *Sphaerotylus vanhoeffeni*, identification dubious; black trefoil, *Polymastia umbraculum*.

tyle 6.5–7.3–8.1 μm, proximal diameter of shaft 5.6–6.2–7.0 μm, maximum diameter of shaft 11.5–14.8–19.0 μm.

- Small spicules – straight or gently curved, slender tylostyles (Figure 5D). Length 125–152–180 μm, diameter of tyle 2.5–4.0–5.0 μm, proximal diameter of shaft 2.3–2.7–3.1 μm, maximum diameter of shaft 3.1–4.0–5.0 μm.
- Exotyles gently curved, slender, 350–463–555 μm long and 5.0–5.5–6.0 μm in diameter (Figure 5E, F). Their proximal extremities rounded, usually without tyles or more rarely with weakly developed tyles (Figure 5G, I). Distal ornamentations irregular, usually with four to eight more or less prominent short protuberances or claws directed towards the proximal tips, umbrelliform or occasionally grapnel-shaped (Figure 5H). Width of ornamentation with protuberances 4.0–4.9–6.3 μm. Some ornamentations with reduced protuberances and slightly displaced along the shafts (Figure 5J). Surface of ornamentations tuberculated or granulated.

OCCURRENCE

(Figure 3)

Known only from the type locality near SW Africa, SE Atlantic.

REMARKS

Proteleia sollasi is known only from the holotype. The presence of an extra palisade of intermediary spicules in the cortex and grapnel-like ornamentations on the exotyles were considered as the main distinctive features of this species (Dendy & Ridley, 1886; Boury-Esnault, 2002). Meanwhile, we have revealed that the shape of the exotyle ornamentations in *P. sollasi* is irregular and varies from grapnel-like to umbrelliform. Very similar exotyles are recorded in *Proteleia tapetum* (Kelly-Borges & Bergquist, 1997) and *Polymastia umbraculum* Kelly-Borges & Bergquist, 1997. Furthermore, irregular ornamentations with short protuberances are present on some exotyles of *Sphaerotylus antarcticus* and *S. borealis*, although their exotyles are much longer than those in *Proteleia* spp. Grapnel-like exotyle ornamentations with very long claws are typical of *Koltunia burtoni*, a species previously placed into *Proteleia*. However, its giant exotyles are several times larger than those of of *P. sollasi*. Moreover, *K. burtoni* is distinguished from *Proteleia* spp. by a single-layered cortex and a thick surface hispidation. The extra palisade layer in cortex has not been recorded in any other polymastiid with exotyles other than *P. sollasi*. But among other polymastiids *Polymastia corticata* Ridley & Dendy, 1886 and *P. littoralis* Stephens, 1915 do have such an extra palisade of intermediary spicules lying under the superficial palisade of small spicules.

Proteleia tapetum (Kelly-Borges & Bergquist, 1997) comb. nov.

(Figures 35 & 36)

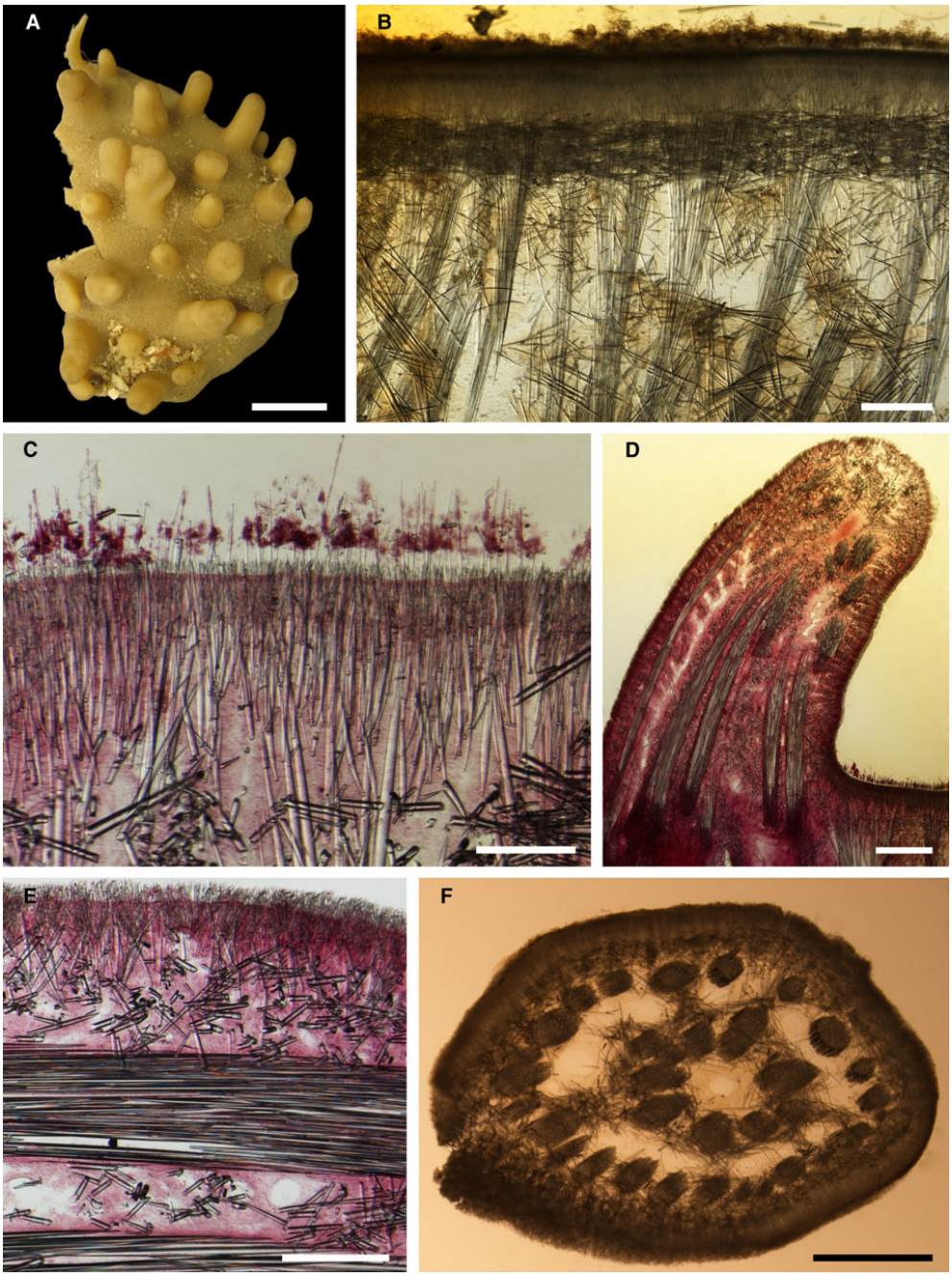

Fig. 4. *Proteleia sollasi*, holotype BMNH 1887.5.2.62: (A) habitus; (B) unstained longitudinal section through the body, general view; (C) longitudinal section through the body stained with carmine, detail of cortical palisade; (D) longitudinal section through a papilla stained with carmine, general view; (E) the same section, detail of the papilla wall; (F) unstained transversal section through a papilla. Scale bars: A, 10 mm; B, 0.5 mm; C, 0.2 mm; D, 1 mm; E, 0.3 mm; F, 1 mm.

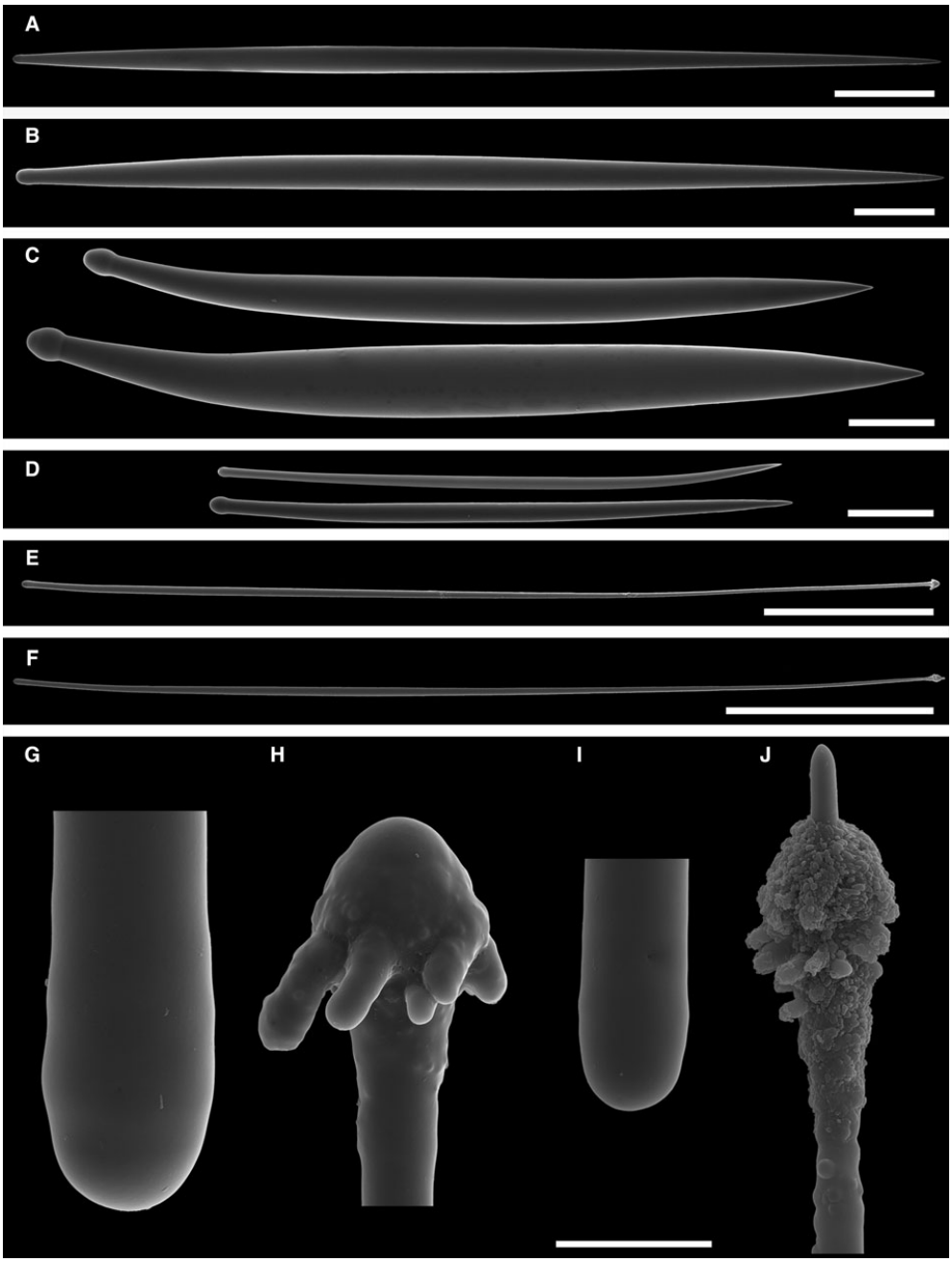

Fig. 5. *Proteleia sollasi*, spicules: (A) larger principal strongyloxea; (B) smaller principal strongyloxea; (C) intermediary subtylostyles; (D) small tylostyles; (E) exotyle with a prominent grapnel-like distal ornamentation, general view; (F) exotyle with a reduced distal ornamentation, general view; (G) proximal tip of the exotyle depicted in E, detailed view; (H) grapnel-like distal ornamentation of the exotyle depicted in E, detailed view; (I) proximal tip of the exotyle depicted in F, detailed view; (J) distal ornamentation of the exotyle depicted in F, detailed view. Scale bars: A, 0.1 mm; B, 0.04 mm; C and D, 0.02 mm; E and F, 0.1 mm; G–J, 0.004 mm.

Original description: *Polymastia tapetum* Kelly-Borges & Bergquist, 1997, p. 372, figures 4 & 5A–C.

TYPE MATERIAL

Holotype: NZNM Por 65 (specimen in alcohol, a fragment studied), BMNH 1996.2.22.10 (fragment of holotype in alcohol, studied), Castor Bay, east Coast of North Island, New Zealand, 36°45′S 174°46′E, mid low-tide, 12.09.1988.
Paratype: NZNM Por 557 (one specimen, not studied), from the same sample as the holotype.
Paratype: NZNM Por 558 (one specimen, not studied), Goat Island, Leigh, New Zealand, 36°16′S 174°48′E, shallow subtidal, 08.03.1991.

DESCRIPTION

External morphology

(According to Kelly-Borges & Bergquist, 1997)
Encrusting sponges growing in circular to oblong patches, ~ 6 × 3 cm wide and 0.2 × 1 cm thick. Surface golden yellow to bright orange in life and cream in alcohol, with microscopically smooth, generally flattened triangular-shaped papillae, 3–15 mm long and 3–6 mm wide at base. Inhalant papillae separate from exhalant papillae, the latter with 2–3 wide exhalant canals and several narrower inhalant canals. Surface areas between the papillae obscured by silt and sand trapped by projecting spicules.

Skeleton

(Our observations)
Main choanosomal skeleton composed of longitudinal tracts (220–370 μm thick) of principal spicules which radiate in the cortex and terminate under a superficial palisade (Figure 6A). Auxiliary choanosomal skeleton comprises intermediary and small spicules scattered singly or arranged in randomly oriented groups, each of 3–5 spicules. These groups are accumulating in the base of the sponge, forming a layer along the substratum. Cortex made of two intermingled layers – a superficial palisade (180–270 μm thick) of bouquets of small tylostyles with single filiform subtylostyles interspersed in between and an inner layer (440–510 μm thick) of intermediary spicules (Figure 6B). Sparsely scattered exotyles cross the cortex with their distal extremities projecting above the surface. Papilla walls comprise the palisade of small tylostyles and a loose network of intermediary spicules.

Spicules

(Our observations, N = 8 for exotyles and N = 10 for other categories)

- Principal spicules – strongyloxeas to fusiform subtylostyles, often polytylote (Figure 6C). Length 393–578–814 μm, proximal diameter of shaft 2.7–5.0–6.9 μm, maximum diameter of shaft 6.1–12.1–16.1 μm.
- Intermediary spicules – straight, occasionally curved, fusiform, often sabre-shaped subtylostyles (Figure 6D). Length 150–218–336 μm, diameter of tyle 5.3–6.2–8.1 μm, proximal diameter of shaft 3.9–4.6–6.0 μm, maximum diameter of shaft 6.6–8.5–11.8 μm.
- Small tylostyles gently curved, slender (Figure 6E). Length 74–85–98 μm, diameter of tyle 3.1–3.7–4.4 μm, diameter of shaft 2.4–2.8–3.2 μm.
- Filiform subtylostyles or styles extremely thin, considerably curved or bent (Figure 6F). Length 73–79–83 μm, diameter of shaft 0.8–1.2–1.6 μm.
- Exotyles gently curved, slender, 472–561–671 μm long, ~ 5 μm in diameter (Figure 6G). Their proximal extremities rounded, usually without tyles or more rarely with little swellings (Figure 6H). Distal ornamentations almost regular, umbrelliform to fungiform, with numerous short protuberances directed towards the proximal tips, 7.4–8.0–8.6 μm in width including the protuberances (Figure 6I).

OCCURRENCE

(Figure 3)
Known only from the type locality near New Zealand, SW Pacific.

REMARKS

Extremely thin exotyles with umbrelliform or fungiform distal ornamentations of *Proteleia tapetum* strongly resemble those of the type species of *Proteleia, P. sollasi*. The two species also exhibit very similar external morphology, both possessing a velvety surface with prominent papillae. However, the authors of *P. tapetum* (Kelly-Borges & Bergquist, 1997) considered these similarities as insufficient for the affiliation of their new species with *Proteleia*, emphasized the main difference between their species and *P. sollasi* (presence of an extra cortical palisade in the latter) and placed *tapetum* into *Polymastia*. At the same time the number and structure of cortical layers vary greatly among *Polymastia* spp. while the overwhelming majority of them including the type species *P. mamillaris* Müller, 1806 lack ornamented exotyles. Hence we propose the assignment of *tapetum* to *Proteleia*.

Genus *Sphaerotylus* Topsent, 1898

TYPE SPECIES

Polymastia capitata Vosmaer, 1885 (by original designation).

DIAGNOSIS

Encrusting sponges of spherical, hemispherical, dome, cushion or button shape. Some species with a single papilla, others possess up to several tens of papillae. Main choanosomal skeleton made of radial or longitudinal tracts of principal monactines. These tracts ascend into the papillae. Auxiliary choanosomal skeleton comprises free-scattered, small and intermediary monactines, occasionally exotyles. A superficial cortical palisade composed of either exotyles with sparse small monactines or small monactines reinforced by exotyles. An inner layer of criss-cross intermediary monactines may be also present. Both cortical layers extend to the walls of prominent papillae. In less prominent papillae the walls are reinforced only by the palisade of small monactines. No exotyles present in the papillae. Small monactines are usually tylostyles. Intermediary and principal monactines vary from styles to tylostyles, the principal spicules often being polytylote. Distal extremities of exotyles rough, spined, granulated, tuberculated or wrinkled, often with knobs varying from spherical to hemispherical, fungiform, umbrelliform or lobate.

Sphaerotylus antarcticus Kirkpatrick, 1907
(Figures 7 & 8)

Original description: *Sphaerotylus antarcticus* Kirkpatrick, 1907, p. 272.

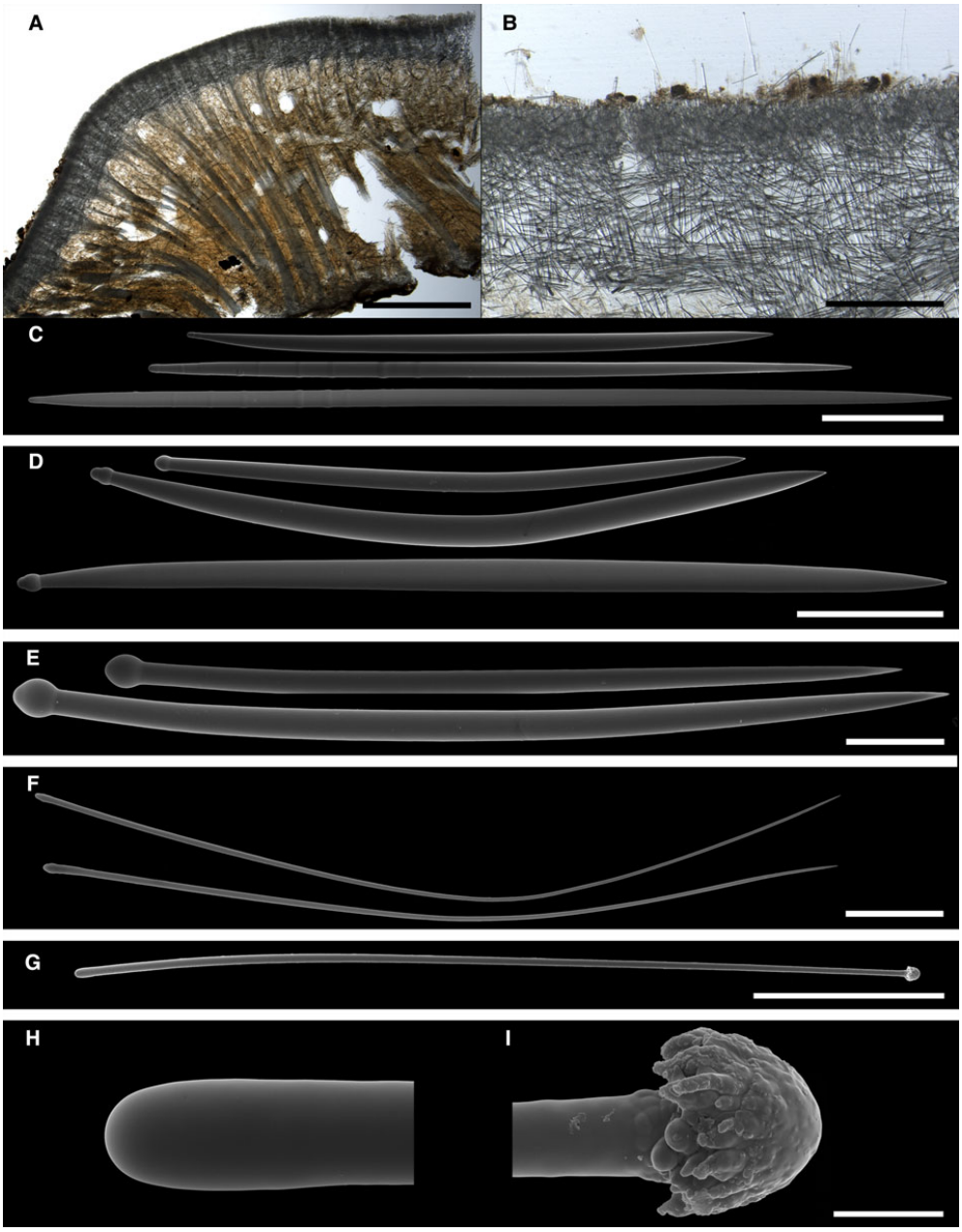

Fig. 6. *Proteleia tapetum*, holotype NZNM Por 65: (A) longitudinal section through the body, general view; (B) the same section, detail of cortex; (C) principal strongyloxeas; (D) intermediary subtylostyles; (E) small tylostyles; (F) filiform styles; (G) exotyle, general view; (H) proximal tip of the exotyle depicted in G, detailed view; (I) distal ornamentation of the exotyle depicted in G, detailed view. Scale bars: A, 5 mm; B, 0.5 mm; C, 0.1 mm; D, 0.05 mm; E and F, 0.01 mm; G, 0.1 mm; H and I, 0.002 mm.

SYNONYMS AND CITATIONS

Sphaerotylus antarcticus (Kirkpatrick, 1908, p. 16, pl. XII figures 1a–16 and pl. XIII figures 1–7; Burton, 1929, p. 446, 1932, p. 339; Koltun, 1964, p. 27, pl. V figures 14–20; Vacelet & Arnaud, 1972, p. 14; Desqueyroux-Faúndez, 1989, p. 107; Barthel *et al.*, 1990, p. 122).

Sphaerotylus borealis antarcticus (Koltun, 1976, p. 168; Sarà *et al.*, 1992, p. 568).

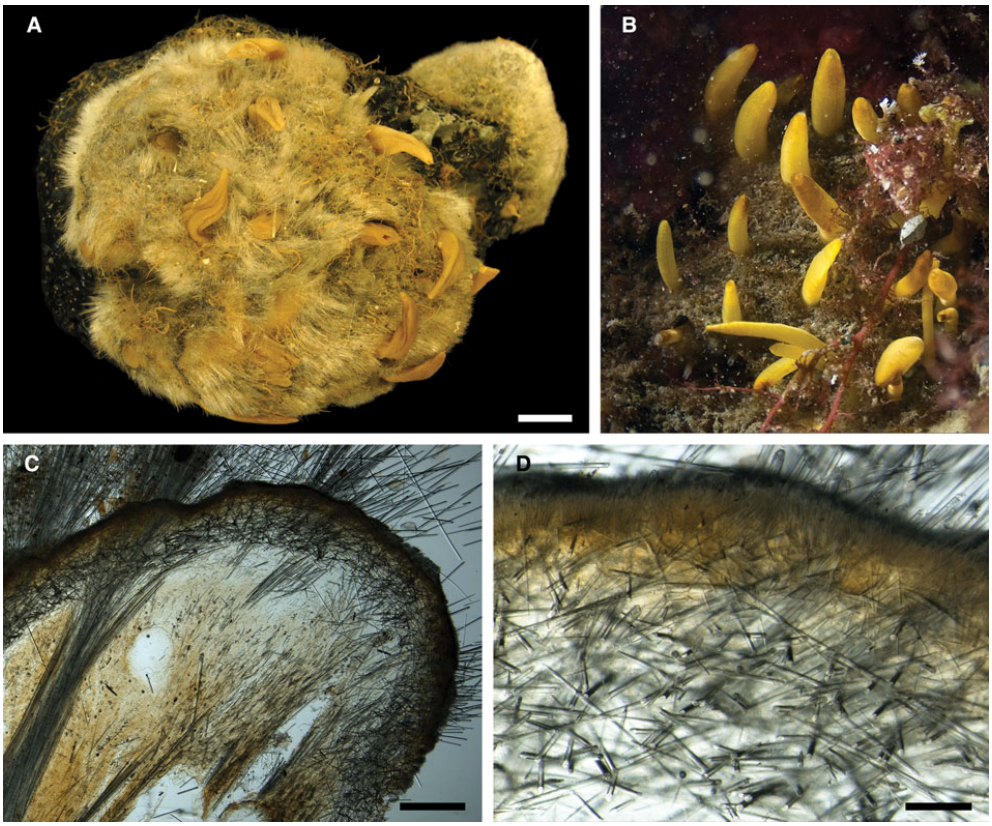

Fig. 7. *Sphaerotylus antarcticus*: (A) lectotype BMNH 1908.2.5.90, habitus; (B) specimen *in situ* in the Paradise Bay, Antarctic Peninsula (courtesy of N. Chervyakova, Moscow State University); (C) longitudinal section through the body of the lectotype, general view; (D) the same section, detail of cortex. Scale bars: A, 10 mm; C, 1 mm; D, 0.2 mm.

TYPE MATERIAL

Lectotype (designated herein, see Figure 7A, specimen preserved in alcohol and depicted by Kirkpatrick (1908) in pl. XII, figure 1A): BMNH 1908.2.5.90, Flagon point of Winter Quarters, Winter Quarters Bay, McMurdo Sound, Ross Sea, Southern Ocean, 77°50′42.77″S 166°39′1.41″E, 18–36.5 m (10–20 fathoms), British National Antarctic Expedition on RV 'Discovery' in 1901–1904, 21.01.1903.

Paralectotypes: BMNH 1908.2.5.91–96 and 1908.2.5.99–99A (10 specimens in alcohol), BMNH 1908.2.3.109 (one dry specimen), BMNH 1908.2.3.100–108 (23 slides prepared from the type series), BMNH 1908.2.5.97, 98 and 110 (specimens considered lost), Winter Quarters Bay, McMurdo Sound, Ross Sea, Southern Ocean, 77°50′42.77″S 166°39′1.41″E, 18–54.5 m (10–30 fathoms), British National Antarctic Expedition on RV 'Discovery' in 1901–1904.

COMPARATIVE MATERIAL EXAMINED

USNM (no number), NW side of New Rock, vicinities of the Palmer US research station, Antarctic Peninsula, Bellingshausen Sea, Southern Ocean, 12.2 m, scuba diving survey, station 103H74, 12.01.1974 (six specimens). USNM (no number), Cape Bellue, vicinities of the Palmer US research station, Antarctic Peninsula, Bellingshausen Sea, Southern Ocean, 66°18′S 65°53′W, 13.7 m, scuba diving survey, station 299H74 (one specimen). ZMBN 98045, Almirante Brown Antarctic Base, Paradise Bay, Bellingshausen Sea, Southern Ocean, 64°54.4′S 62°52.0′W, 21 m, 06.03.2010, coll. N. Chervyakova (one specimen). ZIN RAS (no number), 'Molodezhnaya' Russian research station, Cosmonaut Sea, Southern Ocean, 67°40.3′S 45°23′E, 3 m, The 11th Soviet Antarctic Expedition, scuba diving survey, transect II, station 3, 06.03.1966, coll. Propp (three specimens).

DESCRIPTION

External morphology

Lectotype (Figure 7A) thickly encrusting, 8 × 8 × 2.5 cm in size, overgrowing a volcanic concretion together with the specimen BMNH 1908.2.5.75 (syntype of *Polymastia invaginata*). Surface shaggy, dirty grey, with 15 light-coloured papillae. Most papillae well-defined, conical, 0.9–2.5 cm long, 0.3–1 mm in diameter at base, bearing oscula on the tops. Some papillae damaged. One of these sectioned transversally

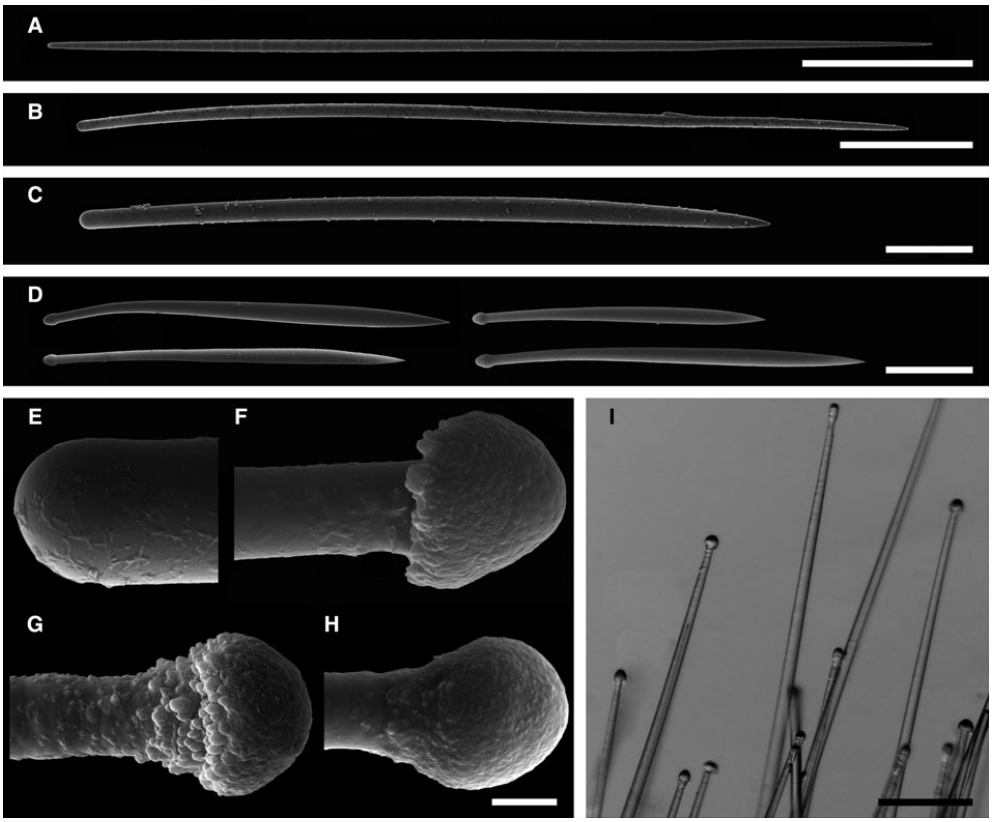

Fig. 8. *Sphaerotylus antarcticus*, spicules: (A) principal style; (B) longer intermediary subtylostyle; (C) shorter intermediary subtylostyle; (D) small spicules; (E) proximal tip of an exotyle, detailed view; (F) distal knob of the same exotyle, detailed view; (G) and (H) distal knobs of other exotyles, detailed view; (I) exotyles echinating the surface, view on a section. Scale bars: A, 0.3 mm; B, 0.1 mm; C and D, 0.03 mm; E–H, 0.01 mm; I, 0.2 mm.

demonstrating a wide central canal with several narrow peripheral canals. Three papillae considerably contracted. Paralectotypes vary greatly in shape, size and prominence of papillae. Larger sponges usually flattened, encrusting. Smaller sponges may be dome-shaped or subspherical. In the smallest specimens the length of papilla may exceed the body dimensions by up to three times. Other studied sponges thickly encrusting or cushion-shaped, the largest specimens up to 200 cm^2. Surface shaggy and heavily dusted with sediment making it dirty greyish or brownish. In life the sponges are often covered by sediment with erect papillae protruding above the sediment (Figure 7B). After sampling and fixation the papillae contract and invaginate into the surface hispidation. Sponges may have up to 50 papillae which are usually slender and cylindrical, more rarely stout and conical, with oscula visible on their summits, colouration yellowish in life and more pale in alcohol.

Skeleton

Main choanosomal skeleton composed of radial or longitudinal tracts of principal spicules crossing the cortex and making up a dense and thick surface hispidation (Figure 7C). Auxiliary choanosomal skeleton comprises singly scattered small, occasionally intermediary, spicules. Cortical palisade (165–170 μm thick) of small spicules (Figure 7D), lying directly on a layer (700–800 μm thick) of tangentially arranged intermediary spicules. Exotyles cross the cortex and join the superficial hispidation (Figure 8I).

Spicules

(measurements based on five specimens, N = 5 for exotyles, N = 10 for other categories):

- Principal spicules – straight, slender, often polytylote subtylostyles to styles (Figure 8A). Length 900–1870–2900 μm, proximal diameter of shaft 17.0–19.5–23.0 μm, maximum diameter of shaft 20.0–32.3–41.0 μm.
- Intermediary spicules – straight, stout subtylostyles to tylostyles (Figure 8B, C). Length 240–490–630 μm, diameter of tyle 8.0–14.8–20.0 μm, proximal diameter of shaft 7.0–9.0–10.0 μm, maximum diameter of shaft 10.0–14.2–20.0 μm.
- Small spicules – straight or gently curved, strongly fusiform, sabre-shaped tylostyles to subtylostyles (Figure 8D). Length 100–123–150 μm, diameter of tyle 3.0–3.2–3.5 μm, proximal diameter of shaft 2.5–2.6–3.0 μm, maximum diameter of shaft 5.5–6.2–7.0 μm.

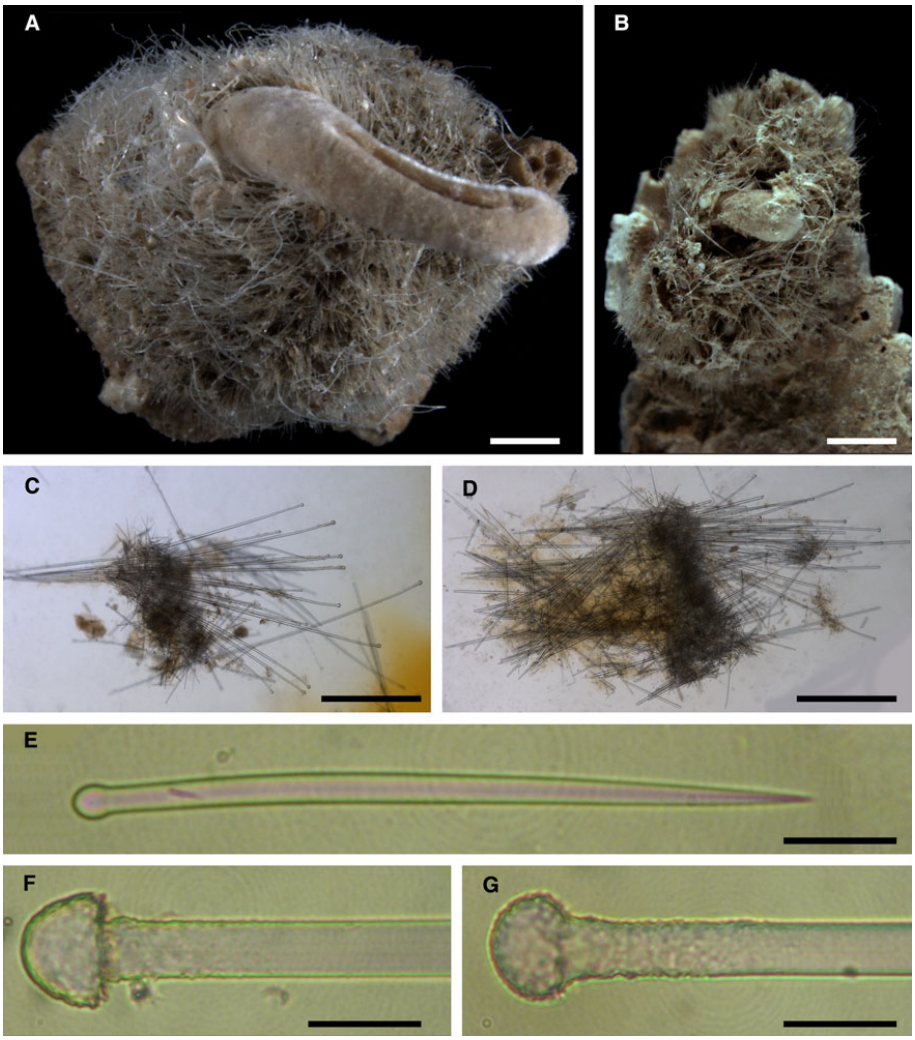

Fig. 9. *Sphaerotylus antarcticus drygalskii*: (A) lectotype ZMB 4836, habitus; (B) paralectotype ZMB 4836, habitus; (C) and (D) longitudinal sections through the body of the type specimens; (E) small tylostyle; (F) and (G) distal knobs of exotyles, detailed view. Scale bars: A and B, 1 mm; C and D, 0.5 mm; E–G, 0.02 mm.

- Exotyles slender, 1000–4656–8000 µm long, shaft diameter 20.0–23.6–30.0 µm. Proximal tyles usually weakly developed or absent (Figure 8E). Distal knobs 24.0–29.9–40.0 µm in diameter, irregular, varying from subspherical to hemispherical, fungiform or umbrelliform, occasionally with short protuberances on the edges (Figure 8F–H). Surface of the knobs and the adjacent portions of the shaft rough, granulated, tuberculated or wrinkled.

OCCURRENCE
(Figure 3)

Southern Ocean: continental sectors 2, 3 (Davis Sea), 4 (Adélie Land), 5 (Ross Sea), 8 (Bellingshausen Sea, Antarctic Peninsula), 9 (Weddell Sea) (sectors numbered according to Sarà *et al.*, 1992), 3–437 m, South Shetland Islands, 20–60 m (data by Desqueyroux-Faúndez, 1989).

REMARKS

Sphaerotylus antarcticus is very similar to *S. borealis* from the northern hemisphere. Both species are characterized by a shaggy surface, two-layered cortex and extremely long exotyles with irregular distal knobs varying from subspherical to fungiform and umbrelliform, the features distinguishing them from the type species of *Sphaerotylus, S. capitatus* (Vosmaer, 1885). The similarities between *S. antarcticus* and *S. borealis* led Koltun (1976) to the assumption that they were subspecies of a single species with a bipolar distribution. The only obvious difference between these two is the sabre-like shape of the small tylostyles in *S. antarcticus*. The

shaggy surface and extremely long exotyles like in *S. antarcticus* and *S. borealis* are also recorded in *Koltunia burtoni*. However, the latter species is distinguished by the cortex lacking the ordinary superficial palisade and the inner spicule layer, and by the unique shape of its exotyles bearing huge grapnel-like ornamentations on the distal extremities.

Sphaerotylus antarcticus drygalskii Hentschel, 1914
(Figure 9)

Original description: *Sphaerotylus antarcticus* var. *drygalskii* Hentschel, 1914, p. 51.

TYPE MATERIAL

Lectotype (designated herein, see Figure 9A): ZMB 4836 (specimen in alcohol), Gauss-Station, Davis Sea, Southern Ocean, 66°02′S 89°38′E, 385 m, Deutschen Südpolar-Expedition, 17.12.1902.
Paralectotype (Figure 9B): ZMB 4836 (one specimen in alcohol), from the same sample as the holotype.
Paralectotype (considered lost): ZMB 4836, the same expedition and locality as for the holotype, 380 m, 22.01.1903.

DESCRIPTION

External morphology

Both lectotype and paralectotype cushion-shaped. Lectotype 0.8 × 0.6 × 0.2 cm in size, detached from substratum (Figure 9A). Paralectotype 0.4 × 0.4 × 0.1 cm in size, attached to a piece of dead bryozoan skeleton (Figure 9B). Surface of both sponges strongly hispid and heavily dusted with sediment making it dirty greyish in colour. Each sponge with a prominent, almost regularly cylindrical central papilla (~ 0.5 cm long in the lectotype and 0.1 cm long in the paralectotype) and few contracted and damaged pin-like peripheral papillae. Oscula not visible.

Skeleton

Main choanosomal skeleton composed of radial or longitudinal tracts of principal spicules which cross the cortex and make up a dense surface hispidation (Figure 9C, D). Auxiliary choanosomal skeleton comprises singly scattered small, occasionally intermediary, spicules. In cortex a palisade (~ 140 μm thick) of small spicules is intermingled with an internal layer (~170 μm thick) of tangentially arranged intermediary spicules. Exotyles cross the cortex and join the superficial hispidation.

Spicules

(measurements based on lectotype and paralectotype, N = 5 for exotyles, N = 10 for other categories)

- Principal spicules – straight, slender, occasionally polytylote subtylostyles to styles. Length 600–723–900 μm, diameter of shaft 10.0–10.4–11.0 μm.
- Intermediary spicules – gently curved or straight subtylostyles to tylostyles. Length 365–440–520 μm, diameter of the shaft 8.0–9.2–10 μm.
- Small spicules – straight or gently curved, slightly fusiform tylostyles (Figure 9E). Length 100–117–132 μm, diameter of shaft 5.0–5.6–6.0 μm.
- Exotyles slender, 750–817–900 μm long, shaft 9.0–10.1–11.0 μm in diameter. Proximal tyles usually weakly developed or absent. Distal knobs 18.0–19.6–21.0 μm in diameter, often regularly fungiform, occasionally subhemispherical, always with granulated surface (Figure 9F, G).

OCCURRENCE

(Figure 3)
Known only from the type locality near Gauss Station, Davis Sea, Southern Ocean.

REMARKS

The only apparent difference between *Sphaerotylus antarcticus drygalskii* and typical *S. antarcticus* is that all three categories of spicules are shorter in the former.

Sphaerotylus borealis (Swarczewsky, 1906)
(Figures 19 & 20)

Original description: *Proteleia borealis* Swarczewsky, 1906, p. 315, pl. X figure 1, pl. XIII figure 2.

SYNONYMS AND CITATIONS

Proteleia borealis (Boury-Esnault, 2002, p. 204).
Sphaerotylus borealis (Rezvoj, 1928, p. 78, figures 4 & 5; Koltun, 1966, p. 83, pl. XXX figures 1 & 5, text-figure 55; Plotkin, 2004, p. 543, figures 1I, 2I, 4B).
Sphaerotylus schoenus var. *borealis* (Hentschel, 1929, p. 925).

TYPE MATERIAL

Holotype (small fragment, considered lost): Small Pir'yu Inlet, near Umba, Kandalaksha Bay, White Sea, ~ 66°40.37′N 34°19.7′E, 5.5 m, coll. Varpakhovsky.

Neotype (designated herein, see Figure 10A): ZIN RAS 11194 (specimen in alcohol), Sredny Island, Keret' Inlet, Kandalaksha Bay, White Sea, 66°17.391′N 33°38.025′E, 10–13 m, 12.07.2000, coll. Plotkin.

COMPARATIVE MATERIAL EXAMINED

Arctic Ocean (one specimen):
ZIN RAS 11178 (one specimen, slides 6084, 6082, 7136–7141), between Svalbard and Franz Josef Land, 82°00′N 42°00′E, 415 m, RV 'Litke', station 26, 18.09.1955, coll. Koltun.
Barents Sea (21 specimens):
ZIN RAS 11145 (one specimen), 72°30′N 23°01′E, 342 m, RV 'Dalnie Zelentsy', cruise 16, station 25, 05.10.1982. ZIN RAS 11146 (one specimen), 73°00′N 35°14′E, 219 m, RV 'Dalnie Zelentsy', cruise 24, station 14, 22.08.1984. ZIN RAS 11156 (one specimen, slide 5527), 73°02′N 25°58′E, 420 m, Expedition of PMNI, station 660, 12.06.1927. ZIN RAS 11157 (one specimen, slide 7882), 75°38′N 30°00′E, 331 m, Expedition of PMNI, station 966, 22.06.1928. ZIN RAS 11158 (one specimen, slide 5523), 72°00′N 35°00′E, 256 m, Expedition of PMNI, station 1062, 17-18.08.1928. ZIN RAS 11159 (one specimen, slide 7884), 70°55′N 37°33′E, 249 m, Expedition of PMNI, station 631, 29.05.1927. ZIN RAS 11160 (one specimen), 69°35′N 33°40′E, 180 m, Expedition of PINRO, RV 'Persey', cruise 53, station 3064, 10.05.1935. ZIN RAS 11163 (one specimen), 70°39′N 33°30′E, 243 m, Expedition of ENPIM, RV 'St. Andrew Pervozvanny', station 467, 16(29).05.1900, coll. Breitfuss. ZIN RAS 11166 (one specimen), 70°45′N 33°30′E, 260 m, RV 'Maslov', cruise 1, station 7/183, 29.11.1968. ZIN RAS 11167 (one specimen), 72°30′N 33°30′E, 142 m, trawl 15, sample 12, 29.05.1924, coll. Ushakov. ZIN RAS 11170 (one specimen), 69°26.5′N 36°34′E, 200 m, RV 'Prof. Derugin', cruise 8, station 155,

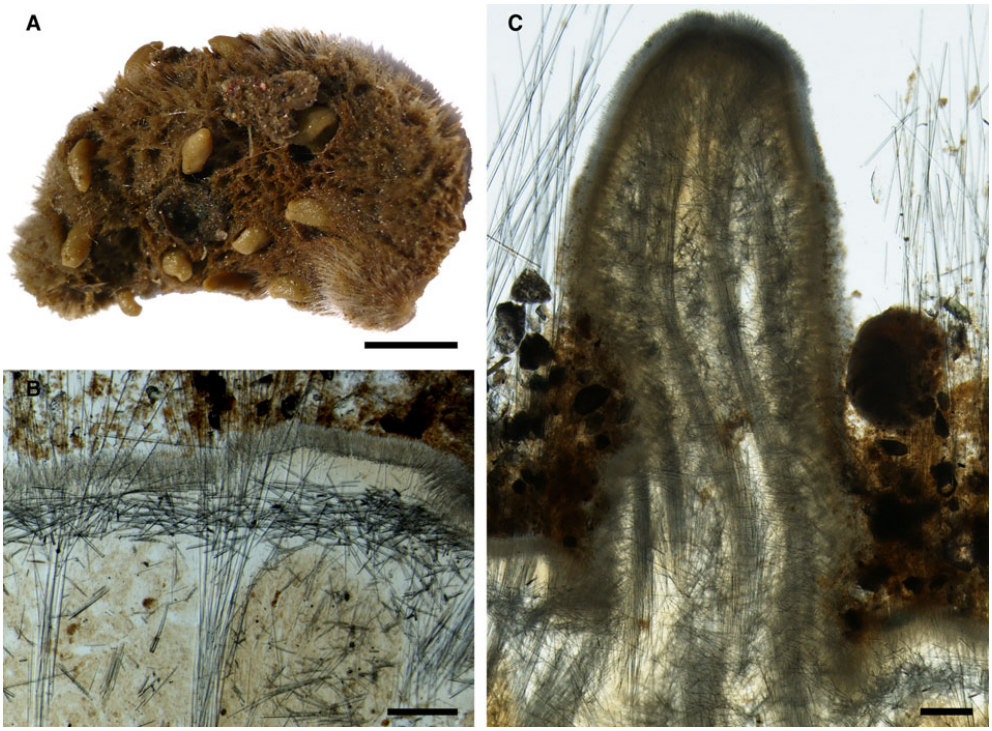

Fig. 10. *Sphaerotylus borealis*: (A) neotype ZIN RAS 1194, habitus; (B) longitudinal section through the body of a White Sea specimen; (C) longitudinal section through a papilla of the White Sea specimen. Scale bars: A, 10 mm; B and C, 0.3 mm.

09.10.1959, coll. Galkin. ZIN RAS 11171 (one specimen), 69°00′N 38°00′E, 175 m, RV 'RT61-Vodnik', cruise 26, station 105, 10.07.1968. ZIN RAS 11174 (one specimen, slide 13403), 69°23.1′N 34°29′E, 130 m, Expedition of Murmansk Biological station, RV 'Diana', station 27, 25.09.1953. ZIN RAS 11176 (one specimen, slide 13597), 69°20′1″N 35°12′8″E, 153 m, Expedition of Murmansk Biological station, station 37, 29.03.1954. ZIN RAS 11177 (one specimen, slides 13309, 13311), 69°11.4′N 36°11′E, 170–165 m, RV 'Prof. Derugin', cruise 8, station 153, 10.10.1958, coll. Galkin. ZIN RAS 11181 (one specimen), 71°00′N 35°40′E, 215 m, Expedition of Murmansk Biological station, station 117a, 28.06.1958, coll. Vilenkin. ZIN RAS 11183 (one specimen, slide 13428), 69°01′N 36°41′E, 128 m, Expedition of Murmansk Biological station, RV 'Diana', station x-1, 14.07.1955. ZIN RAS 11168 (one specimen, slide 5519), Gavrilovo, near the entrance to the bight, Murman Coast, 69°10′56.88″N 35°51′10.45″E, 91 m, station 154/72, 02.08.1894, coll. Knipovich. ZIN RAS 11164 (one specimen, slide 5511), Kildin Straight, Murman Coast, 69°18′49.02″N 34°07′17.13″E R/V 'Alexander Kovalevsky', cruise 43, 31.07.1924, coll. Derugin. ZIN RAS 11173 (one specimen, slide 9131), Kola Bay, Murman Coast RV 'Alexander Kovalevsky', 1908–1909, coll. Derugin. ZIN RAS 11165 (one specimen, slide 0095), Rybachy Peninsula, Murman Coast, 69°55′N 32°38.75′E, 124 m, Expedition of ENPIM, RV 'St. Andrew Pervozvanny', station 716, 04(17).08.1900, coll. Breitfuss.

Between Kara and Laptev Sea (one specimen):
ZIN RAS 11179 (one specimen, slides 5524, 12299), Shokalsky Straight, 78°48.3′N 99°26′E, 43 m, RV 'Rusanov', station 9 (iii, i), 19.08.1932, coll. Vagin & Kondakov.
Norwegian Sea (two specimens):
ZIN RAS 11169 (one specimen, slide 8614), 64°45.8′N 12°31.1′E, 157 m, RV 'Sebastopol', cruise 8, station 1427, 09.04.1958, coll. Zatsepin. ZIN RAS 11184 (one specimen, slide 10258), 66°52′N 14°E, 240 m, RV 'SRT4225′, cruise 1, station 61/127, 21.06.1955, coll. Kobyakova.
White Sea (31 specimens):
ZIN RAS 11148 (one specimen), Basin of the White Sea, 66°08′N 37°31.3′E, 24–31 m, RV 'Pomor', station 20(36), 30.05.1983, coll. Gudimov. ZIN RAS 11149 (one specimen), Dvina Bay, 65°10′N 37°10′E, 37 m, RV 'Pomor', station 11, 29.05.1983, coll. Gudimov. ZIN RAS 11144 (one specimen), near White Sea Biological Station of ZIN RAS, Chupa Inlet, Kandalaksha Bay, 19–22 m, station, 20.10.1967, coll. Golikov. ZIN RAS 11151 (one specimen, slide 21068), Chupa Inlet, Kandalaksha Bay, 66°18.3′N 33°49.5′E, 20 m, RV 'Onega', station 17/361, 19.07.1964, coll. Kunin. ZIN RAS 11152 (one specimen, slide 21069), Chupa Inlet, Kandalaksha Bay, 21–26 m, RV 'Onega', station 33/15, 21.07.1961, coll. Kunin. ZIN RAS 11153 (one specimen, slide 21070), Chupa Inlet, Malaya Klyuschikha Bight, Kandalaksha Bay, 5–20 m, RV 'Onega', station 5/347, 10.07.1964, coll. Kunin. ZIN RAS 11180 (one specimen), Chupa Inlet, Levaya Bight, Kandalaksha Bay, 20 m, station

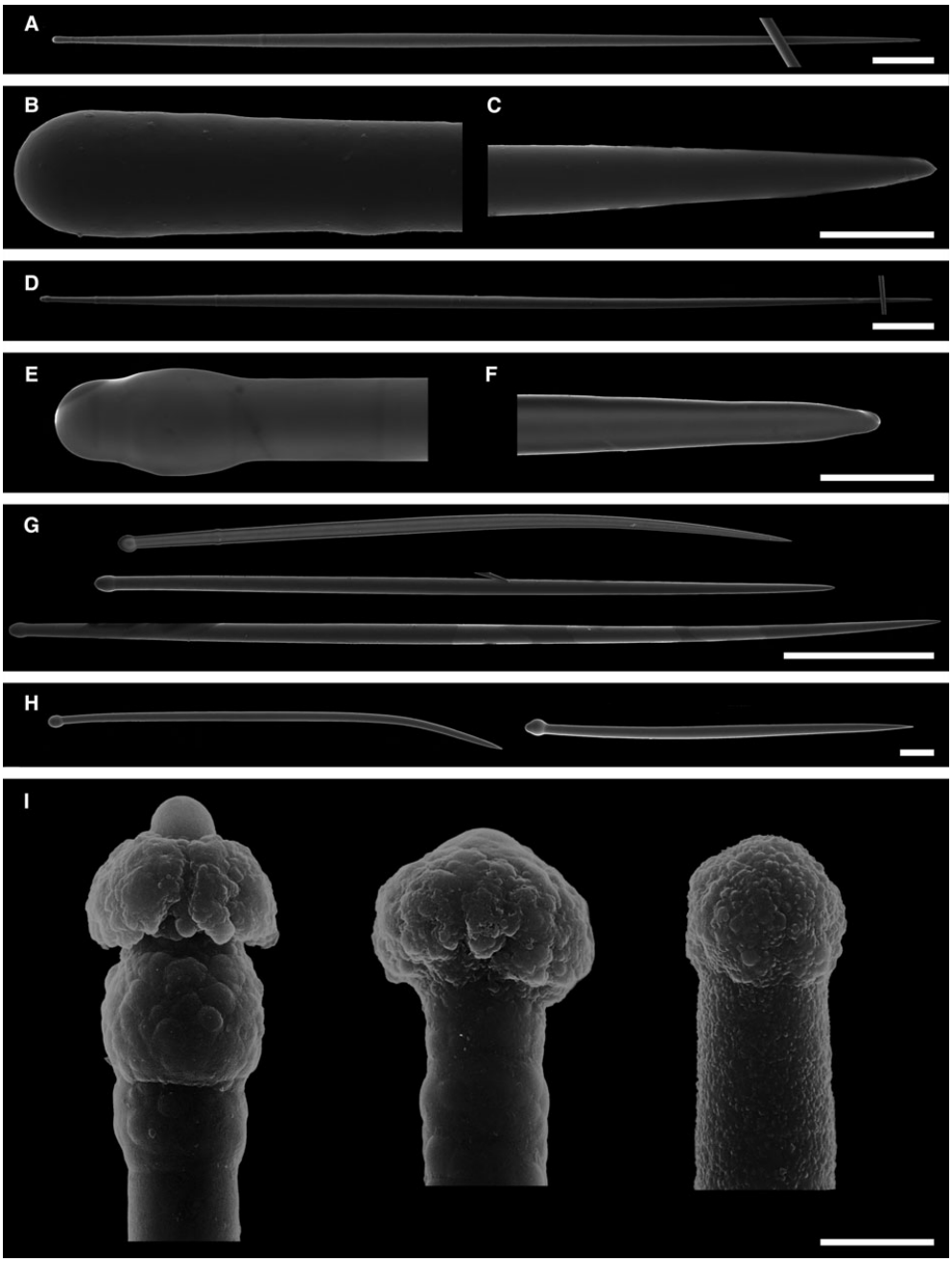

Fig. 11. *Sphaerotylus borealis*, spicules: (A) principal style, general view; (B) proximal tip of the style depicted in A, detailed view; (C) distal tip of the style depicted in A, detailed view; (D) principal subtylostyle, general view; (E) proximal tip of the subtylostyle depicted in D, detailed view; (F) distal tip of the subtylostyle depicted in D, detailed view; (G) intermediary tylostyles; (H) small tylostyles; (I) distal ornamentations of exotyles, detailed view. Scale bars: A, 0.1 mm; B and C, 0.01 mm; D, 0.1 mm; E and F, 0.01 mm; G, 0.1 mm; H and I, 0.01 mm.

9, transect 3, square 0.1 m², 21.07.1977, coll. Golikov. ZIN RAS 11194 (one specimen), Keret' Inlet, Sredny Island, Nagovitsa Harbour, Black Rock, Kandalaksha Bay, 66°17.391′N 33°38.025′E, 10–13 m, station, 12.07.2000, coll. Plotkin. ZIN RAS 11195 (16 specimens), Keret' Inlet, Sredny Island, Nagovitsa Harbour, Black Rock, Kandalaksha Bay, 66°17.391′N 33°38.025′E, 10–13 m, station, 12.07.2000, coll. Plotkin. ZIN RAS 11150 (one specimen, slide 21064), Kolvitsa Inlet, Kandalaksha Bay, 67°05.1′N 32°54.4′E, 20–30 m, RV 'Prof. Mesyatsev', station 856/5, 27.10.1961, coll. Kunin. ZIN RAS 11161 (one specimen, slide 5874), Kovda Inlet, Startseva Bight, Kandalaksha Bay Expedition of Voronezh University, 27.06.1917, coll. Sent-Iler. ZIN RAS 11162 (one specimen, slide 5609), Kovda Inlet, between Oleniy Island and Medvezhiy Island, Kandalaksha Bay, 10–12 m, Expedition of Voronezh University, 1917 or 1921, coll. Sent-Iler. ZIN RAS 11182 (one specimen, slide 9138), Umba Inlet, Kandalaksha Bay, 32 m, station 31(195), 27.06.1895, coll. Knipovich. ZIN RAS 11147 (one specimen), Neck of the White Sea, 65°45′N 39°00′E, 57 m, RV 'Pomor', station 51(15), 02.06.1983, coll. Gudimov. ZIN RAS 11155 (one specimen, slide 5525), Neck of the White Sea, 65°36′N 39°25′E, 54 m, Expedition of PMNI, station 57, 26.09.1921. ZIN RAS 11175 (one specimen, slide 9123), Onega Bay, 64°44′N 35°42.5′E, 30 m, Expedition of PMNI, station 448, 09.06.1926.

DESCRIPTION

External morphology

Holotype was a 3 × 1.5 × 1 cm piece torn from a large encrusting sponge during sampling. Surface was shaggy, with several whitish cylindrical or conical papillae up to 1 cm in length, some with visible oscula on the summits (description according to Swarczewsky, 1906). Neotype is a flattened thickly encrusting sponge measuring 4.5 × 2 × 1 cm (Figure 10A). Surface shaggy, dirty dark brown, overgrown with two ascidians. Twelve cylindrical yellowish papillae up to 0.7 cm long and 0.2 cm wide. Other specimens thickly encrusting or cushion-shaped, the largest up to 100 cm². Surface shaggy, silted with sediment making it dirty greyish or brownish in colour. Up to 50 cylindrical or conical papillae, whitish in life, but usually becoming pale yellow, brownish or pinkish in alcohol. On soft bottoms living sponges are often completely covered by sediment with only erect papillae protruding above the sediment. On hard bottoms the sponges may contract the papillae. After sampling and fixation the papillae always considerably contract and invaginate into the surface hispidation. Oscula not visible in preserved sponges.

Skeleton

Main choanosomal skeleton composed of longitudinal tracts of principal spicules which cross the cortex and make up a dense and thick surface hispidation (Figure 10B). Auxiliary choanosomal skeleton comprises small, occasionally intermediary, spicules often arranged in bundles, 3–7 spicules each. Cortex composed of a 115–120 μm thick palisade of small spicules and an internal layer (~ 210 μm thick) of tangentially arranged intermediary spicules (Figure 10B). In areas around papillae these layers are separated by an intermediate, aspicular zone (~ 100 μm thick) (Figure 19B). Exotyles cross the cortex and join the surface hispidation. Walls of papillae lack the tangential cortical layer. Single intermediary spicules scattered both in the walls and in the bulkheads between canals (Figure 10C).

Spicules

(measurements based on 10 specimens, N = 5 for exotyles, N = 10 for other categories)

- Principal spicules – straight, slender, often polytylote styles to subtylostyles (Figure 11A–F). Length 1100–2423–5000 μm, diameter of shaft 12.0–16.2–19.0 μm.
- Intermediary spicules – usually straight, occasionally curved, slightly fusiform tylostyles (Figure 11G). Length 200–502–796 μm, diameter of tyle 6.9–9.2–11.1 μm, proximal diameter of shaft 5.0–7.1–9.0 μm, maximum diameter of shaft 6.9–10.8–14.3 μm.
- Small spicules – straight or curved, usually slender tylostyles (Figure 11H). Length 94–125–160 μm, diameter of tyle 3.9–4.6–5.1 μm, diameter of shaft 3.0–3.5–4.0 μm.
- Exotyles slender, 5100–6117–7520 μm long, usually with weakly developed or completely reduced proximal tyles. Shafts 13.8–17.2–20 μm in maximum diameter. Distal knobs (14.1–19.9–27.0 μm in diameter) usually irregularly fungiform or umbrelliform, more rarely hemispherical or spherical, occasionally with short protuberances on the edges, sometimes slightly displaced along the shaft or comprising several swellings (Figure 11I). Surface of the knobs and the adjacent portions of the shafts rough, wrinkled, granulated or tuberculated.
- In their material, Swarczewsky (1906) and Koltun (1966) recorded infrequent thick and short fusiform strongyles (length 464–1300 μm, maximum diameter 40–59 μm) in the cortex, but in the sponges examined in the present study this category of spicules has not been observed.

OCCURRENCE

(Figure 12)

Arctic Ocean: between Svalbard and Franz Jozef Land, 415 m, between Kara and Laptev Sea, 43 m, Barents Sea, 91–420 m, White Sea, 5–100 m. North Atlantic: Norwegian Coast – Nord-Trøndelag, 157–240 m.

REMARKS

Sphaerotylus borealis (Swarczewsky, 1906) was originally assigned to *Proteleia* Dendy & Ridley, 1886, due to the similarity between the umbrelliform distal knobs of some exotyles in S. *borealis* and the grapnel-like distal ornamentations of the exotyles in the type species of *Proteleia, P. sollasi*. This placement was later followed by Boury-Esnault (2002). However, *P. sollasi* differs from *S. borealis* by a velvety surface, a three-layered cortex comprising two palisade layers and an inner layer of criss-cross spicules, and much shorter exotyles (not exceeding 0.6 mm). Substantial affinities between *Sphaerotylus borealis* and *S. antarcticus* along with their differences from the type species of *Sphaerotylus, S. capitatus*, and their similarities to *Koltunia burtoni* are discussed above in the Remarks section for *S. antarcticus*.

Sphaerotylus capitatus (Vosmaer, 1885)
(Figures 13 & 14)

Original description: *Polymastia capitata* Vosmaer, 1885, p. 16, pl. IV figures 25–29.

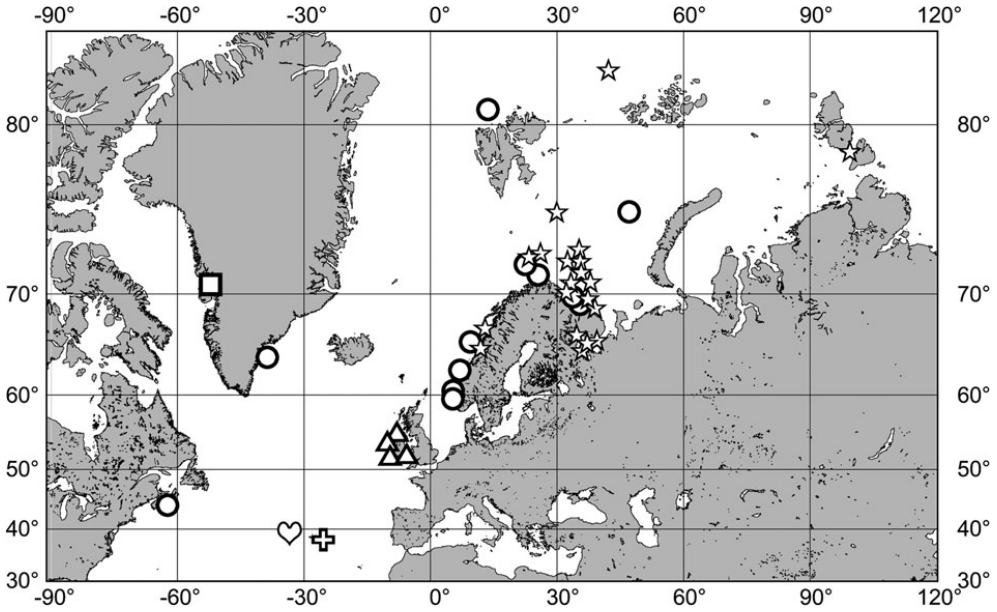

Fig. 12. Distribution of Polymastiidae with ornamented exotyles in the North Atlantic and Arctic: stars, *Sphaerotylus borealis*; circles, *Sphaerotylus capitatus*; triangles, *Sphaerotylus renoufi*; square, *Sphaerotylus tjalfei*; cross, *Trachyteleia stephensi*; heart, *Tylexocladus joubini*.

SYNONYMS AND CITATIONS

Polymastia capitata (Breitfuss, 1911, p. 218).
Polymastia schoenus (Dendy & Ridley, 1886, p. 155, text-fig.).
Radiella schoenus (Sollas, 1882, p. 162, considered as *nomen nudum* by Kirkpatrick, 1908, p. 18).
Sphaerotylus capitatus (Topsent, 1898, p. 244; Boury-Esnault, 2002, p. 206, figure 4; Plotkin, 2004, p. 543, figures 1H, 2H, 4A).
Sphaerotylus schoenus (Topsent, 1913, p. 23, pl. II figure 6; 1928, p. 154; Koltun, 1966, p. 85, pl. XXX figures 6 & 7, text-figure 56; Desqueyroux-Faúndez & Van Soest, 1997, p. 421).
Nec *Sphaerotylus capitatus* (Kirkpatrick, 1908, p. 18; Boury-Esnault & Van Beveren, 1982, p. 39; Uriz, 1988, p. 43; Sarà *et al.*, 1992, p. 568).
Nec *Sphaerotylus schoenus* (Burton, 1929, p. 447; Koltun, 1964, p. 28; Sarà *et al.*, 1992, p. 568).

TYPE MATERIAL

Lectotype (Figure 13A, specimen in alcohol) and one paralectotype (specimen in alcohol) (Figure 13B): RMNH 704, Barents Sea, 72°14.8′N 22°30.9′E, ~ 300 m (165 fathoms), 'Willem Barentz' Expedition, station 28, 30.06.1881.
Paralectotype: BMNH 1910.1.1.612 (specimen in alcohol) and BMNH 1910.1.1.1196-1200 (slides), from the same sample as the lectotype.
Paralectotype: ZMA 1841 (specimen, not studied), from the same sample as the lectotype.

COMPARATIVE MATERIAL EXAMINED

Barents Sea (six specimens):
ZIN RAS 1186 (slide 5445), at the traverse of Bolshaya Voronukha Island, Kola Bay, Murman Coast, 69°16′31.43″N 33°27′23.31″E, 213–235 m, RV 'Alexander Kovalevsky', station 93, 26.06.1909, coll. Derugin (one specimen). ZIN RAS 1187 (slide 5573), Cape Teriberka, Murman Coast, 69°15′08.45″N 35°09′03.95″E, depth unknown, 1880, coll. Hertzenstein (one specimen). ZIN RAS 1188 (slide 5957), near the exit from the Kola Bay to the Ekaterininskaya Harbour, Murman Coast, 69°12′33.96″N 33°26′52.23″E, 55–31 m, station 21, 21.06.1893, coll. Knipovich (one specimen). ZIN RAS 1189, 75°42′N 47°05′E, 309 m, expedition of ENPIM, RV 'St. Andrew Pervozvanny', station 705, 13.08.1902 (one specimen). ZIN RAS 1190, 71°30′N 25°30′E, 275 m, RV 'RT61-Vodnik', cruise 25, station 39, 10.06.1968 (one specimen). ZIN RAS 1191 (slides 7550–7551), 69°43′N 34°10′E, 142 m, Expedition of PMNI, station 295, 10.07.1925 (one specimen).
Svalbard (two specimens):
ZIN RAS 1185 (slides 6058, 12298, 12300), North from Svalbard, 80°35′N 13°35′E, 819 m, RV 'Litke', station 49, 11.10.1955, coll. Koltun (one specimen). ZIN RAS 1192 (slide 6844), SW from Svalbard, precise locality unknown, 608 m, RV 'Lena', station 1a, 11.03.1958, coll. Gorunova & Petrovskaya (one specimen).
Greenland (one specimen):
ZIN RAS 1193 (slide 14714), East Greenland, 64°13′N 38°48′W, 420–450 m, RV 'RT 97′, cruise 21, 1964.
Norwegian Coast (six specimens):
ZMBN 98042, Hordaland, Korsfjorden, North of Stora Skorpa, 60°09.702′ N 5°10.4832′ E, 500–200 m, 10.03.2006, coll. Rapp (one specimen). ZMB 10855, Hordaland, Byfjorden near Bergen, depth unknown, coll. Schaudinn, 1891 (one specimen, misidentified as *Polymastia uberrima* (Schmidt, 1870) by Arndt). HTR, Hordaland, Bømlafjorden,

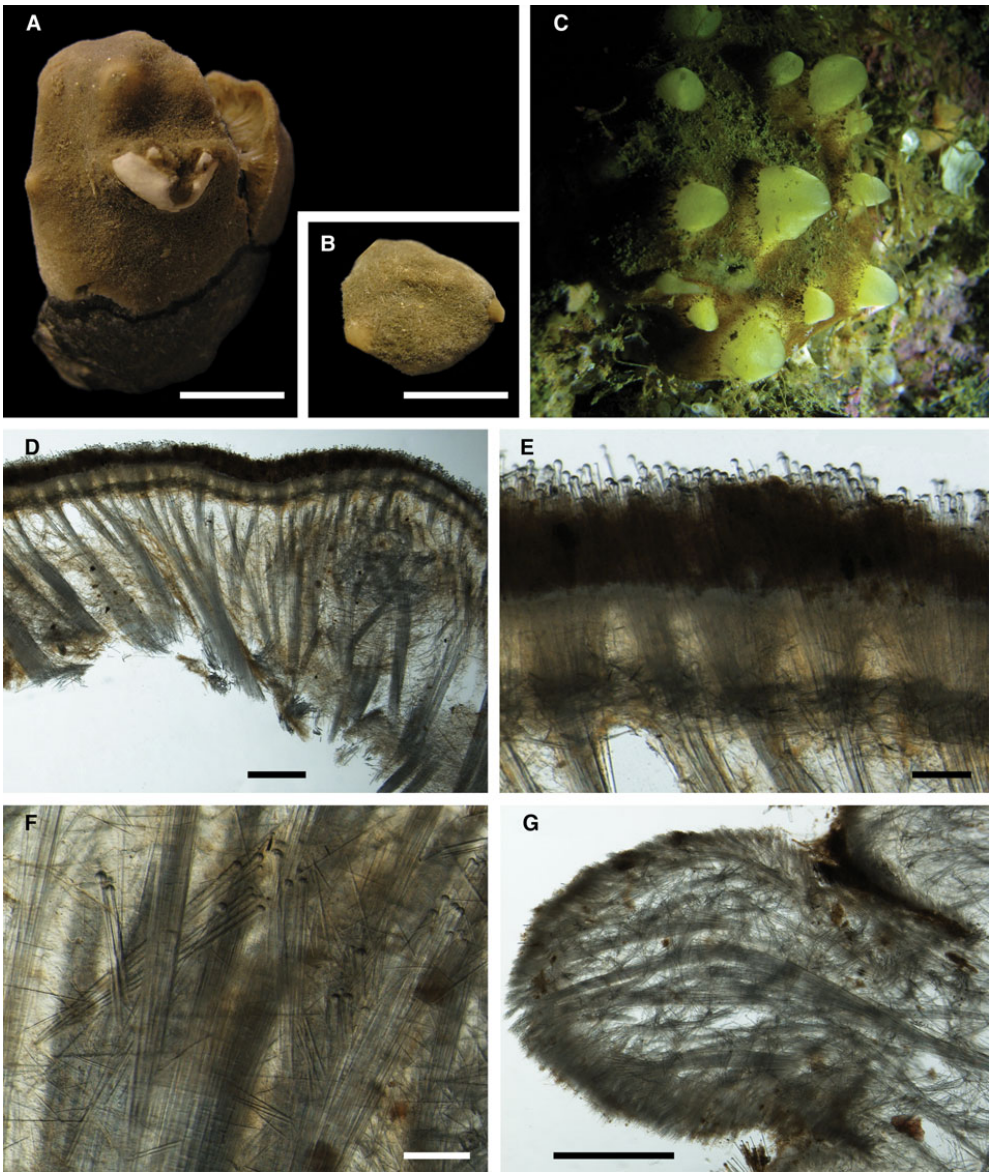

Fig. 13. *Sphaerotylus capitatus*: (A) lectotype RMNH 704, habitus; (B) paralectotype RMNH 704, habitus; (C) specimen ZMBN 98075 *in situ* near Haugbergnes, Troms, Norwegian Sea (courtesy of B.T. Dragnes, OMNIMAR Dragnes, Tromsø); (D) longitudinal section through the body of the lectotype, general view. E, the same section, detail of cortex; (F) the same section, detail of choanosome with exotyles; (G) longitudinal section through a papilla of a specimen from Hordaland, Norway. Scale bars: A and B, 10 mm; D, 1 mm; E, 0.2 mm; F and G, 0.2 mm.

SE from Store Bleikja, 59°36.700–36.750′N 05°15.785–15.450′E, 300–78 m, RV 'Hans Brattstrøm', station 13, 04.07.2006, coll. Rapp (one specimen). HTR, Møre & Romsdal, 62°43.81′N 06°57.80′E, depth unknown, RV 'Håkon Mosby', station 33(329), 12.10.2005, coll. Rapp (one specimen). HTR, Møre & Romsdal, 62°54.12′N 06°50.53′E, 130–190 m, RV 'Håkon Mosby', station 38, 12.10.2005, coll. Rapp (one specimen). ZMBN 98075, Tromsø, Haugbergnes, 69°31.16′ N 19°00.68′ E, 25 m, 20.06.2012, coll. Plotkin (one specimen).

Swedish Coast (four specimens):

GNM 899, 58°28.357–28.308′N 10°29.646–29.289′E, 239–314 m, Expedition of the Swedish marine inventories, station SK 119, 29.08.2007, coll. Hansson (one specimen). GNM 900, 58°26.336–26.447′N 10°31.041–30.852′E, 265–309 m, Expedition of the Swedish marine inventories,

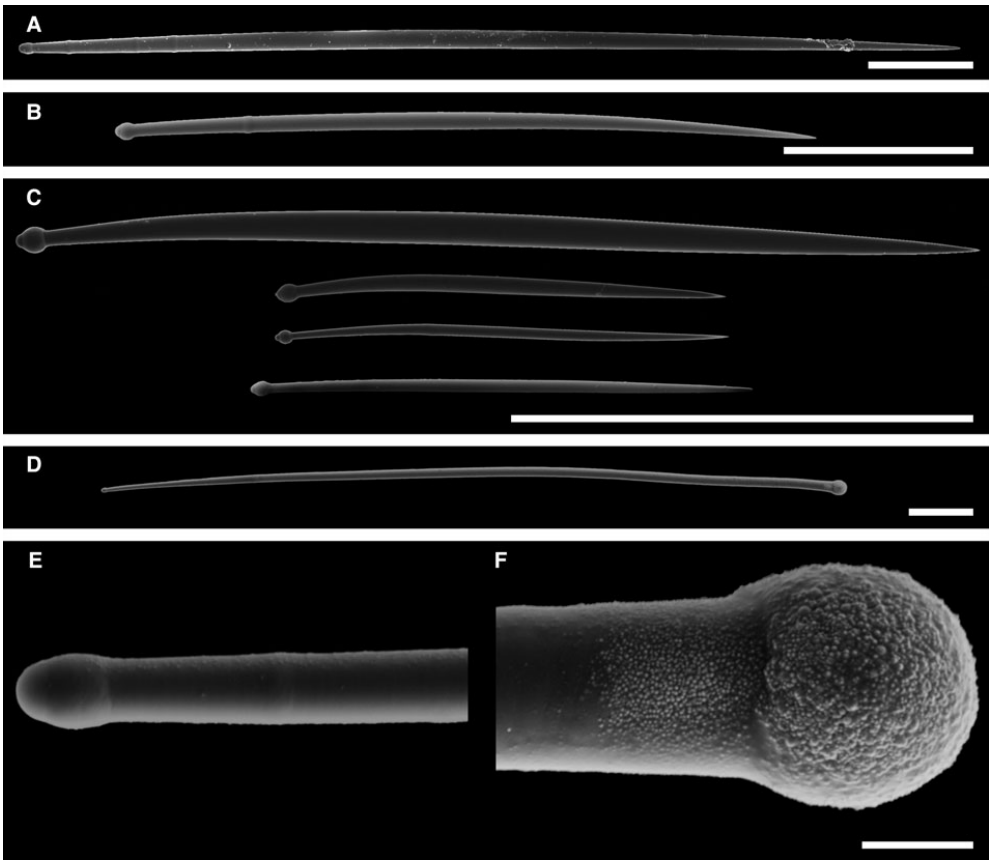

Fig. 14. *Sphaerotylus capitatus*, spicules: (A) principal subtylostyle; (B) intermediary tylostyle; (C) small tylostyles; (D) exotyle, general view; (E) proximal tip of the exotyle depicted in D, detailed view; (F) distal knob of the exotyle depicted in D, detailed view. Scale bars: A–D, 0.1 mm; E and F, 0.01 mm.

station SK 121, 29.08.2007, coll. Hansson, (two specimens). GNM 902, 58°24.530–24.678′N 10°29.877–29.537′E, 266–317 m, Expedition of the Swedish marine inventories, station SK 159, 14.06.2008, coll. Hansson (one specimen).

DESCRIPTION

External morphology

Lectotype fist-shaped sponge, 2–2.5 cm in diameter, attached to a stone and incorporating a piece of a hard coral skeleton (Figure 13A). Surface rough, knobbly and brownish. Several weakly developed or contracted pale papillae. Paralectotype RMNH 704 dome-shaped, 1.4 cm high (Figure 13B). Surface slightly hispid, with a single well-developed but invaginated papilla. Other sponges thickly encrusting, cushion-shaped or massive, fist- and dome-shaped, the largest up to 50 cm^2. Surface velvety, knobbly, dark brown in colour, with up to 30 papillae. Papillae of living sponges whitish or pale yellow in colour, conical, with small scarcely visible oscules on the summits (Figure 13C). In alcohol-preserved specimens the papillae may be considerably contracted looking like tubercles, while their colour does not change much.

Skeleton

Main choanosomal skeleton composed of radial or longitudinal tracts of principal spicules which enter the cortex (Figure 13D, E). Auxiliary choanosomal skeleton comprises small and intermediary spicules usually scattered singly or sometimes arranged in small groups. Some specimens including the lectotype and paralectotype BMNH 10.1.1.1199–1200 also possess exotyles between the choanosomal tracts (Figure 13F). Cortex composed of an outer palisade (~ 110 μm thick) of small spicules, an inner layer (~ 170 μm thick) of tangentially arranged intermediary spicules and an intermediate layer (180–190 μm thick) with a low concentration of spicules. Exotyles cross the cortex forming a dense superficial layer with their distal knobs rising above the palisade (Figure 13E). Papillae walls without the inner cortical layer (Figure 13G). Single intermediary spicules scattered both in the papillae walls and in the bulkheads between the canals.

Spicules

(measurements based on five specimens, N = 10)

- Principal spicules – straight, slightly fusiform or slender, often polytylote subtylostyles to styles (Figure 14A). Length

650–998–1505 μm, diameter of tyle if present 10.0–12.8–16.0 μm, proximal diameter of shaft 8.9–11.5–15.1 μm, maximum diameter of shaft 14.0–19.5–26.0 μm.
- Intermediary spicules – straight or gently curved, slender or slightly fusiform tylostyles (Figure 14B). Length 314–484–650 μm, diameter of tyle 9.1–11.4–14.0 μm, proximal diameter of shaft 6.9–8.8–11.0 μm, maximum diameter of shaft 9.0–13.0–16.5 μm.
- Small spicules – straight or curved, usually slender tylostyles (Figure 14C). Length 96–155–221 μm, diameter of tyle 2.9–4.6–6.1 μm, proximal diameter of shaft 1.1–2.3–3.2 μm, maximum diameter of shaft 2.0–5.0–7.0 μm.
- Exotyles straight or gently curved, slender, 650–974–1250 μm long (Figure 14D). Proximal tyles varying from well-developed (6.8–11.0–14.0 μm in diameter) to reduced (Figure 14E). Distal knobs usually regularly spherical, occasionally hemispherical or elongated, 18.0–22.8–30.0 μm in diameter. Surface of the knobs and the adjacent portions of the shafts usually rough, spined or granulated (Figure 14F). Shafts gradually expanding towards the distal knobs.

OCCURRENCE

(Figure 12)

Arctic Ocean: Barents Sea, 31–309 m, North Svalbard, 608–819 m. North Atlantic: Norwegian Coast – from Troms in the north to Sunnhordland in the south, 25–440 m, Swedish Western Coast, 239–317 m, East Greenland, 420–450 m, Canadian Coast – Nova Scotia, 75 m (data from Topsent, 1928).

REMARKS

This well-defined and widely known North Atlantic species has a confused synonymy. In 1882 Sollas mentioned very briefly his new species *Radiella schoenus* when discussing the characters of *Tetilla* and *Rhaphidotheca*: 'The rounded swelling of the distal ends of projecting spicules is not confined to *Rhaphidotheca*; I have it in a less marked form in a suberite to which I give the name of *Radiella schoenus* (σχοίνος, a bullrush) . . . The swollen terminations of the spicules of *R. schoenus* suggest the possibility of a polyphyletic origin for the Tetractinellida.' (pp. 162–163). In 1885 Vosmaer described a very similar species as *Polymastia capitata*. After examination of Sollas's material, Dendy & Ridley (1886) synonymized *P. capitata* with *R. schoenus*, the latter becoming the senior synonym, but retained this species in *Polymastia*. Despite the act by Dendy and Ridley, Topsent (1898) erected a new genus, *Sphaerotylus*, for *P. capitata* but not for *R. schoenus*. However, later (Topsent, 1913) he acknowledged the seniority of *R. schoenus*. Meanwhile, Kirkpatrick (1908) considered *R. schoenus* as a *nomen nudum*. Since then both names, *S. schoenus* and *S. capitatus* (occasionally allocated to *Polymastia*), have been used in different papers (e.g. Topsent, 1928; Koltun, 1966; Boury-Esnault, 2002; Plotkin, 2004). Moreover, sponges found in the southern hemisphere (including the Antarctic) that have similar morphologies, have also been identified under the same names, *S. capitatus* or *S. schoenus* (Burton, 1929; Koltun, 1964; Boury-Esnault & Van Beveren, 1982; Uriz, 1988; Barthel *et al.*, 1990; Sarà *et al.*, 1992). Formally *R. schoenus* cannot be regarded as *nomen nudum* since Sollas mentioned at least one feature of it, although his description is extremely poor. Nevertheless, for stability reasons we follow Boury-Esnault (2002) and accept the name *S. capitatus* as valid since it has been used more frequently than *S. schoenus* in the last decades. We also agree with her that the records of *S. capitatus*/*S. schoenus* from the southern hemisphere should be regarded as another species. These records are gathered under the species name *S. vanhoeffeni* Hentschel, 1914 below.

Sphaerotylus exospinosus Lévi, 1993
(Figure 15)

Original description: *Sphaerotylus exospinosus* Lévi, 1993, p. 25, figure 6c.

TYPE MATERIAL

Holotype: MNHN D-CL 3583 (specimen in alcohol), New Caledonia, SW Pacific, 22°53.05′S 167°17.08′E, 570–610 m; BIOCAL campaign on RV 'Jean Charcot' in 1985, station DW 46. Lévi based his description on a small sponge fragment which was completely used for making preparations. We have examined these microscopic slides.

DESCRIPTION

External morphology

(according to Lévi, 1993)

Holotype was a piece of a cushion-shaped sponge. Its surface was greyish-pale yellow, hispid because of protruding knobs of exotyles, without papillae.

Skeleton

(according to Lévi, 1993)

Main choanosomal skeleton was composed of longitudinal tracts of principal spicules which extended to the cortex. The cortex comprised a palisade of small spicules and an inner layer of transversal bundles of intermediary spicules. Exotyles rose from the choanosome, crossed the cortex and formed a superficial hispidation actually composing the major portion of the sponge skeleton.

Spicules

(our data, N = 3 for not fully developed exotyles, N = 10 for other categories)

- Principal spicules – straight, slightly fusiform subtylostyles (Figure 15A). Length 418–484–622 μm, diameter of tyle 6.5–7.8–9.1 μm, proximal diameter of shaft 3.9–5.1–5.2 μm, maximum diameter of shaft 10.4–12.7–15.6 μm.
- Intermediary spicules – gently curved or straight, fusiform tylostyles (Figure 15B). Length 244–307–449 μm, diameter of tyle 7.8–9.6–13.0 μm, proximal diameter of shaft 5.2–6.0–7.8 μm, maximum diameter of shaft 11.7–13.1–15.6 μm.
- Small spicules – gently curved, fusiform tylostyles (Figure 15C). Length 93–103–117 μm, diameter of tyle 5.2–5.8–6.5 μm, proximal diameter of shaft 2.6–2.9–3.9 μm, maximum diameter of shaft 3.9–4.7–5.2 μm.
- Fully developed exotyles (Figure 15D) 745–926–1041 μm long, with well-developed proximal tyles

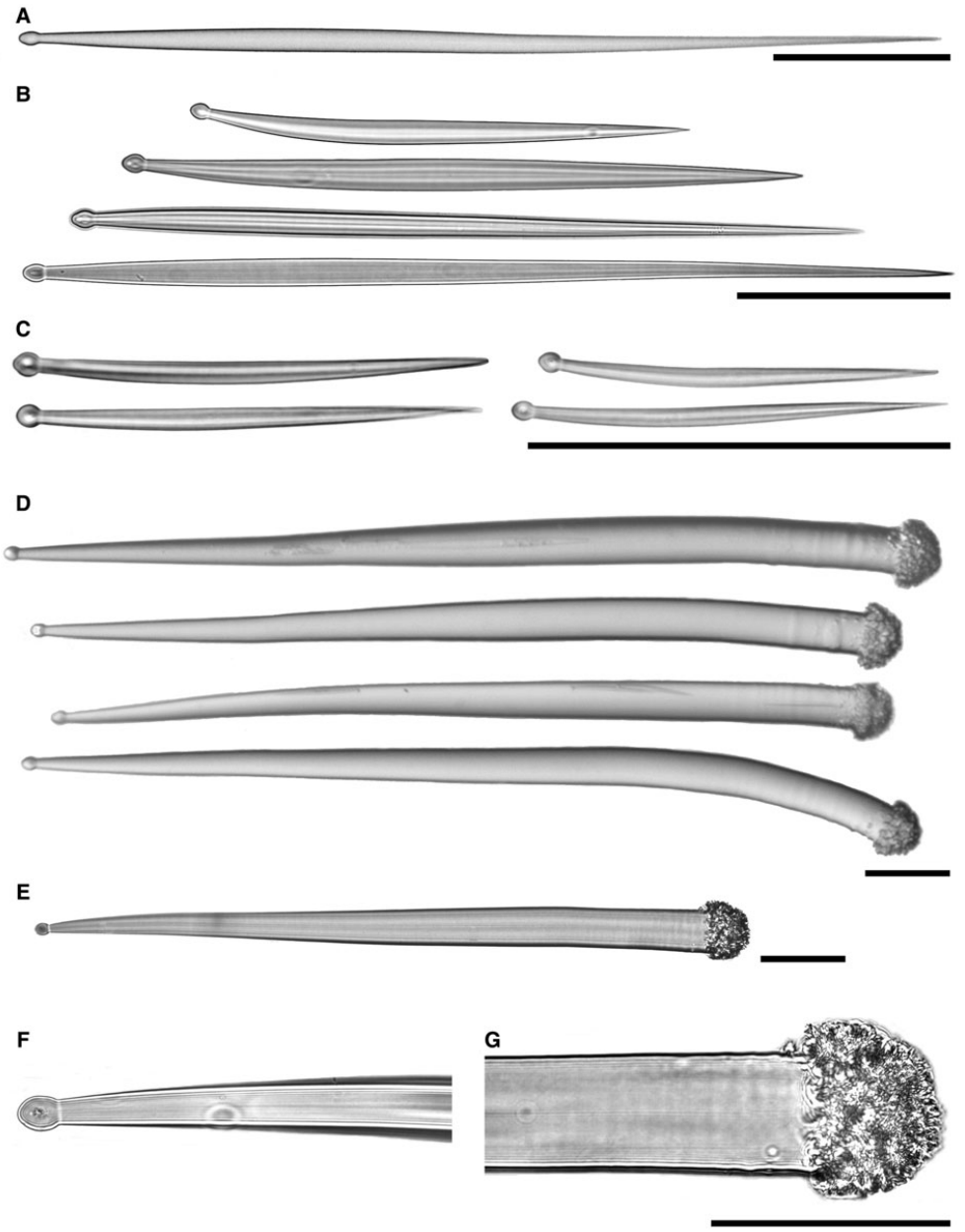

Fig. 15. *Sphaerotylus exospinosus*, spicules on the type slide MNHN D-CL 3583: (A) principal subtylostyle; (B) intermediary tylostyles; (C) small tylostyles; (D) fully developed exotyles; (E) not fully developed exotyle, general view; (F) proximal tip of the exotyle depicted in E, detailed view; (G) distal knob of the exotyle depicted in E, detailed view. Scale bars: A–G, 0.1 mm.

(13.0–15.3–18.2 μm in diameter, Figure 31F), gradually expanding from 7.8–10.8–13.0 μm (shaft diameter near tyle) to 39.0–46.5–51.9 μm (shaft diameter near distal knob). Distal knobs (62.3–72.2–80.5 μm in diameter) cauliflower-shaped, i.e. the widened distal tip is ornamented by a dense crown of branching protuberances. Shaft under the main ornamentation often with small tubercules.

- Not fully developed exotyles of the same shape as the fully developed ones, but smaller. Length 500–571–633 μm,

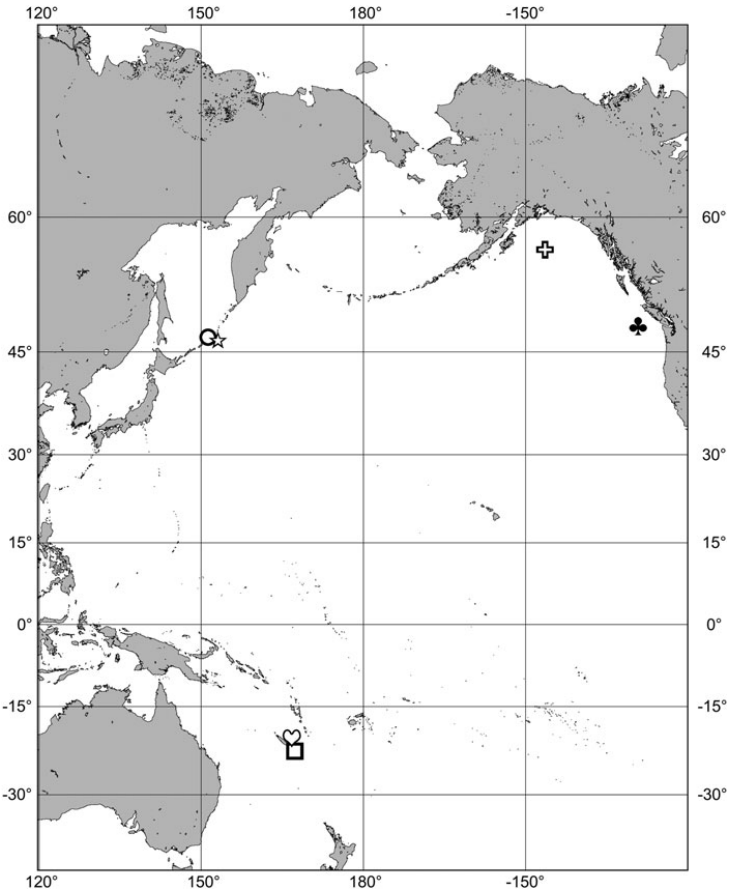

Fig. 16. Distribution of Polymastiidae with ornamented exotyles in the Pacific: square, *Sphaerotylus exospinosus*; circle, *Sphaerotylus exotylotus*; cross, *Sphaerotylus raphidophora*; star, *Sphaerotylus sceptrum*; trefoil, *Sphaerotylus verenae*; heart, *Tylexocladus hispidus*.

diameter of tyle 10.4–11.7–13.0 μm, proximal diameter of shaft ~ 8 μm, distal diameter of shaft 20.8–27.7–31.2 μm, diameter of distal knob 33.8–44.1–51.9 μm (Figure 15E–G).

OCCURRENCE

(Figure 16)

Known only from the type locality off New Caledonia, SW Pacific.

REMARKS

Lévi (1993) established *Sphaerotylus exospinosus* based on the uniqueness of the cauliflower-shaped ornamentations of its exotyles. However, except for this feature no data on its similarities to and distinctions from other *Sphaerotylus* spp. can be obtained because of the lack of tissue material.

Sphaerotylus exotylotus Koltun, 1970
(Figures 17 & 18)

Original description: *Sphaerotylus exotylotus* Koltun, 1970, p. 175, pl. VII figures 1 & 2, text-figure 7.

SYNONYMS AND CITATIONS

Sphaerotylus exotylotus (Plotkin, 2002, p. 106, figure 3.)

TYPE MATERIAL

Lectotype (designated herein, see Figure 17A): ZIN RAS 10615 (specimen in alcohol), slide 16160), Simushir Island, Kurile Islands, NE Pacific, 46°38′N 152°03′E, 1440–1540 m, RV 'Vityaz', cruise 39, station 5594, 12.07.1966.

Paralectotypes (Figure 17B, C): ZIN RAS 10615 (two specimens in alcohol), from the same sample as the lectotype.

DESCRIPTION

External morphology

Small, thick, cushion-shaped sponges detached from substrata (Figure 17A–C). Surface for the most part rough or velvety, knobbly and dark brown in colour (Figure 17D). Each specimen with a single exhalant papilla which in the preserved state is considerably contracted and invaginated into the surface. Area surrounding the papilla free of knobs, wrinkled and

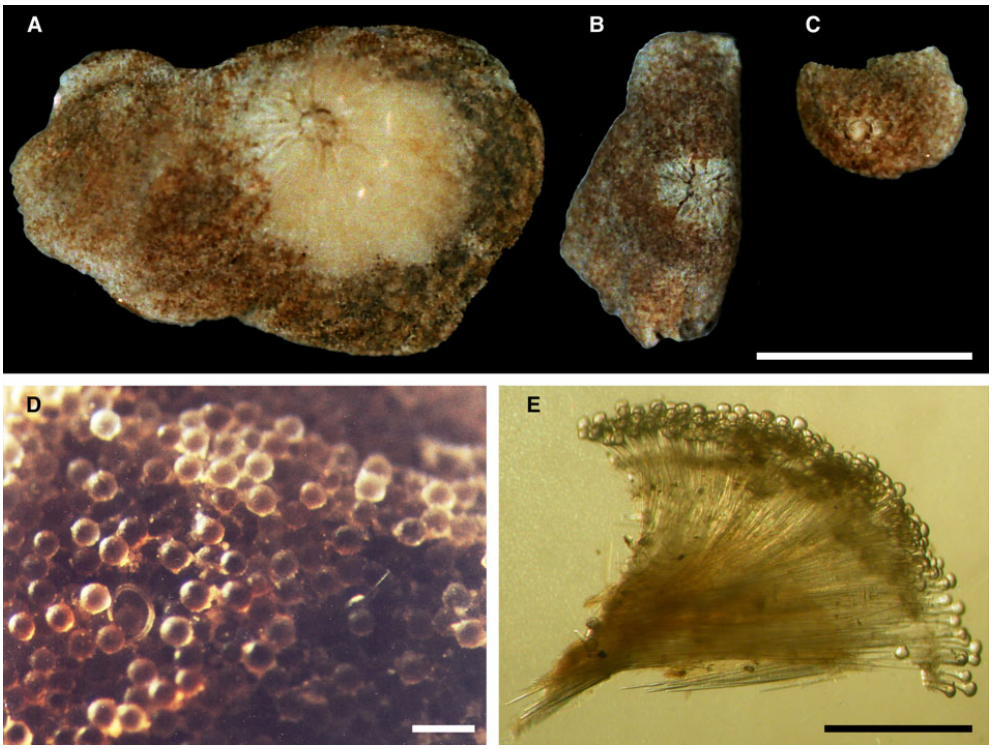

Fig. 17. *Sphaerotylus exotylotus*: (A) lectotype ZIN RAS 10615, habitus; (B) and (C) paralectotypes ZIN RAS 10615, habitus; (D) surface of the lectotype, detailed view; (E) longitudinal section through the body of the lectotype. Scale bars: A–C, 10 mm; D, 0.2 mm; E, 1 mm.

light in colour. Lectotype 2.4 × 1.5 × 0.6 cm in size, with the smooth area around its papilla occupying ~ 1/3 of the surface (Figure 17A). One of the paralectotypes 1.5 × 0.8 × 0.3 cm in size, with the smooth area around its papilla slightly reduced (Figure 17B). The other paralectotype 0.8 × 0.6 × 0.2 cm in size, with the smooth area hardly visible with the naked eye (Figure 17C).

Skeleton

Main choanosomal skeleton composed of radial tracts of principal spicules which enter the cortex (Figure 17E). Auxiliary choanosomal skeleton comprises singly scattered small and intermediary spicules and occasionally exotyles. Dense superficial cortical palisade made of exotyles, between which small spicules are embedded. Internal cortical layer of criss-cross intermediary spicules confused, loose and disrupted by the exotyles.

Spicules

(measurements based on three specimens, N = 30)

- Principal spicules – usually straight, slightly fusiform subtylostyles (Figure 18A–F). Length 700–1183–1700 μm, diameter of shaft 15.0–19.2–25.0 μm.
- Intermediary spicules – gently curved, slightly fusiform tylostyles (Figure 18G). Length 200–326–500 μm, diameter of shaft 8.2–11.3–14.0 μm.
- Small spicules – straight or gently curved, slender tylostyles (Figure 18H). Length 100–138–180 μm, diameter of shaft 5.1–6.8–8.0 μm.
- Exotyles straight, clavate, 500–668–850 μm long (Figure 18I). Proximal tyles usually well-developed, occasionally weakly developed, 18.5–23.6–30.0 μm in diameter. Distal knobs well-developed, regular, bulb- or pear-shaped, with rough, spined or granulated surface, 80.2–97.8–110.0 μm in diameter.

OCCURRENCE

(Figure 16)

Known only from the type locality off the Kurile Islands, NW Pacific.

REMARKS

Sphaerotylus exotylotus resembles *S. vanhoeffeni*, especially in the substitution of the palisade of exotyles for the ordinary palisade of tylostyles and the inner layer of criss-cross spicules in the cortex, but differs by the peculiar clavate shape of the exotyles.

Sphaerotylus isidis (Thiele, 1905) comb. nov.
(Figures 19 & 20)

Original description: *Polymastia isidis* (Thiele, 1905, p. 414, figures 25 and 38a–e).

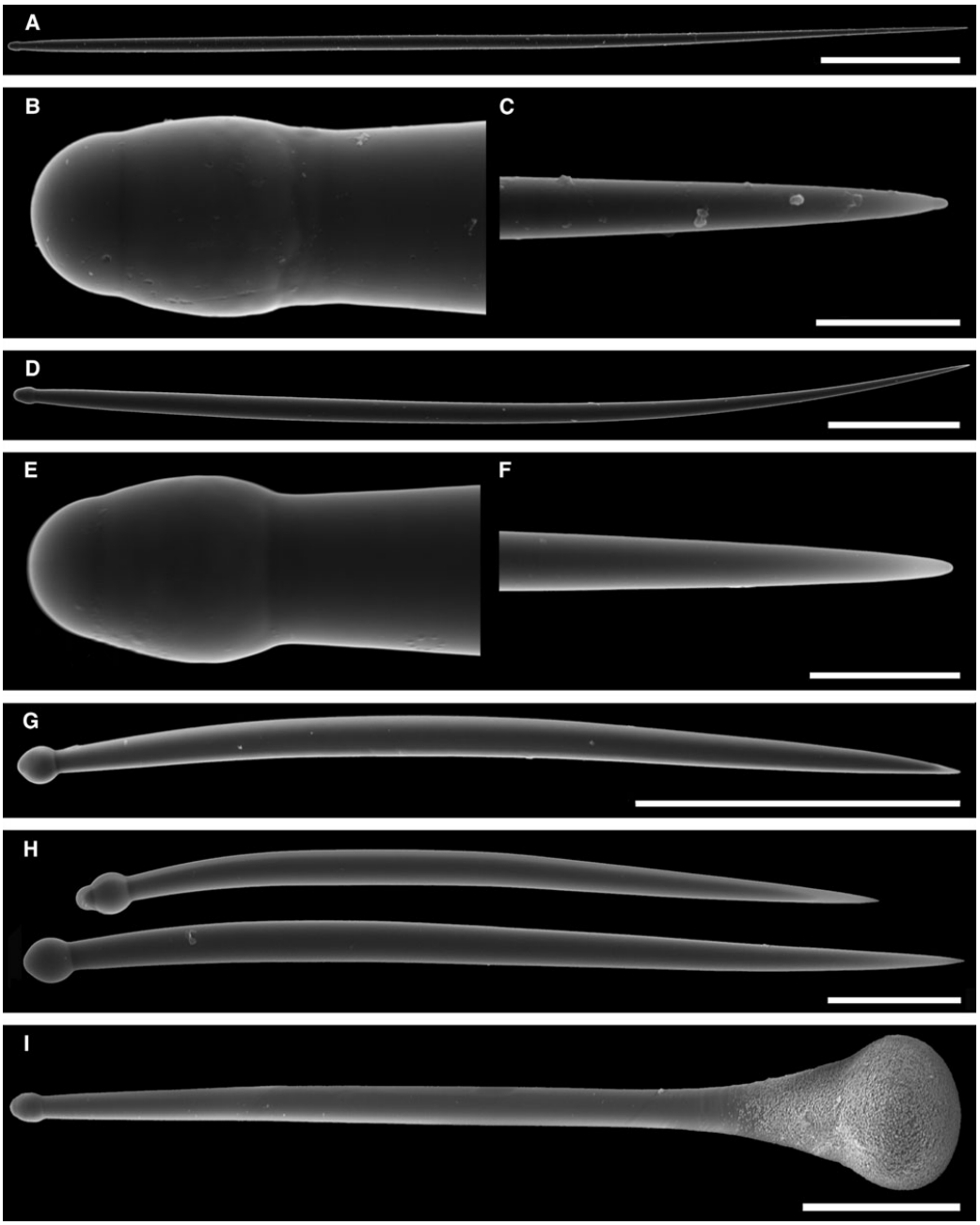

Fig. 18. *Sphaerotylus exotylotus*, spicules: (A) long principal subtylostyle, general view; (B) proximal tip of the subtylostyle depicted in A, detailed view; (C) distal tip of the subtylostyle depicted in A, detailed view; (D) short principal subtylostyle, general view; E, proximal tip of the subtylostyle depicted in D, detailed view; (F) distal tip of the subtylostyle depicted in D, detailed view; (G) intermediary tylostyle; (H) small tylostyles; (I) exotyle. Scale bars: A, 0.2 mm; B and C, 0.01 mm; D, 0.1 mm; E and F, 0.01 mm, G, 0.1 mm; H, 0.02 mm; I, 0.1 mm.

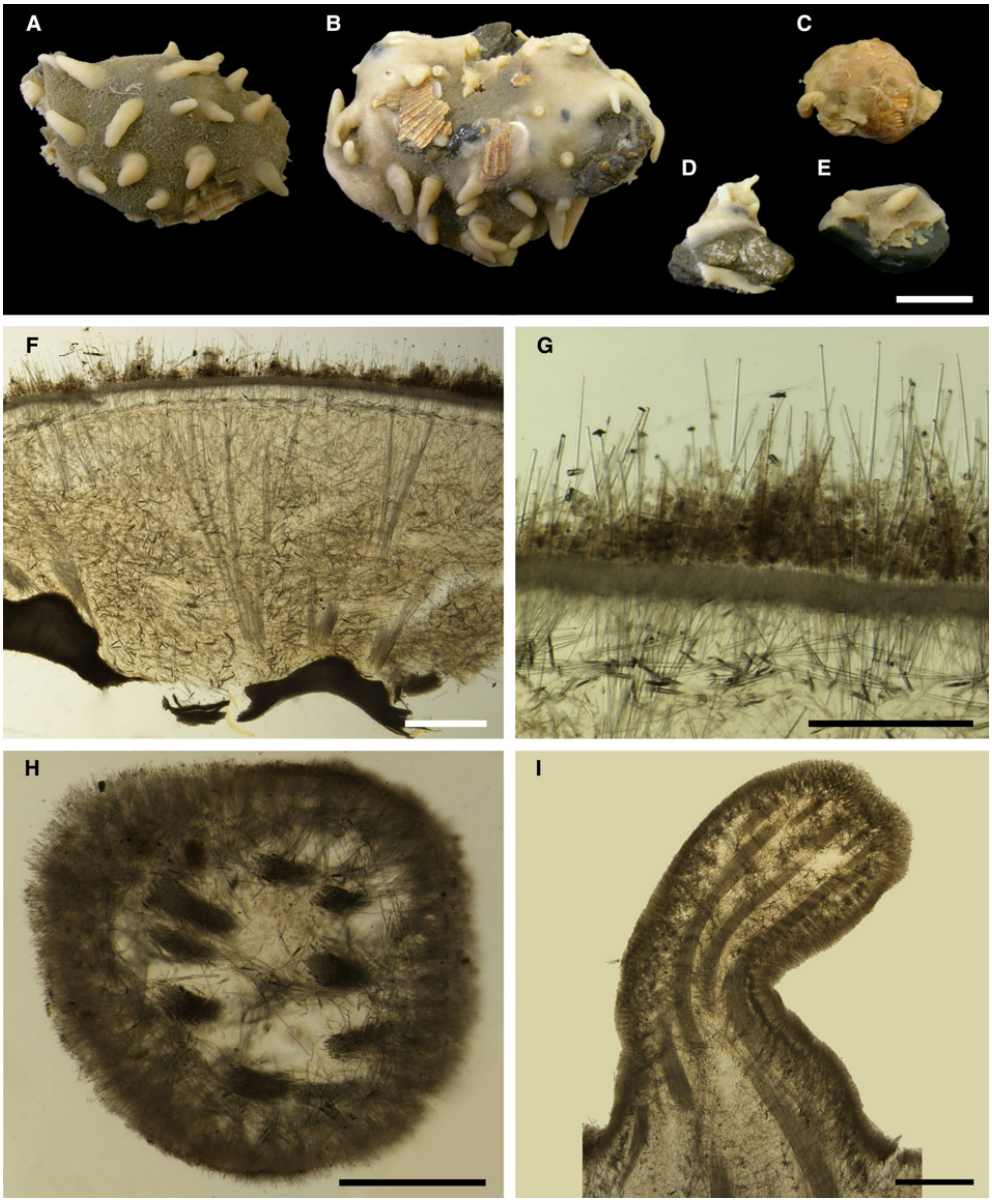

Fig. 19. *Sphaerotylus isidis*: (A) lectotype ZMB 3271, habitus; (B)–(E), paralectotypes, ZMB 3271, habitus; (F) longitudinal section through the body of the lectotype, general view; (G) the same section, detailed view of cortex; (H) transversal section through a papilla of the paralectotype depicted in B; (I) longitudinal section through another papilla of the same paralectotype. Scale bars: A–E, 10 mm; F, 1 mm; G and H, 0.5 mm; I, 1 mm.

SYNONYMS AND CITATIONS

Nec *Polymastia isidis* (Burton, 1932, p. 337; Koltun, 1964, p. 26; Desqueyroux, 1975, p. 57; Boury-Esnault & Van Beveren, 1982, p. 35, pl. 4 figure 15; Uriz, 1988, p. 44, figure 20a–c).

Nec *Polymastia isidis* var. *simplex* Hentschel, 1914, p. 47, pl. V figure 3.

TYPE MATERIAL

Lectotype (designated herein, see Figure 19A): ZMB 3271 (specimens in alcohol), Almirantazgo Sound (Admiralty Sound), Tierra del Fuego, Chilean Coast, SE Pacific, 54°19.0′S 69°30.0′W, 19 m, coll. Plate.

Paralectotypes (Figure 19B–E): ZMB 3271 (four specimens in alcohol), from the same sample as the holotype.

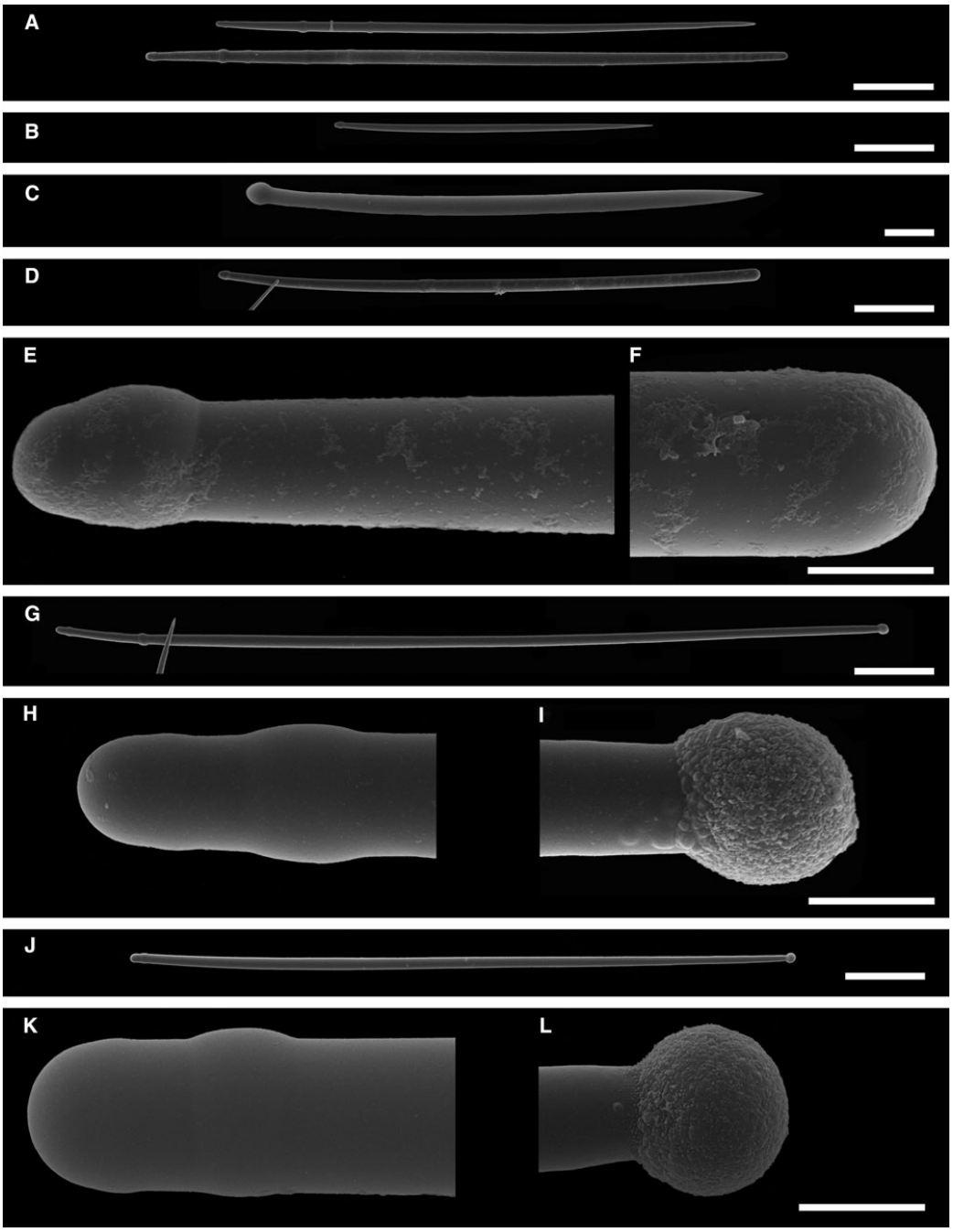

Fig. 20. *Sphaerotylus isidis*, spicules: (A) principal styles; (B) intermediary subtylostyle; (C) small tylostyle; (D) exotyle with rounded distal tip, general view; (E) proximal tip of the exotyle depicted in D, detailed view; (F) distal tip of the exotyle depicted in D, detailed view; (G) exotyle with slightly irregular, spherical distal knob, general view; (H) proximal tip of the exotyle depicted in G, detailed view; (I) distal knob of the exotyle depicted in G, detailed view; (J) exotyle with regularly spherical distal knob, general view; (K) proximal tip of the exotyle depicted in J, detailed view; (L) distal knob of the exotyle depicted in J, detailed view. Scale bars: A and B, 0.1 mm; C, 0.01 mm; D, 0.1 mm; E and F, 0.01 mm; G, 0.1 mm; H and I, 0.01 mm; J, 0.1 mm, K and L, 0.01 mm.

DESCRIPTION

External morphology

Encrusting sponges with prominent cylindrical or slightly conical papillae which lack visible oscula. Surface mostly rough, dirty greyish in colour, but partly smooth and pale. Lectotype 3.6 × 2.3 cm in size, with 17–18 papillae, attached to a bivalve shell (Figure 19A). Paralectotypes with less rough surface, attached to pebbles and/or to shell fragments. The largest paralectotype 4.3 × 2.9 cm in size, with ~ 26 papillae (Figure 19B). Other paralectotypes damaged (Figure 19C–E).

Skeleton

Main choanosomal skeleton composed of longitudinal or radial tracts of principal spicules entering the cortex and partly protruding above it (Figure 19F). Auxiliary choanosomal skeleton formed by scattered intermediary and small spicules, the latter usually arranged in dense bundles of up to 10 spicules each. Cortex comprises a palisade (~ 110 μm thick) of small spicules and an inner layer (70–80 μm thick) of tangentially arranged intermediary spicules, separated by a distinct zone (~ 180 μm thick) with few spicules (Figure 19G). Exotyles sparsely scattered over the cortex rising above the palisade. Both cortical layers extend to the papillae walls (Figure 19H, I). Bulkheads between the canals reinforced by the intermediary spicules (Figure 19H).

Spicules

(measurements based on lectotype and two paralectotypes, N = 15 for exotyles, N = 30 for other categories)

- Principal spicules – straight, slender subtylostyles with displaced tyles, often polytylote and with rounded distal tips (Figure 20A). Length 679–751–818 μm, diameter of tyle 8.8–13.5–17.9 μm, proximal diameter of shaft 7.5–8.6–10.2 μm, maximum diameter of shaft 12.9–15.2–17.9 μm.
- Intermediary spicules – straight subtylostyles to tylostyles (Figure 20B). Length 400–418–448 μm, diameter of tyle 8.2–9.0–9.9 μm, proximal diameter of shaft 5.4–7.5–9.5 μm, maximum diameter of shaft 10.1–11.2–12.3 μm.
- Small spicules – straight or gently curved, slender tylostyles to subtylostyles (Figure 20C). Length 106–160–210 μm, diameter of tyle 4.7–6.8–8.1 μm, proximal diameter of shaft 3.2–4.9–7.2 μm, maximum diameter of shaft 4.0–6.3–8.2 μm.
- Exotyles usually gently curved, slightly fusiform (Figure 20D, G, J). Length 682–863–1085 μm, maximum diameter of shaft 12.9–15.6–18.8 μm. Proximal tyles weakly developed, occasionally displaced or absent (Figure 20E, H, K). Some exotyles with extra tyles along the shafts (Figure 20G). Distal knobs (diameter 11.7–13.8–15.5 μm) mostly of regularly spherical shape, more rarely slightly irregular, with granulated surface (Figure 20I, L). Occasionally the knob is absent, and an exotyle terminates with a gradually expanded blunt distal tip (Figure 20F).

OCCURRENCE

(Figure 3)

Known only from the type locality off the Chilean coast, SE Pacific. Records from other regions need verification.

REMARKS

We transfer *isidis* from *Polymastia* to *Sphaerotylus* since the type specimens possess exotyles with spherical distal knobs, which is the main diagnostic feature of the type species of *Sphaerotylus*. Meanwhile, neither the author of *S. isidis* (Thiele, 1905), nor the early investigators of the type material (Desqueyroux-Faúndez & Van Soest, 1997) noted the exotyles. Evidently they made preparations only from the edge parts of the sponges where the exotyles were damaged. Comparing *S. isidis* with their new species *Polymastia villosa* Desqueyroux-Faúndez & van Soest (1997) wrote: 'We have also examined the holotype (here designated) ZMB 3267, of *Polymastia isidis* Thiele, 1905, from Chile, which is distinct from our new species in the size of the largest tylostyles, which reach only 850 × 15 μm. That species was also reported from Kerguelen (Boury-Esnault & Van Beveren, 1982) with larger tylostyles (up to 1600 μm) and with several papillae; this may turn out to be a separate species' (p. 421). This refers to the designation of the lectotype, but it is unclear which of the syntypes they had examined because there was no picture or text passage indicating which specimen the measurements were based on. Following the original description the species name *Polymastia isidis* appeared repeatedly in the records of sponges from various areas in the southern hemisphere other than the type locality near the Chilean coast, – Wilhelm II coast of the Antarctica (Hentschel, 1914), Palmer Archipelago and Falkland Islands (Burton, 1932), South Shetland Islands (Desqueyroux, 1975), Kerguelen (Boury-Esnault & Van Beveren, 1982), Namibian coast (Uriz, 1988) and eastern Weddell Sea (Barthel *et al.*, 1990). However, none of these authors mentioned the exotyles in their sponges and it therefore remains uncertain whether they belong to *S. isidis* or not. For the moment we can only confirm the absence of exotyles in one of the syntypes of *Polymastia isidis* var. *simplex* Hentschel, 1914 (ZMB 4829) which we have studied. Other records need verification.

Sphaerotylus raphidophora Austin, Ott, Reiswig, Romagosa & McDaniel, 2014

Original description: *Sphaerotylus raphidophora* Austin, Ott, Reiswig, Romagosa & McDaniel, 2014, p. 36, figures 12 & 13.

TYPE MATERIAL

(not studied)

Holotype: USNM 1231336, Giacomini Seamount, Gulf of Alaska, NE Pacific, 56°25.43′N 146°22.28′W), 862 m, NOAA 2004 Exploring Alaska's Seamounts Expedition, Alvin Dive 4040, 16.08.2004.

DESCRIPTION

(according to Austin *et al.*, 2014)

External morphology

Irregular button-shaped sponge ~ 1.6–1.7 cm in diameter and 0.69 cm thick. Surface yellow-brown in alcohol. No papillae observed.

Skeleton

Main choanosomal skeleton composed of longitudal tracts of principal spicules. Auxiliary choanosomal skeleton comprises singly scattered intermediary spicules and occasional trichodragmata of raphides. Cortex formed by a palisade of small spicules reinforced by exotyles.

Spicules

(see Austin *et al.* (2014) for number of spicules measured)

- Principal spicules – straight, fusiform subtylostyles or strongyloxeas, occasionally with rounded distal extremities. Length 711–1107–1615 μm, diameter 10.3–20.4–25.4 μm.
- Intermediary spicules – gently curved, fusiform tylostyles. Length 228–418–613 μm, diameter 10.5–13.4–17.8 μm.
- Small spicules – gently or considerably curved, fusiform tylostyles to styles. Length 104–172–271 μm, diameter 2.0–3.6–6.6 μm.
- Raphides often with furcate extremities and numerous procumbent processes along the shaft. Length 60.8–72.4–80.
- Exotyles straight, with rounded smooth proximal extremities and rounded granulated distal extremities, occasionally with weakly developed distal knobs. Length 568–890–1374 μm, diameter 26.0–38.9–49.9 μm.

OCCURRENCE

(Figure 16)

Known only from the type locality, Gulf of Alaska, NE Pacific.

REMARKS

Sphaerotylus raphidophora is distinguished from all other *Sphaerotylus* spp. by the presence of raphides in trichodragmata that is in fact the main diagnostic feature of *Spinularia* Gray, 1867. *Sphaerotylus raphidophora* and the type species of *Spinularia, S. spinularia* (Bowerbank, 1866), also possess the similar architechure of cortex formed by a single layer, a palisade of small spicules. At the same time *Spinularia* spp. lack exotyles and possess a marginal spicule fringe (Plotkin *et al.*, 2012) that is absent in *S. raphidophora*. External morphology of *S. raphidophora* and its exotyles with rounded tuberculated distal extremities resemble those of *S. capitatus* and *S. isidis*, although the distal swellings on the exotyles of the latter two species are more prominent. For a full description of *S. raphidophora* see Austin *et al.* (2014).

Sphaerotylus renoufi sp. nov.

(Figures 21 & 22)

TYPE MATERIAL

Holotype (Figure 21A): BELUM MC5015 (in alcohol), Glannafeen Cliff, Lough Hyne, Co Cork, SW Ireland, 51°30.03′N 09°18.12′W, 10 m, 25.05.2009, coll. B.E. Picton.

Paratype: BELUM MC5010 (one specimen in alcohol, Figure 21B), from the same locality as the holotype.

Paratype: BELUM MC5013 (one specimen in alcohol), from the same locality as the holotype.

COMPARATIVE MATERIAL EXAMINED

South-West Ireland (eight specimens):

BELUM MC7695, Mc7696 and MC7697 (three specimens), Co Cork, Lough Hyne, Glannafeen Cliff, 51°30.03′N 09°18.12′W, 6–10 m, 02.–03.08.1993, coll. C.C. Morrow & B.E. Picton. BELUM MC3708 and MC3711 (two specimens), Co Cork, Lough Hyne, Glannafeen Cliff, 51°30.03′N 09°18.12′W, 10 m, 09.04.2007, coll. B.E. Picton. BELUM MC7698 (one specimen), Co Cork, Bantry Bay, S of Black Ball Head, 51°35.31′N 10°02.22′W, 35 m, 05.06.1993, coll. B.E. Picton. BELUM MC7699 (one specimen), Co Kerry, Kenmare River, Kilmakillogue Harbour, 51°46.64′N 09°49.77′W, depth 20 m BCD; coll. B.E. Picton, 12.08.1995. BELUM MC7700 (one specimen), Co Kerry, Kenmare River, NE of Inishkeragh, 51°47.94′N 09°53.29′W, 21 m, 13.08.1995, coll. E.M. Sides.

West Ireland (four specimens):

BELUM MC7701 (two specimens), Co Galway, Mannin Bay, Carrigeenbeg, 53°26.75′N 10°12.75′W, 40 m, coll. C.C. Morrow, 16.06.1995. BELUM Mc7702 (one specimen), Co Galway, Clifden Bay, SSW of Carrickana Rocks, 53°28.98′N 10°09.93′W, 38 m, coll. B.E. Picton, 11.06.1995. BELUM Mc7703 (one specimen), Co Galway, Friar Island, N of Malthooa, 53°33.23′N 10°13.57′W, 34 m, coll. B.E. Picton, 22.06.1995.

North-West Ireland (10 specimens):

BELUM Mc7705 (one specimen), Co Mayo, Inishkea Island, 54°04.36′N 10°11.98′W, 43 m, coll. B.E. Picton, 08.08.1994. BELUM Mc7706 (one specimen), Co Sligo, Mullaghmore, Thumb Rock, 54°28.31′N 08°26.71′W, 22 m, 16.05.1994, coll. C.C. Morrow. BELUM Mc7707 (one specimen), Co Donegal, St. John's Point, Black Rock, 54°34.69′N 08°25.64′W, 19 m, 22.05.1994, coll. C.C. Morrow. BELUM Mc7708 (one specimen), Co Donegal, SE Deegagh Point, 55°09.23′N 07°41.55′W, 12 m, 13.07.1993, coll. C.C. Morrow. BELUM Mc5056, Mc5061, Mc5068, Mc5073, Mc5076 and Mc5080 (six specimens), Co Sligo, Mullaghmore, Thumb Rock, 54°28.31′N 08°26.71′W, 22 m, 8.–10.07.2009, coll. B.E. Picton & C.C. Morrow.

North-East Ireland (one specimen):

BELUM Mc3761, Co Antrim, Rathlin Island, Duncan's Bay, 55°18.70′N 06°15.09′W, 34 m 22.06.2007, coll. B.E. Picton.

Irish Sea, Welsh Coast (six specimens):

BELUM Mc5428, Mc5435, Mc5440 and Mc5441 (four specimens), Pembrokeshire coast, Huw's Reef, 51°57.84′N 05°07.54′W, 17.4 m, coll. B.E. Picton, 04.08.2009. BELUM Mc5757 and Mc5760 (two specimens), Pembrokeshire coast, Skomer, Thorn Rock, 51°43.80′N 5°15.95′W, 18.8 m, 06.08.2009, coll. B.E. Picton.

ETYMOLOGY

Named after Professor Louis Renouf of University College, Cork, the first biologist to note the unique character of Lough Hyne, Co Cork and to begin marine research there in 1923.

DESCRIPTION

External morphology

Cushion-shaped sponges with a convex upper surface (Figure 21A–C). Surface shaggy, dark in colour because of the covering silt, with bright yellow papillae (in life, Figure 21C). Papillae with oscula on the summits. Holotype 1.6 × 1.5 × 0.4 cm in size, with four papillae which are 3–6 mm long and ~ 2 mm in diameter (Figure 21A). Other specimens up to 12 cm², with one to five papillae per cm² of the surface. Papillae 1–11 mm long and 1.5–3.5 mm in diameter.

Skeleton

Main choanosomal skeleton composed of radial or longitudinal tracts (~ 110 μm thick) of principal spicules which cross the cortex and make up a surface hispidation that is up to 2200 μm thick (Figure 21D). Auxiliary choanosomal skeleton comprises singly scattered small and intermediary spicules. Cortex up to 300 μm thick composed of an outer layer of small spicules arranged in bouquets and a slightly thinner, loose inner layer of tangentially arranged intermediary spicules (Figure 21E, F). Exotyles cross the cortex

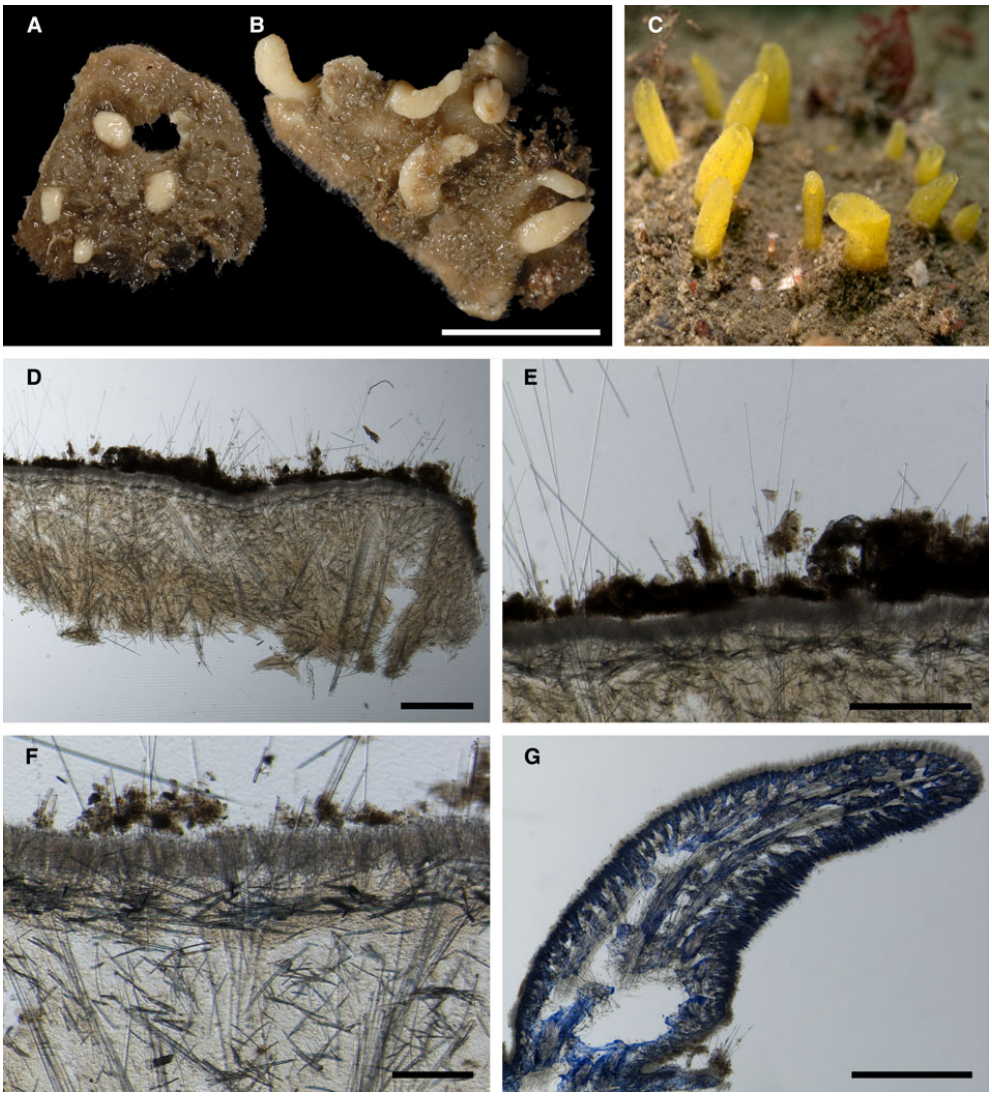

Fig. 21. *Sphaerotylus renoufi*: (A) holotype BELUM MC5015, habitus; (B) paratype BELUM MC5010, habitus; (C) specimen BELUM MC5068 *in situ* on Thumb Rock, Mullaghmore, Co Sligo, NW Ireland (courtesy of B. Picton, Ulster Museum, Belfast); (D) longitudinal section through the body of paratype BELUM MC5013, general view; (E) the same section, detail of cortex echinated by an exotyle; (F) longitudinal section through the body of holotype BELUM MC5015, detailed view of cortex; (G) longitudinal section through a papilla of the holotype stained with toluidine. Scale bars: A and B, 10 mm;D, 1 mm; E, 0.5 mm; F, 0.2 mm; G, 1 mm.

(Figure 21E). Both cortical layers extend to the papillae walls (Figure 21G). Central exhalant canal in papilla surrounded by ascending tracts of principal spicules. Several inhalant canals located in the periphery. Bulkheads between the canals reinforced by a network of intermediary spicules.

Spicules

(measurements based on holotype and two paratypes, N = 19 for exotyles, N = 70 for other categories)

- Principal spicules – usually straight, slightly fusiform, polytylote subtylostyles, often with blunt distal tips (Figure 22A–D). Length 560–796–1030 μm, diameter of shaft 7.5–14.8–16 μm.
- Intermediary spicules – straight, slender tylostyles to subtylostyles (Figure 22E). Length 200–415–650 μm, diameter of shaft 5.0–9.7–13.8 μm.
- Small spicules (Figure 22F) – straight, slightly fusiform tylostyles. Length 70–132–210 μm, diameter of shaft 2.0–4.1–6.5 μm.

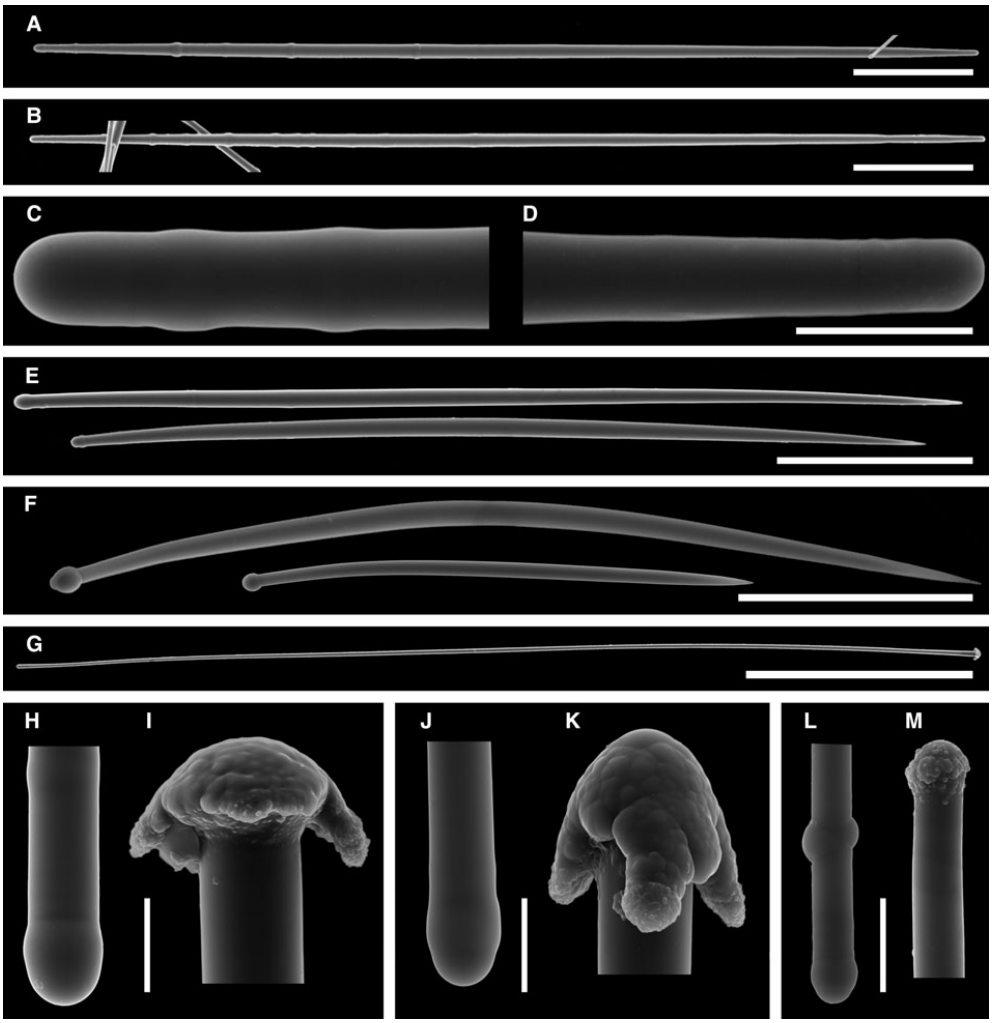

Fig. 22. *Sphaerotylus renoufi*, spicules: (A) and (B) subtylostyles, general view; (C) proximal tip of the subtylostyle depicted in B, detailed view; (D) distal tip of the subtylostyle depicted in B, detailed view; (E) intermediary subtylostyles; (F) small tylostyles; (G) exotyle, general view; (H) proximal tip of the exotyle depicted in G, detailed view; (I) fungiform distal ornamentation of the exotyle depicted in G, detailed view; (J) proximal tip and (K) grapnel-like distal ornamentation of another exotyle, detailed view; (L) proximal tip and (M) rounded distal ornamentation of one more exotyle, detailed view. Scale bars: A and B, 0.1 mm; C and D, 0.01 mm; E, 0.1 mm; F, 0.05 mm; G, 0.5 mm; H–M, 0.01 mm.

- Exotyles gently curved or straight, almost cylindrical, slender (Figure 22G–M). Length 1110–1755–2460 μm, diameter of shaft 5.0–8.0–10.0 μm. Proximal tyles are weakly developed (Figure 22H, J, L) or absent. Distal knobs (7.0–19.4–25.3 μm) fungiform (Figure 22I) or lobate (Figure 22K), occasionally subspherical (Figure 22M), with granulated surface.

OCCURRENCE

(Figure 12)

NE Atlantic: widely distributed around Ireland (western coast and Irish Sea) and along western Wales (Pembrokeshire coast), 6–42 m.

REMARKS

Sphaerotylus renoufi resembles *S. antarcticus* and *S. borealis* in several features – a thick superficial hispidation composed of the ascending tracts of principal spicules, several prominent papillae and a two-layered cortex, but differs from the latter two species by shorter principal spicules and exotyles, as well as by the presence of lobate distal knobs on some exotyles.

Sphaerotylus sceptrum Koltun, 1970

(Figures 17 & 18)

Original description: *Sphaerotylus sceptrum* Koltun, 1970, p. 177, pl. V figure 4, text-figure 8.

SYNONYMS AND CITATIONS

Sphaerotylus sceptrum (Plotkin, 2002, p. 106, figure 2).

TYPE MATERIAL

Holotype: ZIN RAS 10614 (specimen in alcohol, slide 16132), Simushir Island, Kurile Islands, NE Pacific, 46°38′N 152°03′E, 1440–1540 m, RV 'Vityaz', cruise 39, station 5594, 12.07.1966.

DESCRIPTION

External morphology

Several fragments of a cushion-shaped, crumby sponge detached from substratum. Surface bears tiny papillae with oscula on the summits. Surface areas surrounding the papillae pale and almost smooth. Peripheral surface rough or velvety and brownish in colour. Largest fragment 4 × 3.5 × 1.5 cm in size, with three papillae.

Skeleton

Main choanosomal skeleton composed of radial tracts of principal spicules which ascend and fan in the cortex (Figure 23A). Auxiliary choanosomal skeleton comprises singly scattered small spicules, pairs of exotyles and occasionally intermediary spicules. Cortex around the papillae 1700–2100 μm thick, composed of a superficial layer (150–200 μm thick) of dense bouquets of small spicules reinforced by the branching tracts ascending from the choanosome, a loose inner layer (300–750 μm thick) of criss-cross intermediary spicules and a space with aquiferous cavities in between the spicule layers (Figure 23B, C). The cavities connected with ostia scattered between the superficial spicule bouquets. Bulkheads between the cavities reinforced by the ascending choanosomal tracts of principal spicules and single intermediary spicules. Peripheral cortex is a dense palisade of exotyles, occasionally encrusted with the small spicules and underlain by tufts of the intermediary spicules (Figure 23B, D).

Spicules

(N = 10)

- Principal spicules – straight, slightly fusiform styles (Figure 23E). Length 600–1254–1400 μm, proximal diameter of shaft 9.2–12.9–15.1 μm, maximum diameter of shaft 15.0–20.3–25.0 μm.
- Intermediary spicules – straight, slender or occasionally stout tylostyles to subtylostyles (Figure 23F). Length 200–411–524 μm, proximal diameter of shaft 5.5–9.3–11.2 μm, maximum diameter of shaft 8.0–11.1–13.9 μm.
- Small spicules – usually straight, slender tylostyles (Figure 23G). Length 101–128–160 μm, diameter of tyle 4.1–4.4–5.5 μm, proximal diameter of shaft 2.8–3.4–4.5 μm, maximum diameter of shaft 3.7–4.3–5.7 μm.
- Exotyles stout, sceptre-shaped (Figure 23E, H). Length 195–219–250 μm. Well-developed proximal tyles, 13.4–16.1–20.1 μm in diameter. Shafts gradually expanding from 10.2–11.4–13.0 μm near the proximal tyles to 28.5–31.9–35.0 μm at the distal extremities. Surface of the distal extremeties turberculated or granulated. No distal knobs.

OCCURRENCE

(Figure 16)

Known only from the type locality off the Kurile Islands, NW Pacific.

REMARKS

Sphaerotylus sceptrum is distinguished from its congeners by the remarkably heterogeneous cortex. In the areas around the papillae it is composed of a superficial palisade of small tylostyles and an inner layer of criss-cross spicules, bears ostia and aquiferous cavities and lacks exotyles that is architecture typical of many other polymastiids. However, in the peripheral zones the palisade of exotyles completely substitutes for the layers of tylostyles that resemble the cortex in *Sphaerotylus exotylotus* and *S. vanhoeffeni*. The exotyles of *S. sceptrum* are most similar to those of *S. vanhoeffeni*, but in the former species they are shorter and expand much more towards the distal extremities which do not bear any knobs and are covered by the remarkably large tubercules.

Sphaerotylus strobilis sp. nov.

(Figures 24 & 25)

TYPE MATERIAL

Holotype: BMNH 1926.4.14.86.7.517 (specimen in alcohol), South Africa, depth unknown, coll. J.D.F. Gilchrist.

Paratype (one specimen in alcohol)): BMNH 1926.4.14.86.7.519, South Africa, depth unknown, coll. J.D.F. Gilchrist.

Both sponges are labelled *Proteleia sollasi*, presumably by Kirkpatrick.

ETYMOLOGY

The name refers to the shape of the distal knobs of exotyles (Latin *strobilus* = a strobile, a cone).

DESCRIPTION

External morphology

Both sponges cushion-shaped, attached to bivalves. Holotype (Figure 24A) ~ 3.5 × 3.5 × 1.5 cm in size. Surface minutely hispid, mostly covered by sediment, with sparse clean yellowish areas and nine conical yellowish papillae, 0.4–1.6 cm long, 0.2–0.8 cm wide at base. Paratype (Figure 24B) ~ 3 × 3 × 0.7 cm in size. Surface mostly velvety, free of sediment, yellowish in colour, with a narrow marginal hispidation and eight yellowish papillae. Papillae conical or cylindrical, 1–1.8 cm long, 0.3–0.5 cm wide at base. Considerably contracted oscula visible on the summits of most papillae in both specimens.

Skeleton

Main choanosomal skeleton (Figure 24C) composed of radial or longitudinal tracts (220–450 μm thick) of principal spicules. Tracts radiate and cross the cortex, few of them forming a surface hispidation. Auxiliary choanosomal skeleton mainly of intermediary spicules which are often grouped in dense bundles, each bundle consisting of up to 10 spicules. These bundles are highly abundant in the subcortical area where they cross each other (Figure 24D). Tiny sediment particles and foraminiferans are commonly incorporated in the choanosome. Cortex comprises three layers

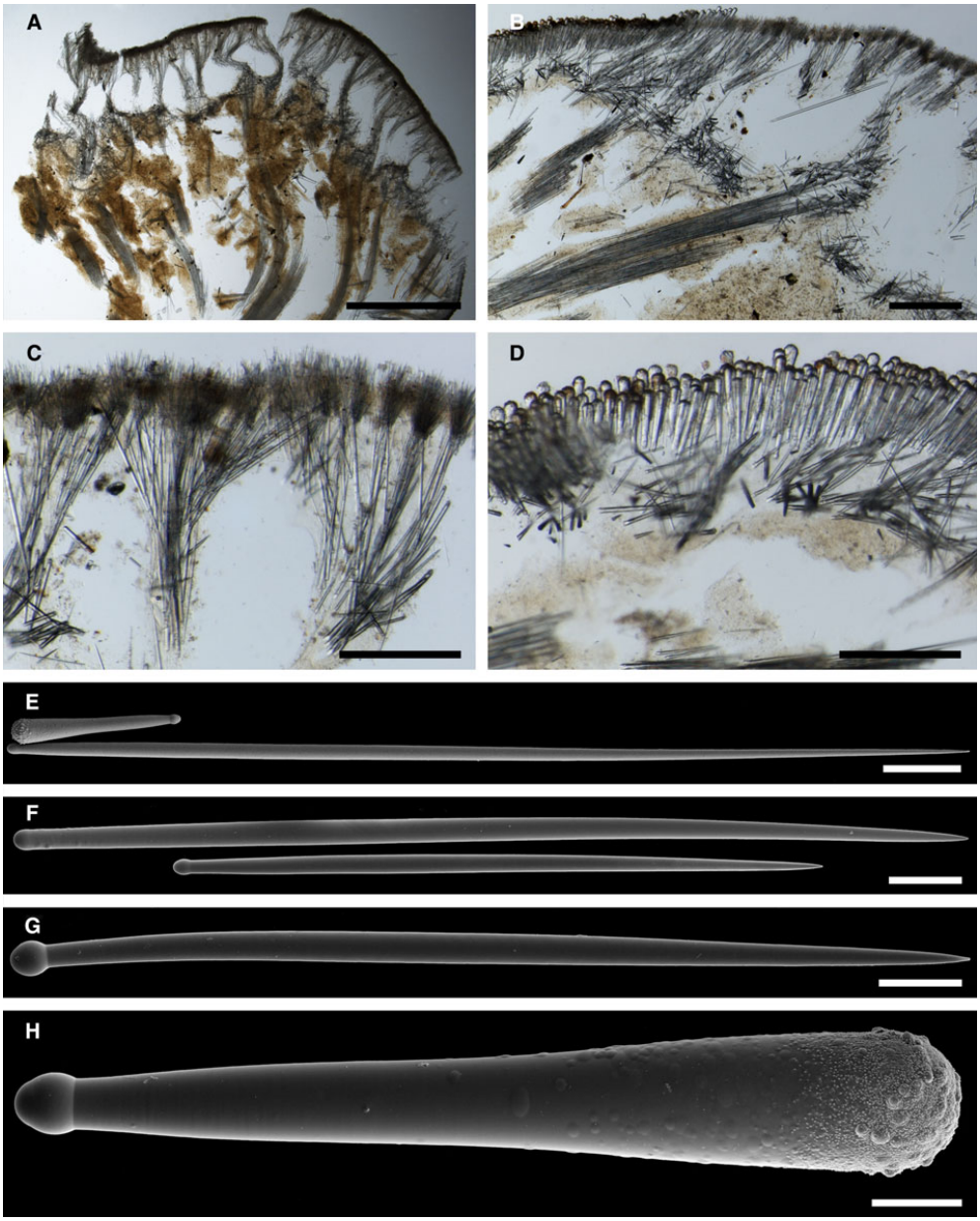

Fig. 23. *Sphaerotylus sceptrum*, holotype ZIN RAS 10614: (A) longitudinal section through the body, general view; (B) another longitudinal section through the body showing the transitional area between the peripheral and central cortex; (C) the same section, detail of the central cortex showing bouquets of small spicules reinforced by the tracts ascending from choanosome; (D) the same section; detail of the peripheral cortex showing a palisade of exotyles; (E) principal style and exotyle; (F) intermediary subtylostyles; (G) small tylostyle; (H) exotyle. Scale bars: A, 3 mm; B, 0.5 mm; C and D, 0.3 mm; E, 0.1 mm; F, 0.04 mm; G, 0.01 mm; H, 0.02 mm.

(Figure 24D) – a superficial palisade (170–290 μm thick) of small spicules, an inner well-defined layer (120–330 μm thick) of densely lying criss-cross intermediary spicules and an intermediate layer (230–400 μm thick) where intermediary spicules are sparsely scattered. Single exotyles scattered among the small spicules in the palisade join the surface hispidation. Skeleton of papillae walls made of the cortical palisade and the inner layer where the criss-cross intermediary spicules distributed more sparsely than in the cortex (Figure 24E).

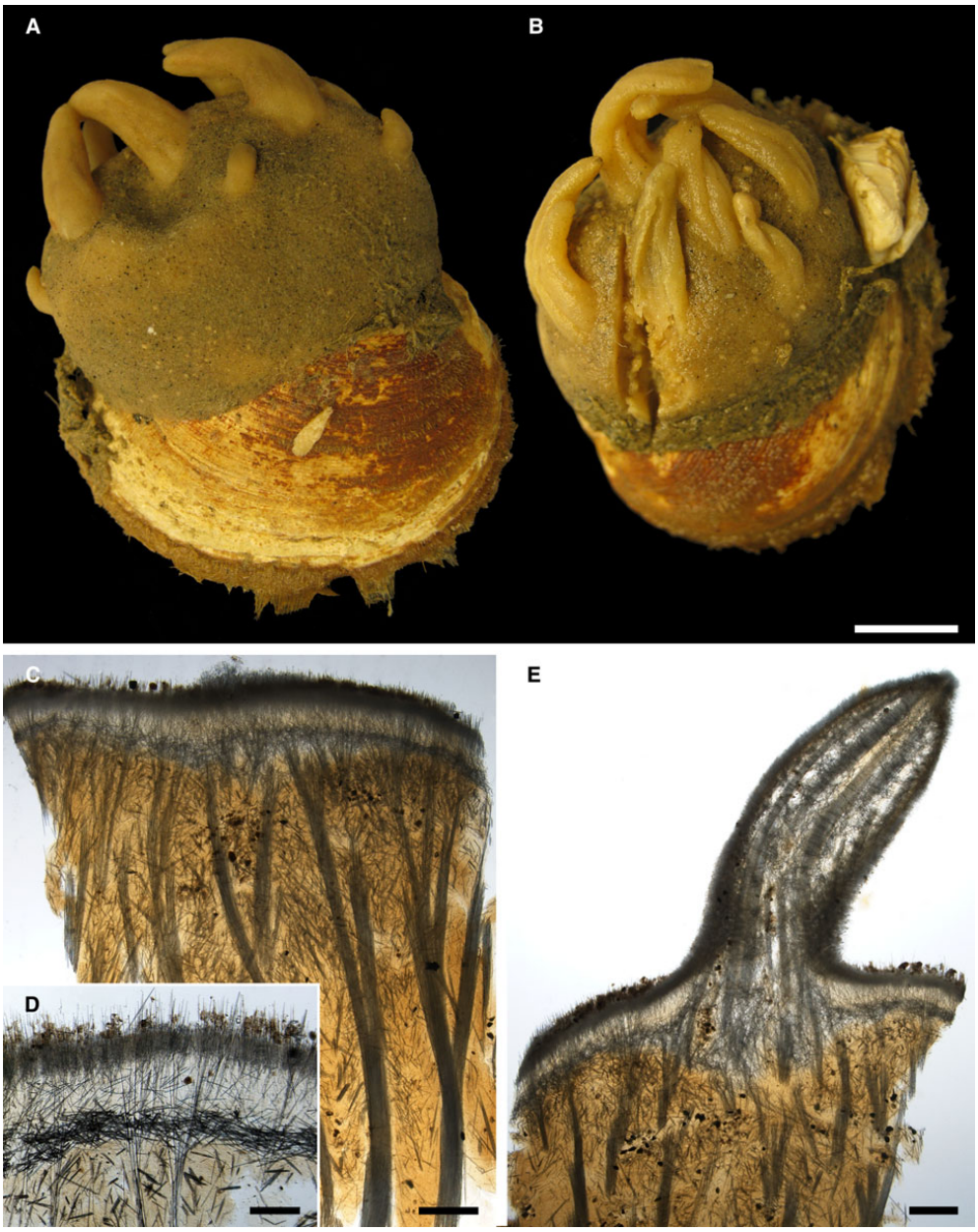

Fig. 24. *Sphaerotylus strobilis*: (A) holotype BMNH 1926.4.14.86.7.517, habitus; (B) paratype BMNH 1926.4.14.86.7.519, habitus; (C) longitudinal section through the body of the holotype, general view; (D) the same section, detail of cortex; (E) longitudinal section through a papilla of the holotype. Scale bars: A and B, 10 mm; C, 1 mm; D, 0.4 mm; E, 1 mm.

Spicules
(measurements based on holotype, N = 9 for exotyles, N = 30 for other categories)

- Principal spicules – straight, slender, subtylostyles to styles (Figure 25A–C). Length 860–1007–1100 μm, proximal diameter of shaft 7.2–8.4–9.1 μm, maximum diameter of shaft 19.5–21.7–24.3 μm.
- Intermediary spicules – styles and subtylostyles resembling principal spicules in shape (Figure 25D–F). Length 490–543–585 μm, proximal diameter of shaft 5.8–6.9–7.3 μm, maximum diameter of shaft 9.8–12.2–14.0 μm.

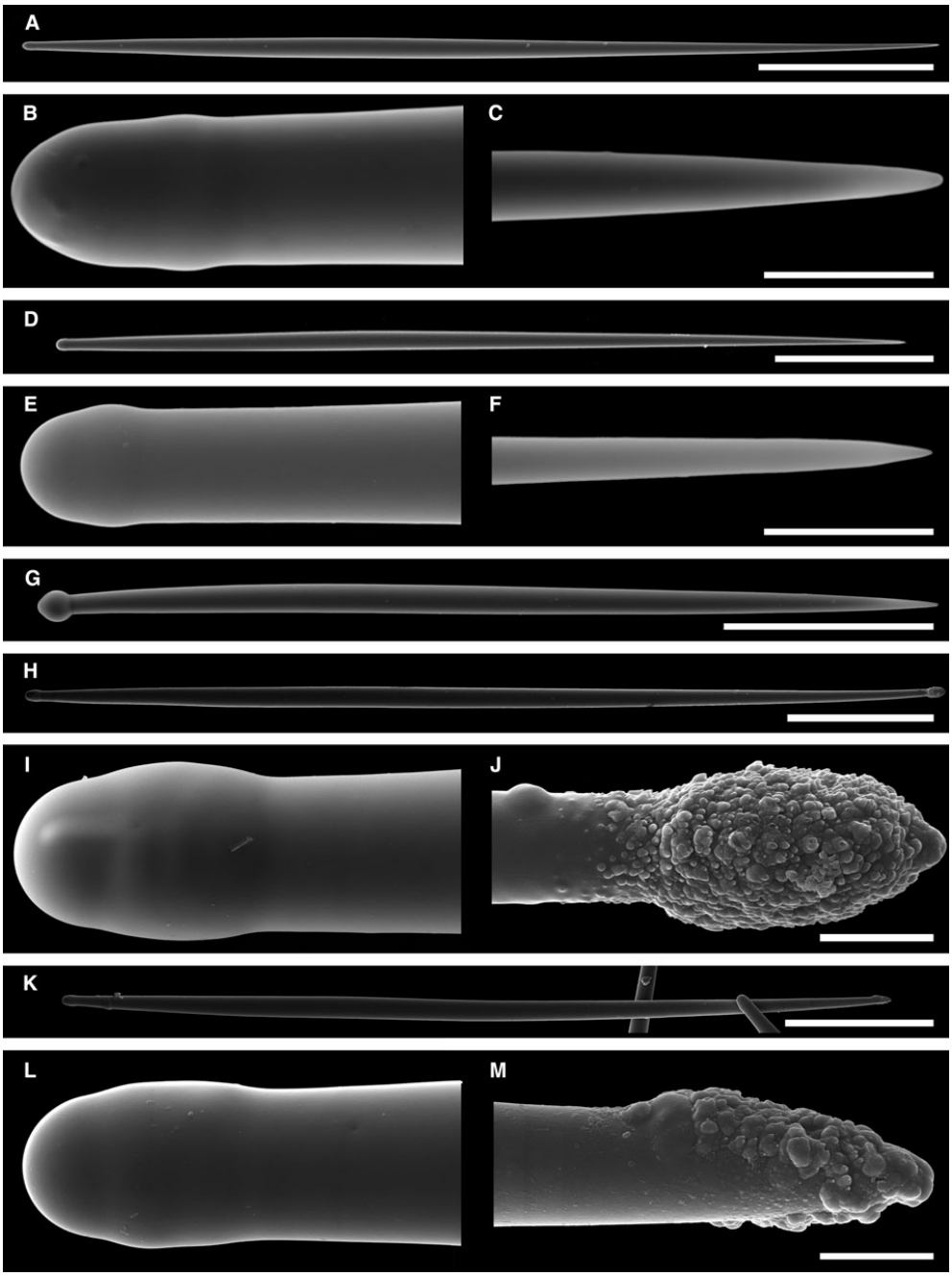

Fig. 25. *Sphaerotylus strobilis*, spicules: (A) principal subtylostyle, general view; (B) proximal tip of the subtylostyle depicted in A, detailed view; (C) distal tip of the subtylostyle depicted in A, detailed view; (D) intermediary subtylostyle, general view; (E) proximal tip of the subtylostyle depicted in D, detailed view; (F) distal tip of the subtylostyle depicted in D, detailed view; (G) small tylostyle; (H) exotyle with a regular distal knob, general view; (I) proximal tip of the exotyle depicted in H, detailed view; (J) distal knob of the exotyle depicted in H, detailed view; (K) exotyle with an irregular distal knob; (L) proximal tip of the exotyle depicted in K, detailed view; (M) distal knob of the exotyle depicted in K, detailed view. Scale bars: A, 0.2 mm; B and C, 0.01 mm; D, 0.1 mm; E and F, 0.01 mm; G, 0.04 mm; H, 0.1 mm; I and J, 0.005 mm; K, 0.1 mm; L and M, 0.005 mm.

- Small spicules – straight, usually slender tylostyles (Figure 25G). Length 147–170–195 μm, diameter of tyle 4.8–6.4–8.3 μm, proximal diameter of shaft 2.0–3.7–5.1 μm, maximum diameter of shaft 4.9–6.6–8.2 μm.
- Exotyles straight or gently curved, fusiform (Figure 25H, K), usually with weakly developed proximal tyles (Figure 25I, L). Length 565–599–632 μm, proximal diameter of shaft 6.2–6.8–7.0 μm, maximum diameter of shaft 14.0–14.5–15.0 μm. Distal tips acerated or blunt, covered by numerous tubercles of different size which usually form regular (Figure 25J), occasionally irregular (Figure 25M), strobile-shaped knobs 6.2–6.9–7.3 μm in diameter.

OCCURRENCE

Known only from the type locality near South Africa.

REMARKS

Holotype and paratype of this new species were labelled as *Proteleia sollasi*. Presumably the identification was done by Kirkpatrick who studied the 'Gilchrist collection' from South Africa (Kirkpatrick, 1902, 1903a, b), but did not mention these sponges in his papers. In fact *Sphaerotylus strobilis* lacks at least two main features of *P. sollasi*, grapnel-like ornamentations on the exotyles and an extra palisade of intermediary spicules in the cortex. At the same time our new species shares the presence of a velvety surface, a three-layered cortex including an intermediate layer of low spicule concentration and a relatively small length of exotyles with *S. capitatus* and *S. isidis*. But in contrast to the latter two species in *S. strobilis* some tracts of principal spicules make up a surface hispidation that rather resembles *S. borealis* and *S. antarcticus*, although in the latter two both principal spicules and exotyles are much longer and the hispidation is much more dense and thicker than in our new species. The main distinctive feature of *S. strobilis* is the strobile-shaped knobs of its exotyles.

Sphaerotylus tjalfei sp. nov.
(Figures 29 & 30)

TYPE MATERIAL

Holotype (specimen in alcohol): ZMUC-DEM-243, West Greenland, 70°47′N 52°21′W, 600 m, RV 'Tjalfe', 06.08.1908.
Paratype (one specimen in alcohol): ZMUC-DEM-244 (paratype), from the same sample as the holotype.
Paratype (one specimen in alcohol): ZMUC-DEM-245 (paratype), from the same sample as the holotype.

ETYMOLOGY

'Tjalfe' is the name of the Danish hired vessel and the type material was collected during one of her cruises. These specimens were examined by Lundbeck who labelled them '*Polymastia tjalfi*', but he never described them or mentioned this name in his publications.

DESCRIPTION

External morphology

Dome-shaped sponges with a shaggy surface, dark brown in colour because of the covering silt. Holotype and paratype ZMUC-DEM-244 overgrowing a hard calcareous tube (of a serpulid polychaete or a piece of a hydrocoral skeleton) (Figure 26A). Holotype 2.5 × 2.4 cm in size, bearing a distinct low papilla with an osculum on the summit. Paratype ZMUC-DEM-244 1.9 × 1.6 cm in size, lacking any visible papilla. Paratype ZMUC-DEM-245 1.7 × 1.5 cm in size, detached from substratum and overgrown by an ascidian (Figure 26B). Its single very tiny papilla completely invaginated into the surface hispidation on the body summit.

Skeleton

Main choanosomal skeleton composed of radial tracts of principal spicules which cross the cortex and make up a surface hispidation (Figure 26D). Auxiliary choanosomal skeleton comprises singly scattered small spicules (Figure 26E). In cortex a palisade (~ 170 μm thick) of small spicules lies directly on a layer (~ 140 μm thick) of tangentially arranged intermediary spicules (Figure 26F). Short, stout strongyles sparsely scattered along the cortex (Figure 26G). Exotyles cross the cortex joining the surface hispidation (Figure 26C). Distal portions of many protruding spicules are often broken and hence it is impossible to determine whether they are exotyles or usual principal monactines.

Spicules

(measurements based on holotype and both paratypes, N = 5 for exotyles, N = 4 for cortical strongyles, N = 30 for other categories)

- Principal spicules – straight or gently curved, fusiform, often polytylote subtylostyles to styles (Figure 27A). Length 854–1273–2013 μm, diameter of tyle (if present) 8.3–13.7–20.8 μm, proximal diameter of shaft 7.5–12.6–20.8 μm, maximum diameter of shaft 19.2–28.2–36.4 μm.
- Intermediary spicules – usually straight, slender or slightly fusiform tylostyles to subtylostyles (Figure 27B). Length 378–518–797 μm, diameter of tyle 7.8–11.0–19.5 μm, proximal diameter of shaft 5.5–8.4–13.3 μm, maximum diameter of shaft 7.2–14.5–22.6 μm.
- Small spicules – straight or occasionally gently curved, stout, fusiform tylostyles (Figure 27C). Length 97–145–226 μm, diameter of tyle 4.1–6.0–8.5 μm, proximal diameter of shaft 3.0–4.5–6.1 μm, maximum diameter of shaft 4.8–7.9–13.7 μm.
- Cortical strongyles short, stout, regularly cylindrical or slightly fusiform, occasionally with weakly developed tyles. Length 49–174–314 μm, maximum diameter of shaft 11.9–57.1–90.5 μm.
- Exotyles usually gently curved, slender, almost cylindrical (Figure 27D). Length 1080–1710–2856 μm, proximal diameter of shaft 10.5–16.0–19.2 μm, maximum diameter of shaft 17.9–29.4–37.7 μm. Proximal tyles weakly developed or absent (Figure 27E). Distal knobs (18–28.4–37.6 μm in diameter) usually regularly spherical (Figure 27F, G), occasionally with extra swellings on shafts (Figure 27H). Surface of the knobs tuberculated to a greater or lesser extent. Some exotyles lacking distal knobs and only possessing slightly expanded blunt distal tips.

OCCURRENCE

(Figure 12)

Known only from the type locality in West Greenland, NW Atlantic.

REMARKS

Externally, with its thick surface hispidation and single papilla, *Sphaerotylus tjalfei* is reminiscent of *Polymastia invaginata*.

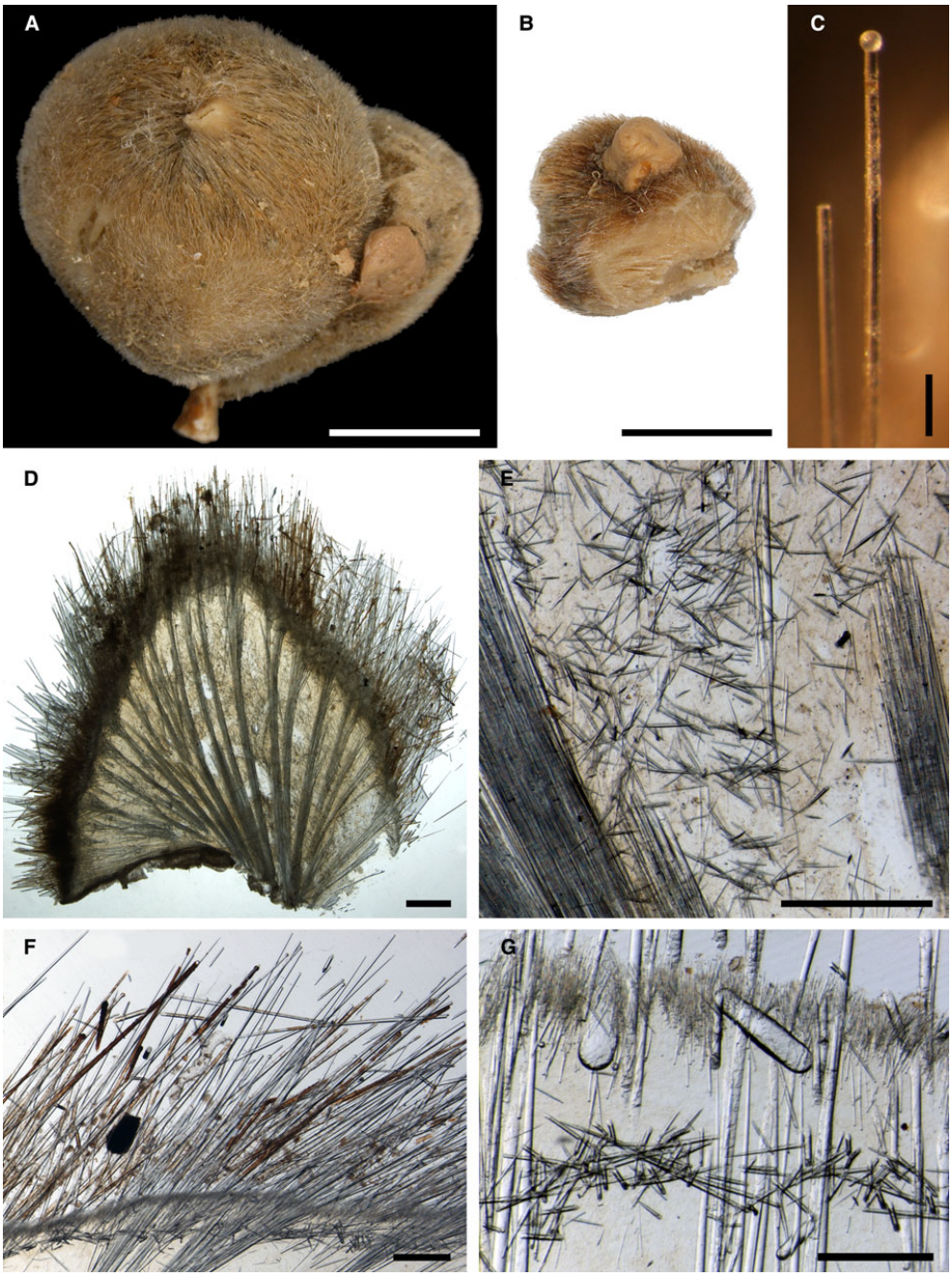

Fig. 26. *Sphaerotylus tjalfei*: (A) holotype ZMUC-DEM-243 and paratype ZMUC-DEM-244 growing together, habitus; (B) paratype ZMUC-DEM-245, habitus; (C) exotyle echinating the surface of paratype ZMUC-DEM-245 under stereomicroscope; (D) longitudinal section through the body of paratype ZMUC-DEM-245, general view; (E) the same section, detail of auxiliary choanosomal skeleton; (F) the same section, detail of cortex; (G) the same section, detail of cortex showing stout strongyles. Scale bars: A and B, 10 mm; C, 0.1 mm; D, 1 mm; E and F, 0.5 mm; G, 0.3 mm.

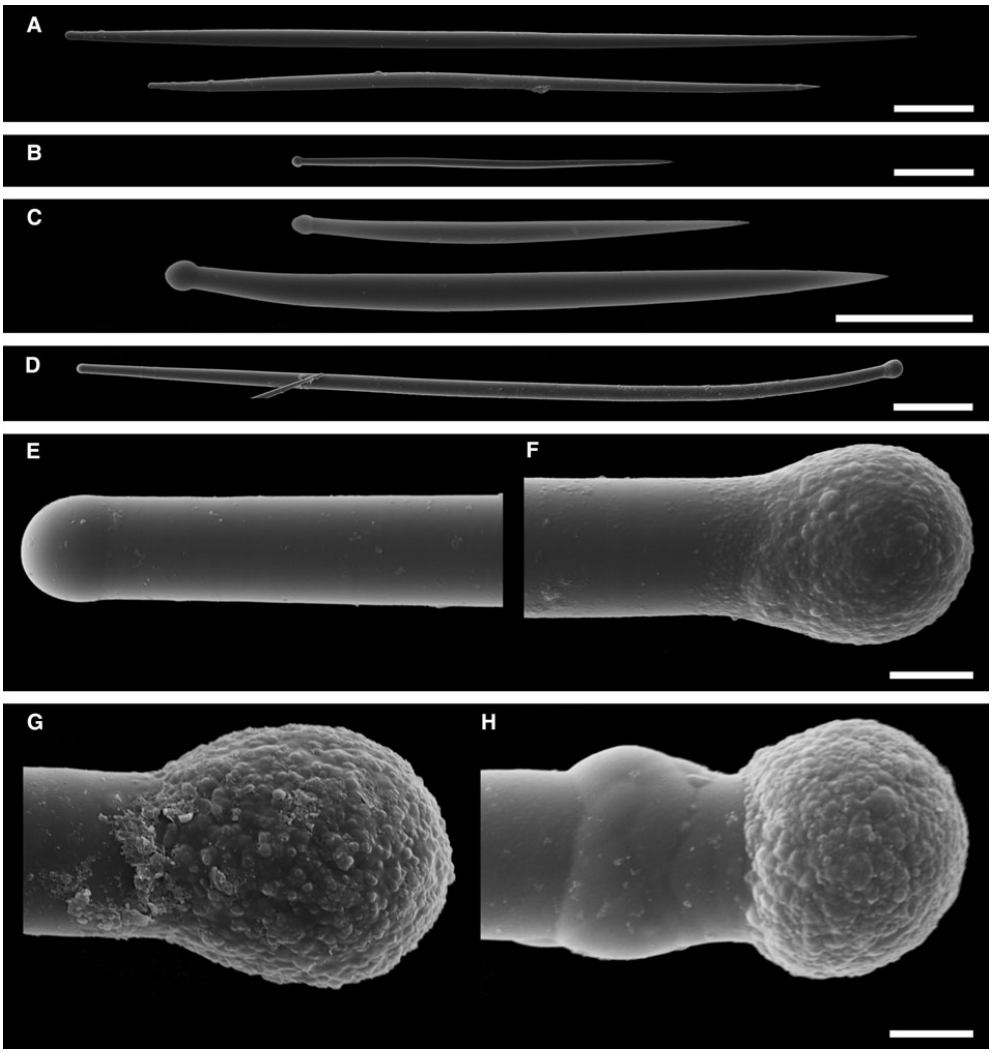

Fig. 27. *Sphaerotylus tjalfei*, spicules: (A) principal styles; (B) intermediary tylostyle; (C) small tylostyles; (D) exotyle, general view; (E) proximal tip of the exotyle depicted in D, detailed view; (F) distal knob of the exotyle depicted in D, detailed view; (G) and (H) distal knobs of other exotyles, detailed view. Scale bars: A and B, 0.1 mm; C, 0.03 mm; D, 0.1 mm; E–H, 0.01 mm.

But *P. invaginata* is distinguished by the lack of ornamented exotyles and a cortex composed solely of a palisade of small spicules. The thick surface hispidation along with the two-layered cortex observed in *S. tjalfei* is also recorded in three other species of *Sphaeroylus* (*S. antarcticus, S. borealis* and *S. renoufi*). However, in contrast to *S. tjalfei* the latter three species possess several papillae and usually irregular distal knobs on the exotyles. Symmetrically spherical distal knobs on the exotyles of *S. tjalfei* rather resemble those in the type species of *Sphaerotylus, S. capitatus*, as well as in *S. isidis*. Conspicuous stout and short strongyles scattered in the cortex of *S. tjalfei* are also recorded in *P. invaginata* by Plotkin & Janussen (2008) and in *S. borealis* by Swarczewsky (1906) and Koltun (1966).

Sphaerotylus vanhoeffeni Hentschel, 1914
(Figures 28 & 29)

Original description: *Sphaerotylus capitatus* var. *vanhöffeni* Hentschel, 1914, p. 50, pl. 5 figure 5.

SYNONYMS AND CITATIONS

Sphaerotylus capitatus (Kirkpatrick, 1908, p. 18, pl. XII figure 1c, pl. XIII figures 8–13, pl. XIV figures 1–4; Barthel *et al.*, 1990, p. 122; Sarà *et al.*, 1992, p. 568).

?*Sphaerotylus capitatus* (Boury-Esnault & Van Beveren, 1982, p. 39, figure 9a–c; Uriz, 1988, p. 43).

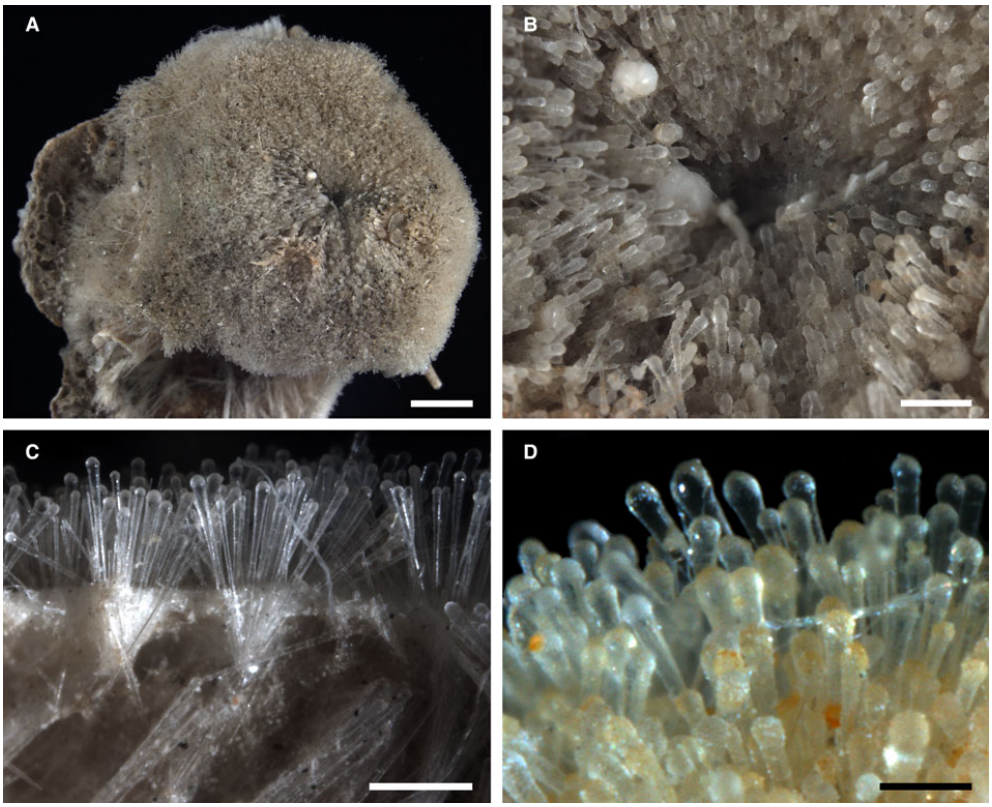

Fig. 28. *Sphaerotylus vanhoeffeni*, lectotype ZMB 4837: (A) habitus, general view; (B) habitus, central area of the surface, detailed view; (C) habitus, cut edge, detailed view of exotyle bouquets; (D) distal extremities of the exotyles protruding above the surface, detailed view. Scale bars: A, 2 mm; B and C, 0.5 mm; D, 0.2 mm.

Sphaerotylus schoenus (Burton, 1929, p. 447; Koltun, 1964, p. 28; Barthel *et al.*, 1990, p. 122; Sarà *et al.*, 1992, p. 568).
Sphaerotylus schoenus vanhöffeni (Koltun, 1976), p. 168.

TYPE MATERIAL

Lectotype (designated herein, see Figure 28A): ZMB 4837 (specimen in alcohol), Gauss-Station, Davis Sea, Southern Ocean, 66°02′S 89°38′E, 380 m, Deutschen Südpolar-Expedition, 22.12.1902.
Paralectotypes: ZMB 4837 (two specimens in alcohol), from the same locality as the lectotype, 385 m, Deutschen Südpolar-Expedition, 28.01.1903.

COMPARATIVE MATERIAL EXAMINED

BMNH 1908.2.5.111–112 (one specimen in alcohol and its buds mounted on slide, identified as *Sphaerotylus capitatus* by Kirkpatrick, 1908), Flagon Point, Winter Quarters Bay, McMurdo Sound, Ross Sea, Southern Ocean, 77°50′42.77″S 166°39′1.41″E, 18–36 m (10–20 fathoms), British National Antarctic Expedition on RV 'Discovery', 21.01.1903.

DESCRIPTION

External morphology

All type specimens cushion-shaped. Lectotype 1.3 × 1.2 × 0.3 cm in size, attached to a concretion fouled by a dead bryozoan (Figure 28A). Surface whitish to dirty greyish in colour, with prominent distal tips of exotyles (Figure 28B–D). A considerable invagination in the central area obviously indicates the position of a papilla in the living sponge (Figure 28B). Paralectotypes considerably damaged in their central areas; one specimen free, 0.3 cm in diameter, the other 0.5 cm in diameter, attached to a pebble. BMNH specimen thickly encrusting, with a roughly velvety, knobbly surface bearing several threads with buds and seven papillae partially invaginated into the surface hispidation.

Skeleton

Main choanosomal skeleton composed of radial tracts of principal spicules which enter the cortex. Auxiliary choanosomal skeleton consists of singly scattered small and intermediary spicules and occasional exotyles. Dense cortex made of exotyle bouquets with sparsely embedded small and intermediary spicules (Figure 28C).

Spicules

(measurements based on three specimens, N = 10)

- Principal spicules – straight, slightly fusiform or slender, occasionally polytylote subtylostyles (Figure 29A). Length 936–1179–1489 μm, diameter of tyle 11.1–13.7–

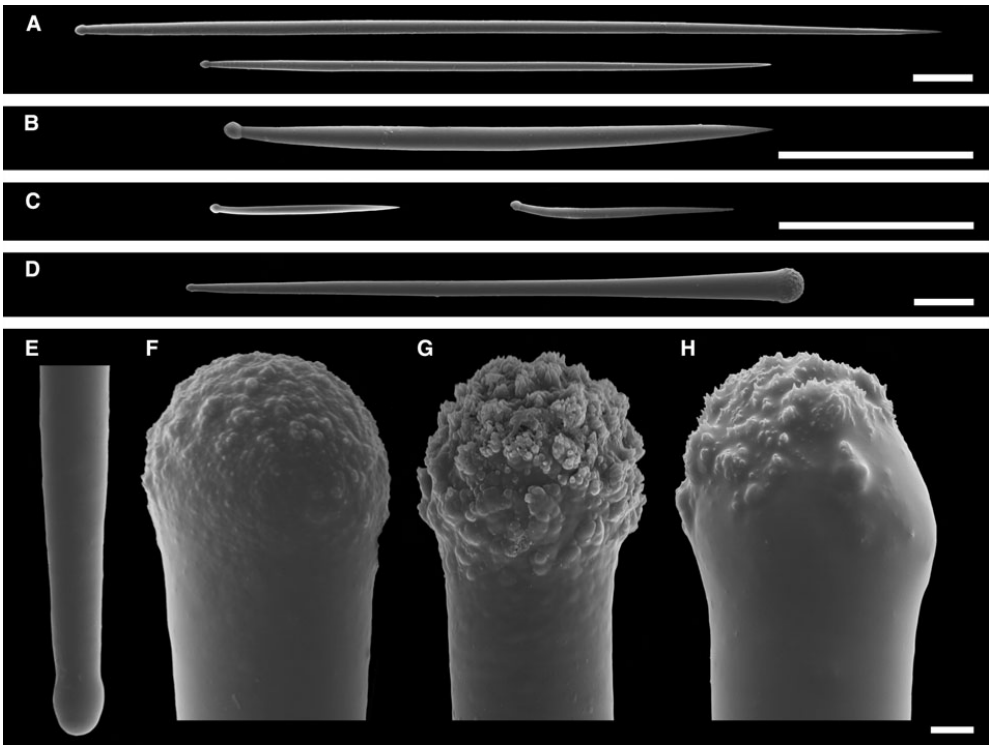

Fig. 29. *Sphaerotylus vanhoeffeni*, spicules: (A) principal subtylostyles; (B) intermediary tylostyle; (C) small tylostyles; (D) exotyle, general view; (E) proximal tip of the exotyle depicted in D, detailed view; (F) distal knob of the exotyle depicted in D, detailed view; (G) and (H) distal knobs of other exotyles, detailed view. Scale bars: A–D, 0.1 mm; E–H, 0.01 mm.

17.5 μm, proximal diameter of shaft 7.2–9.6–13.5 μm, maximum diameter of shaft 15.8–19.7–23.6 μm.

- Intermediary spicules – almost straight, usually fusiform, stout tylostyles (Figure 29B). Length 280–391–601 μm, diameter of tyle 8.7–9.2–10.1 μm, proximal diameter of shaft 5.3–6.1–7.5 μm, maximum diameter of shaft 12.5–13.4–15.1 μm.
- Small spicules – straight or occasionally curved, slender or slightly fusiform tylostyles (Figure 29C). Length 97–123–152 μm, diameter of tyle 4.9–5.7–7.0 μm, proximal diameter of shaft 3.5–4.3–5.3 μm, maximum diameter of shaft 4.5–6.2–8.3 μm.
- Exotyles straight, club-shaped, 671–911–1075 μm long (Figure 29D). Proximal tyles weakly developed (Figure 29E). Shafts gradually expanding from 8.5–11.6–17.2 μm at the proximal ends to 21.7–50.5–62.0 μm at the distal extremities (Figure 29D). Distal knobs not much wider than the shaft but well-recognizable due to their strongly tuberculated surface (Figure 29F–H).

OCCURRENCE

(Figure 3)

Southern Ocean: continental sectors 2, 3 (Davis Sea), 5 (Ross Sea), 9 (Weddell Sea) (sectors numbered according to Sarà *et al.*, 1992), 18–400 m. Indian Ocean: Kerguelen, 234–245 m (data from Boury-Esnault & Van Beveren, 1982, dubious taxonomic status). SE Atlantic: Namibian Coast, 232–403 m (data from Uriz, 1988, dubious taxonomic status).

REMARKS

Sphaerotylus vanhoeffeni is morphologically very similar to *S. capitatus* from the northern hemisphere and hence many authors regarded these two as a single species with a bipolar distribution (Kirkpatrick, 1908; Burton, 1929; Koltun, 1964, 1976; Boury-Esnault & Van Beveren, 1982; Uriz, 1988; Sarà *et al.*, 1992). In fact the Antarctic sponges differ from the typical *S. capitatus* by the substitution of the exotyle bouquets for the ordinary cortical palisade and layer of criss-cross tylostyles and the weaker prominence of the distal knobs on the exotyles. Besides that *S. vanhoeffeni* produces buds that have never been recorded in *S. capitatus*. However, we have not examined the Kerguelen and South African specimens described by Boury-Esnault & van Beveren (1982) and Uriz (1988), and thus we allocate them to *S. vanhoeffeni* with some doubt.

Sphaerotylus verenae Austin, Ott, Reiswig, Romagosa & McDaniel, 2014

Original description: *Sphaerotylus verenae* Austin, Ott, Reiswig, Romagosa & McDaniel, 2014, p. 39, figure 14.

TYPE MATERIAL
(not studied)
Holotype: RBCM (Royal British Columbia Museum in Victoria, British Columbia) 009-00053-001, Endeavour Ridge, off British Columbia/Washington, NE Pacific, 47°48.5′N 129°07.5′W, 2220 m, Alvin Dive A1443, 29.08.1984, coll. V. Tunnicliffe.

Paratype (one specimen): CMNI (Canadian Museum of Nature in Ottawa, Ontario) 2009-0027, Endeavour Ridge, off British Columbia/Washington, NE Pacific, 47°57.6′N 129°06.4′W, 2150 m, KML (Khoyatan Marine Laboratory in North Saanich, British Columbia) 1033, Alvin Dive A1439, 25.08.1984, coll. V. Tunnicliffe.

COMPARATIVE MATERIAL
(not studied)
Two specimens, Endeavour Ridge, off British Columbia/Washington, NE Pacific, 47°57.6′N 129°06.4′W, 2150 m, KML 1033, Alvin Dive A1439, 25.081984, coll. V. Tunnicliffe. One specimen, Rift Valley Floor, 47°55′N 129°06′W, off British Columbia/Washington, NE Pacific, 2196 m, KML 1034, Alvin Dive A1436, 22.08.1984, coll. V. Tunnicliffe.

DESCRIPTION
(according to Austin *et al.*, 2014)

External morphology
Sponges flattened, button-shaped or hemispherical, with single short exhalant papillae, 0.9–2.0 cm in diameter. Surface with smooth central area, white in life and becoming yellowish after preservation, and with a slightly hispid dark brown peripheral band.

Skeleton
Main choanosomal skeleton composed of longitudinal tracts of principal spicules extending to the cortex. Auxiliary choanosomal skeleton unknown. A superficial palisade of small spicules spreads over the entire cortex. In peripheral area it is underlaid by a tangential layer of small and intermediary spicules and reinforced by exotyles.

Spicules
(see Austin *et al.* (2014) for number of spicules measured)

- Principal spicules – straight, slightly fusiform subtylostyles, often with oval tyles. Length 870–1023–1500 μm, diameter 9.6–17.5–21.1 μm.
- Intermediary spicules – straight, slightly fusiform tylostyles. Length 280–531–670 μm, diameter 7.5–11.6–17.5 μm.
- Small spicules – gently curved, slightly fusiform tylostyles, occasionally with oval tyles. Length 96–114–142 μm, diameter 2.4–4.0–5.5 μm.
- Exotyles club-shaped gradually expanding towards the distal ends, with stronger or weaker developed proximal tyles and rounded smooth distal extremities, occasionally with weakly developed distal swellings. Length 1008–1275–1459 μm, medial diameter 19–48–67 μm.

OCCURRENCE
(Figure 16)
NE Pacific: Endeavour hydrothermal vent field, 2150–2220 m.

REMARKS
Sphaerotylus verenae strongly resembles *S. exotylotus* in external morphology and the club-like shape of the exotyles. Taking into account that both species inhabit deep-sea geothermally active mountainous bottoms of the North Pacific (North-east and North-west region respectively) we can assume their close affinities. The differences between *S. verenae* and *S. exotylotus* concern the size and the fine details of exotyles along with the architecture of cortex. Exotyles in the latter species possess well-developed minutely tuberulated distal bulbs and are almost two times shorter than the exotyles in *S. verenae* which have smooth distal extremities often lacking bulbs. Ordinary polymastiid cortical palisade of small tylostyles found in *S. verenae* is substituted by a palisade of exotyles in *S. exotylotus*. For a full description of *S. verenae* see Austin *et al.* (2014).

Genus *Trachyteleia* Topsent, 1928

TYPE SPECIES
Trachyteleia stephensi Topsent, 1928 (by monotypy).

DIAGNOSIS
Thickly encrusting sponges. Papillae unknown. Main choanosomal skeleton made of radial tracts of principal tylostyles. Auxiliary choanosomal skeleton comprises free-scattered intermediary tylostyles. Cortex composed of a palisade of small tylostyles and an inner layer of criss-cross intermediary tylostyles, and reinforced by exotyles which differ from principal tylostyles only by larger size and finely spined distal extremities.

Trachyteleia stephensi Topsent, 1928
(Figure 30)

Original description: *Trachyteleia stephensi* Topsent, 1928, p. 152, pl. VI figure 11.

SYNONYMS AND CITATIONS
Trachyteleia stephensi (Boury-Esnault, 2002, p. 218, figure 15).

TYPE MATERIAL
MNHN D-T 1285 (slides from holotype), Island of Villafranca, Azores, NE Atlantic, 1740 m, Scientific campaigns of the Prince of Monaco, campaign in 1911, station 3150. Topsent based his description on a small sponge fragment which was completely used for preparations. We have examined his microscopy slides.

DESCRIPTION
External morphology
(according to Topsent, 1928)
Holotype was a piece of a cushion-shaped sponge. Its surface was hispid, grey in alcohol, without papillae.

Skeleton
Main choanosomal skeleton composed of radial tracts of principal spicules which cross the cortex (Figure 30A). Auxiliary choanosomal skeleton comprises free-scattered intermediary spicules. Cortex made of a superficial palisade of small spicules and an inner layer of criss-cross intermediary spicules, reinforced by exotyles protruding above the surface.

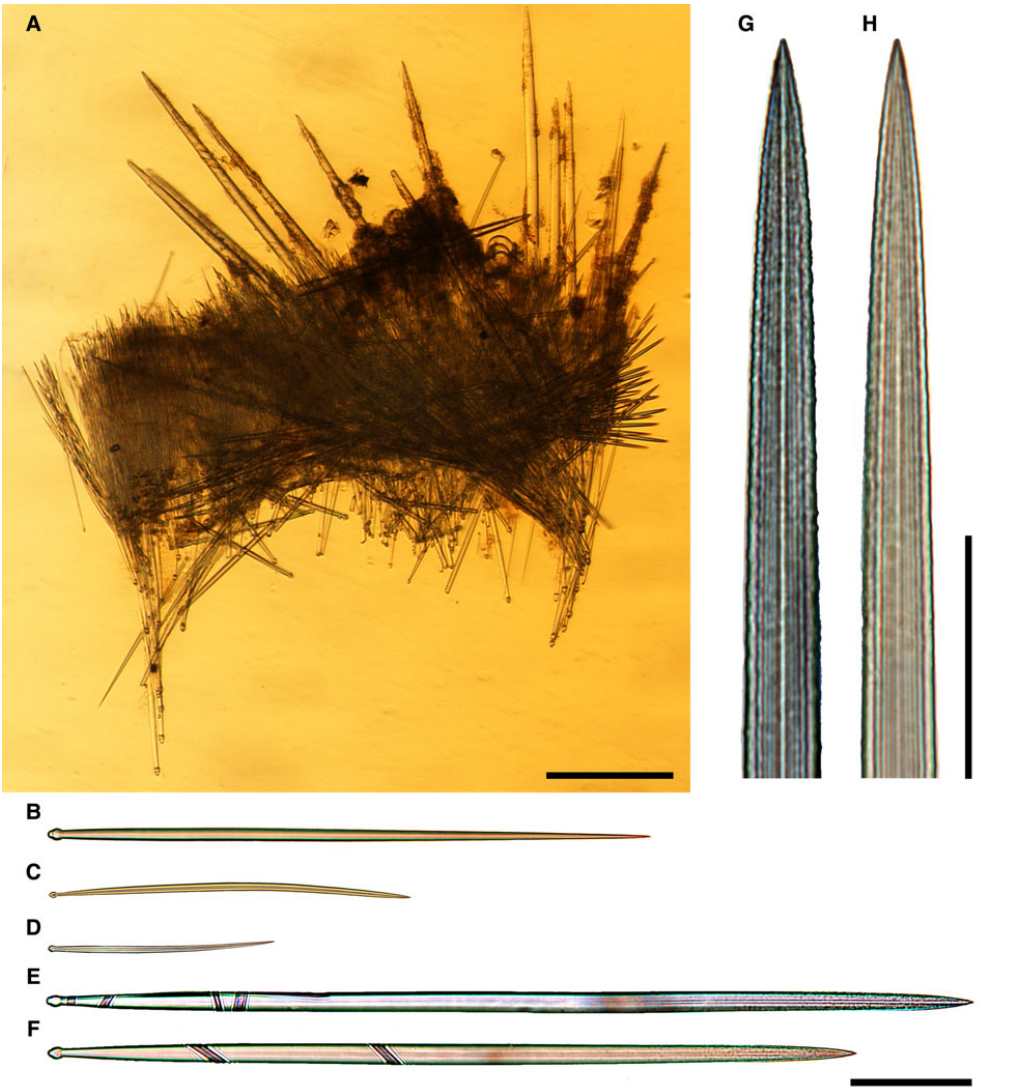

Fig. 30. *Trachyteleia stephensi*, holotype MNHN D-T 1285: (A) longitudinal section through the body; (B) principal spicule; (C) intermediary spicule; (D) small spicule; (E) and (F) exotyles, general view; (G) finely spined distal tip of the exotyle depicted in E, detailed view; (H) finely spined distal tip of the exotyle depicted in F, detailed view. Scale bars: A, 0.2 mm; B–F, 0.1 mm; G and H, 0.05 mm.

Spicules
(N = 13 for exotyles, N = 30 for other categories)

- Principal spicules – straight or more rarely gently curved, slightly fusiform tylostyles (Figure 30B). Length 337–508–602 μm, diameter of tyle 6.5–8.9–11.7 μm, proximal diameter of shaft 3.9–6.1–9.1 μm, maximum diameter of shaft 7.8–9.8–13.0 μm.
- Intermediary spicules resemble the principal tylostyles in shape (Figure 30C). Length 270–296–327 μm, diameter of tyle 3.9–5.3–7.8 μm, proximal diameter of shaft 1.3–2.9–5.2 μm, maximum diameter of shaft 3.9–6.3–10.4 μm.
- Small spicules – gently curved, slender tylostyles (Figure 30D). Length 184–223–265 μm, diameter of tyle 3.9–5.2–6.5 μm, proximal diameter of shaft 2.6–3.5–5.2 μm, maximum diameter of shaft 5.2–5.6–7.8 μm.
- Exotyles – fusiform tylostyles (Figure 30E, F). Length 653–712–770 μm, diameter of tyle 8.9–11.2–13.0 μm, proximal diameter of shaft 6.1–7.9–10.4 μm, maximum diameter of shaft 11.7–15.9–18.2 μm. Among the examined exotyles 10 had distal tips covered with tiny weakly developed spines (Figure 30G, H) and three were entirely smooth.

OCCURRENCE
(Figure 12)
Known only from the type locality near Azores, NE Atlantic.

REMARKS
Trachyteleia stephensi has never been recorded again since it was described by Topsent (1928). The record of this species among the demosponges from the Cape Verde Islands and tropical West Africa (Van Soest, 1993) is an obvious mistake (Van Soest, personal communication). Presence of tiny spines on the distal tips of exotyles is in fact the only distinguishing feature of *Trachyteleia*. This unstable feature seems to be insufficient evidence for the validity of this genus while other characters cannot be carefully examined on the poor material available.

Meanwhile, a number of other polymastiid species possess similar non-ornamented exotyles in addition to a standard set of two to three categories of monactines. Most of these species are currently allocated to *Polymastia*, e.g. *P. invaginata, P. grimaldii* Topsent, 1913 and *P. hirsuta* Kelly-Borges & Bergquist, 1997. But one of them, originally described as *Tethea hispida* Bowerbank, 1864, was placed in a separate genus, *Suberitechinus*, by de Laubenfels (1949). Boury-Esnault (2002) recognized the validity of *Suberitechinus hispidus* as a species but synonymized *Suberitechinus* with *Trachyteleia*, although with some doubt. We have examined the slides prepared from the holotype of *S. hispidus*, BMNH 1868.8.27.18, and found several substantial differences between this species and *T. stephensi*. In *S. hispidus* the exotyles reach 4000 μm in length, several times longer than in *T. stephensi*. All observed exotyles of *S. hispidus* lack spines, while many principal spicules are polytylote, a feature not observed in *T. stephensi*. Thus, following Plotkin *et al.* (2012) we provisionally recognize both *Trachyteleia* and *Suberitechinus* as valid genera. However, detailed and comparative descriptions along with phylogenetic analyses based on molecular and other independent datasets on *Suberitechinus* and other polymastiids with non-ornamented exotyles are required for the definitive classification of these taxa.

Genus *Tylexocladus* Topsent, 1898

TYPE SPECIES
Tylexocladus joubini Topsent, 1898 (by original designation).

DIAGNOSIS
Thickly encrusting, spherical to hemispherical sponges, usually with a single exhalant papilla. Main choanosomal skeleton composed of radial tracts of principal monactines. Auxiliary choanosomal skeleton comprises free-scattered small monactines and may also include smooth centrotylote microxeas. All species with a superficial cortical palisade made either of small monactines reinforced by exotyles or exclusively of exotyles. Some species also with an inner cortical layer of criss-cross monactines. Principal and small monactines are usually tylostyles. Exotyles with denticulate distal ornaments and often with proximal tyles (cladotylostyles).

Tylexocladus hispidus Lévi, 1993
(Figure 31)

Original description: *Tylexocladus hispidus* Lévi, 1993, p. 23, figure 6B.

SYNONYMS AND CITATIONS
Tylexocladus hispidus (Kelly-Borges & Bergquist, 1997, p. 396; Boury-Esnault, 2002, p. 207).

TYPE MATERIAL
Holotype: MNHN D-CL 3582 (specimen in alcohol), New Caledonia, SW Pacific, 20°34.35′S 166°53.90′E, 435 m, campaign BIOCAL on RV 'Jean Charcot' in 1985, station DW 08.

DESCRIPTION
External morphology
Holotype – cushion-shaped crust attached to sand grains, ~ 10 × 10 × 1 mm in size (Figure 31A). Surface whitish in colour, with sparse bristle of large exotyles and undercoat of slightly protruding smaller exotyles, without papillae.

Skeleton
Holotype lacks the major portion of its choanosome. Remnants of the choanosome comprise sparse radial tracts of principal spicules which fan and ascend to the cortex. Cortex better preserved. Major portion of the cortex comprises a dense palisade of small and intermediary exotyles crossed by a layer of criss-cross tylostyles in its medial zone and pierced by large exotyles ascending from the choanosome (Figure 31B). In a tiny spot of the surface without exotyles the ascending tracts of principal spicules form bouquets reinforced by sparse intermediary tylostyles. In the surrounding area the palisade is made of intermediary exotyles and the crossing layer comprising intermediary tylostyles is loose (Figure 31C). In the peripheral cortex the palisade is composed of small exotyles and crossed by a dense layer of small tylostyles (Figure 31D).

Spicules
(N = 7 for large exotyles, N = 10 for other categories)

- Principal spicules – usually straight, slender styles to subtylostyles. Length 450–557–610 μm, diameter of shaft 9.0–10.0–12.0 μm (Figure 31E).
- Intermediary tylostyles usually gently curved, slender (Figure 31F). Length 255–293–334 μm diameter of tyle 3.9–6.4–9.5 μm, diameter of shaft 3.4–8.5–12.1 μm.
- Small tylostyles curved, stout, occasionally fusiform (Figure 31G). Length 104–147–188 μm, diameter of tyle 4.7–9.8–13.0 μm, maximum diameter of shaft 3.3–9.2–12.4 μm.
- Small exotyles – stout club-shaped cladotylostyles (Figure 31H). Length 214–341–510 μm. Well-developed, smooth or occasionally tuberculated proximal tyles, 8.0–15.5–21.3 μm in diameter. Shafts expanding from 3.0–10.4–15.0 μm near proximal tyles to 6.0–21.5–28.8 μm at distal ends. Distal ends usually denticulate, with numerous acerated jags resembling the distal ornamentations of exotyles in *Tylexocladus joubini*. Some exotyles with bowl-like distal ornamentations formed by smooth jags fused together.
- Intermediary exotyles – fusiform cladotylostyles with weakly developed proximal tyles (Figure 31I). Length 800–967–1145 μm, maximum diameter of shaft 33.0–37.6–44.0 μm. Distal extremities looking like the acerated tips of ordinary monactines were cleft.
- Large exotyles – cladotylostyles resembling the intermediary exotyles in shape but appearing more slender. Length 3012–3876–4994 μm, maximum diameter of shaft 32.0–34.6–38.0 μm.

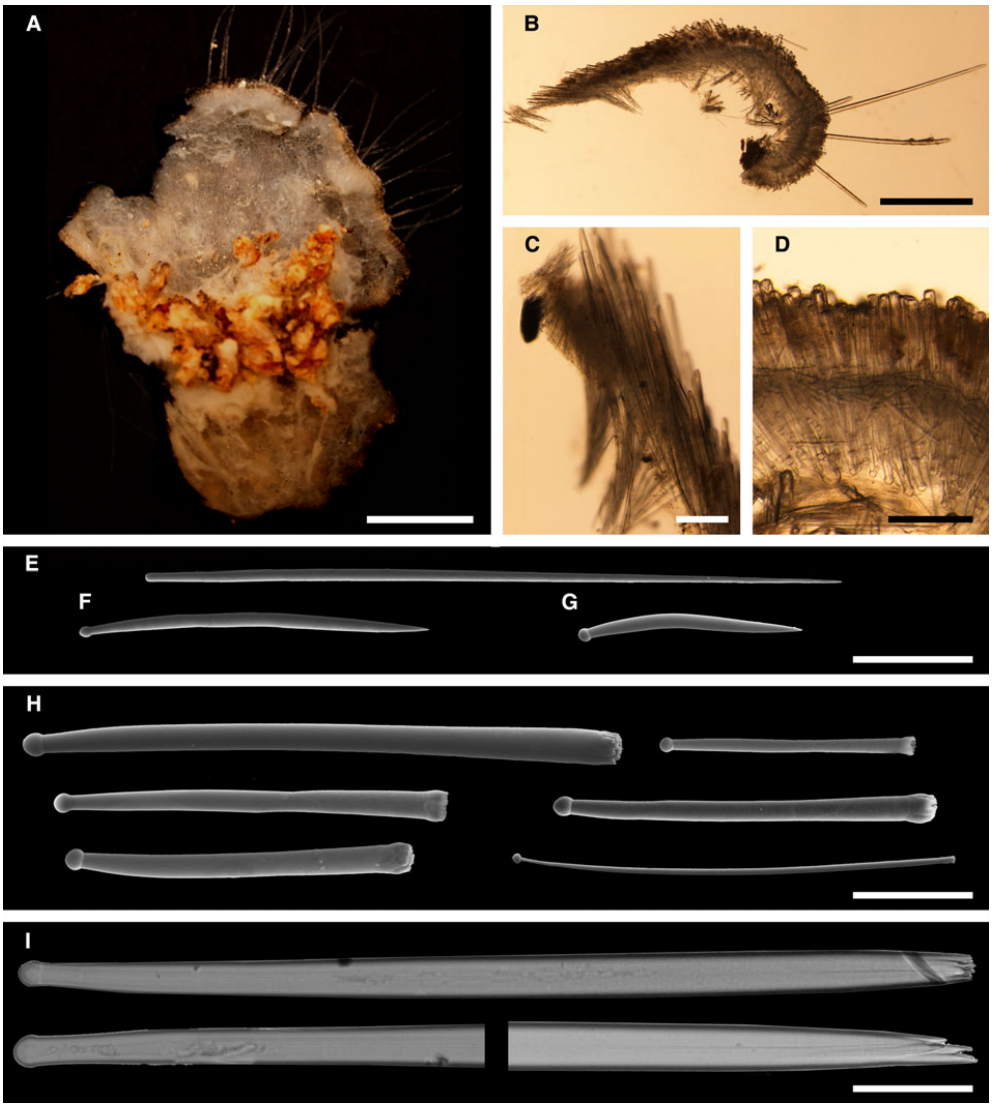

Fig. 31. *Tylexocladus hispidus*, holotype MNHN D-CL 3582: (A) habitus; (B) longitudinal section through the body, general view; (C) the same section, detail of central cortex; (D) the same section, detail of peripheral cortex; (E) principal tylostyle; (F) small tylostyle of central cortex; (G) small tylostyle of peripheral cortex; (H) small exotyles of peripheral cortex; (I) intermediary exotyles of central cortex. Scale bars: A, 2 mm; B, 1 mm; C and D, 0.2 mm; E–I, 0.1 mm.

OCCURRENCE

(Figure 16)

Known only from the type locality near New Caledonia, SW Pacific.

REMARKS

Tylexocladus hispidus differs from the type species of *Tylexocladus, T. joubini*, by the lack of a cortical palisade of small tylostyles and by the presence of three categories of cladotylostyles, the smallest resembling those of *T. joubini* and forming the peripheral palisade, the intermediary with narrowed and cleft distal extremities forming the central palisade and the largest resembling the intermediary ones in shape and making up the surface bristle. The lack of microxeas also discriminates *T. hispidus* from the type specimens of *T. joubini*, although some other specimens of the latter species lack the microxeas as well (see below).

Tylexocladus joubini Topsent, 1898

(Figures 32–34)

Original description: *Tylexocladus joubini* Topsent, 1898, p. 242, figure 2d.

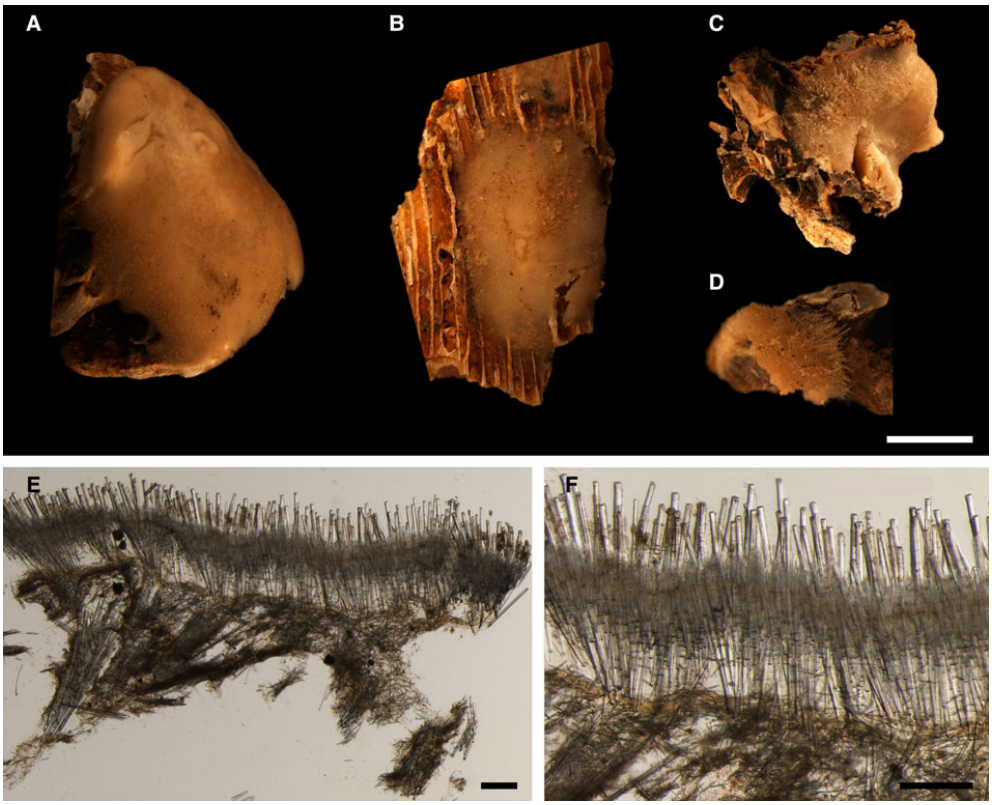

Fig. 32. *Tylexocladus joubini*, type and characteristic specimens: (A) lectotype MOM 04-0526a, habitus; (B) paralectotype MOM 04-0526b, habitus; (C) paralectotype MOM 04-0526c, habitus; (D) specimen MOM 04-1244a, habitus; (E) longitudinal section through the body of the lectotype, general view; (F) the same section, detail of cortex. Scale bars: A–D, 5 mm; E and F, 0.2 mm.

SYNONYMS AND CITATIONS

Tylexocladus joubini (Topsent, 1904, p. 122, pl. I figure 9, pl. XII figures 10–11; Topsent, 1928 (part.): 151, pl. VI figure 4; Kelly-Borges & Bergquist, 1997: p. 395, figure 26A–B; Boury-Esnault, 2002, p. 207, figure 5).

Nec *Tylexocladus joubini* (Boury-Esnault *et al.*, 1994, p. 75, figure 50).

TYPE MATERIAL

Lectotype (designated herein, see Figure 32A, the largest specimen from the sponges depicted by Topsent (1904) in pl. I, figure 9): MOM 04-0526a (in alcohol), Azores, NE Atlantic, 39°21′20″N 33°26′08″W, 1360 m, Scientific campaigns of the Prince of Monaco, campaign in 1896 on yacht 'Princesse Alice', station 702.

Paralectotypes (Figure 32B–C): MOM 04-0526b-c (two specimens in alcohol), from the same sample as the lectotype.

Slides from the type series: MNHN D-T 853 (one slide), BMNH1930.7.1.22 (one slide).

COMPARATIVE MATERIAL EXAMINED

MOM 04-1244a-b (two specimens in alcohol), NE Atlantic, Azores, to the West from Florès, 1229 m, Scientific campaigns of the Prince of Monaco, campaign in 1905, station 2210. MNHN D-T 1242 (one slide): NE Atlantic, Azores, to the West from Florès, 914–650 m, Scientific campaigns of the Prince of Monaco, campaign in 1905, station 2214 (Topsent (1928) recorded one intact specimen from this sample, but only a slide has been found).

DESCRIPTION

External morphology

Thickly encrusting sponges. Surface velvety to hispid, with single weakly developed exhalant papillae. Lectotype cushion-shaped, ~ 2 × 2 × 0.2 cm in size, with uniformly velvety surface (Figure 32A). Paralectotype MOM 04-0526b (Figure 32B) and specimen MOM 04-1244b (Figure 33A) with velvety surface bearing well-defined hispid marginal fringe. Paralectotype MOM 04-0526c (Figure 32C) uniformly hispid. Specimen MOM 04-1244a (Figure 32D) is a poorly preserved hispid fragment.

Skeleton

Main choanosomal skeleton composed of radial tracts of principal spicules entering the cortex, radiating and expanding into bouquets (Figure 32E). In specimen MOM 04-1244b some of principal spicules protrude slightly above the

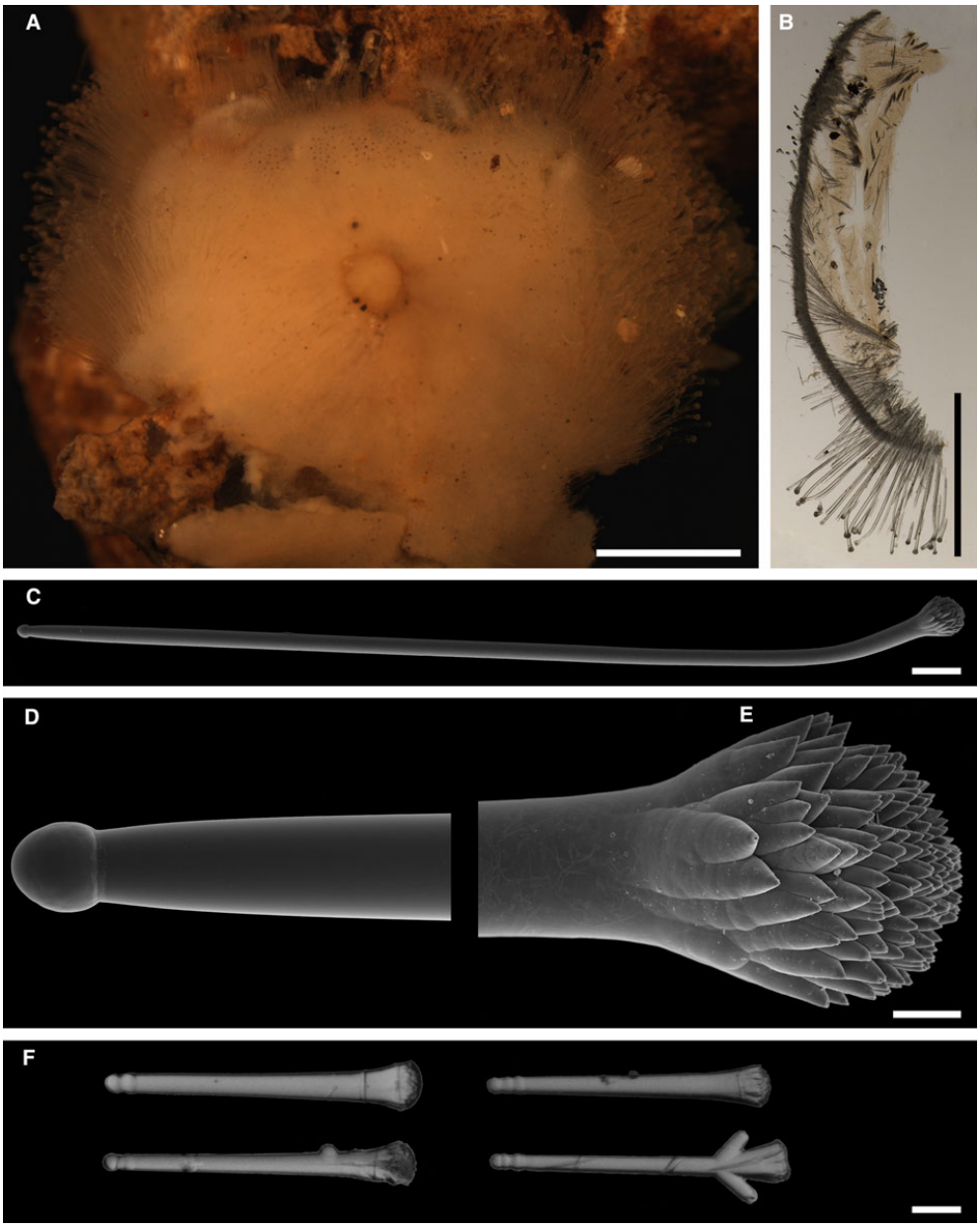

Fig. 33. *Tylexocladus joubini*, aberrant specimens: (A) specimen MOM 04-1244b, habitus; (B) specimen MOM 04-1244b, longitudinal section through the body; (C) specimen MOM 04-1244b, exotyle, general view; (D) proximal tyle of the exotyle depicted in C, detailed view; (E) artichoke-shaped distal extremity of the exotyle depicted in C, detailed view; (F) slide MNHN D-T 1242, polytylote exotyles (some with lateral processes). Scale bars: A and B, 2 mm, C, 0.1 mm; D and E, 0.02 mm; F, 0.1 mm.

cortex. Auxiliary choanosomal skeleton comprises free-scattered small tylostyles and microxeas (in most sponges studied) or only small tylostyles (in specimen MOM 04-1244b and on slide MNHN D-T 1242). Cortex (~ 190–200 μm thick) is a single palisade of small tylostyles, reinforced by exotyles (Figure 32F). In the lectotype and paralectotype MOM 04-0526c exotyles spread uniformly over the surface (Figure 32E, F). In paralectotype MOM 04-0526b and specimen MOM 04-1244b exotyles concentrated mainly at the periphery forming a marginal fringe (Figure 33B).

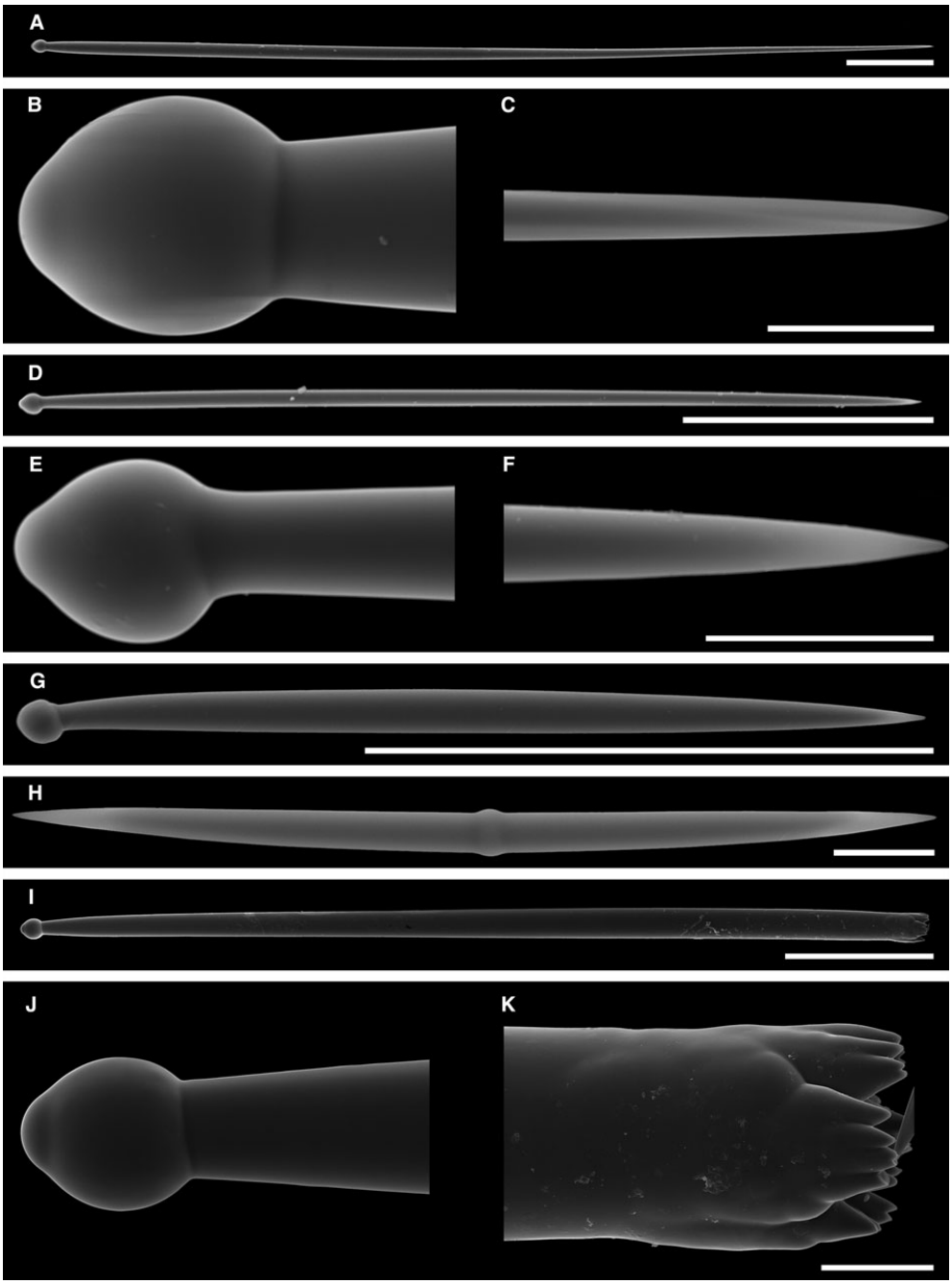

Fig. 34. *Tylexocladus joubini*, spicules of type specimens: (A) principal tylostyle, general view; (B) proximal tip of the tylostyle depicted in A, detailed view; (C) distal tip of the tylostyle depicted in A, detailed view; (D) small tylostyle, one of the largest in its category, general view; (E) proximal tip of the tylostyle depicted in D, detailed view; (F) distal tip of the tylostyle depicted in D, detailed view; (G) small tylostyle, one of the smallest in its caregory; (H) centrotylote microxea; (I) cladotylostyle, general view; (J) proximal tyle of the cladotylostyle depicted in I, detailed view; (K) denticulate distal extremity of the cladotylostyle depicted in I, detailed view. Tylostyles taken from paralectotype MOM 04-0526c, oxea and cladotylostyle taken from lectotype MOM 04-0526a. Scale bars: A, 0.1 mm; B and C, 0.01 mm; D, 0.1 mm; E and F, 0.01 mm; G, 0.1 mm; H, 0.01 mm; I, 0.1 mm; J and K, 0.01 mm.

Table 1. Individual spicule dimensions (in μm) for specimens of *Tylexocladus joubini*.

Specimen	Principal tylostyles (N = 10): length diameter of tyle diameter of shaft	Small tylostyles (N = 10): length diameter of tyle diameter of shaft	Microxeas (N = 10): length diameter of central tyle	Exotyles (N = 8): length diameter of proximal tyle diameter of shaft near tyle diameter of distal ornamentation
MOM 04-0526a (lectotype)	920–1007–1104 12.4–13.9–17.9 13.9–15.8–18	169–221–302 7–9.2–11 8.1–9.7–10.1	89.8–98.6–115 4–4.9–5.5	475–549–604 11.3–13.6–14.3 7.5–8.9–10.2 15.1–19.2–22.1
MOM 04-0526b (paralectotype)	1052–1093–1133 13.3–14.8–17.8 14.7–16.2–17.8	160–258–359 7.3–7.6–8 7.8–8.5–9.8	65–82.9–101 3–3.8–5	575–723–930 11–12.2–13.8 7.9–9.2–10.2 14.7–18.1–20.7
MOM 04-1244b	780–958–1150 15.6–17.6–18.2 13–15.6–18.2	120–201–291 7.8–8.9–10.4 6.5–7.2–7.8	Not found	1452–1689–1959 23.8–25–26.3 20–24.7–29.8 88–90.2–91.8
MNHN D-T 853	550–660–780 12.2–13.6–14.8 10.4–11.1–12.5	148–170–200 6.5–7.8–10.4 5.2–7–7.8	Not found	365–497–590 20.8–31.2–39 18.2–26.5–33.8 46.8–69.2–91

Spicules

(measurements based on four specimens, individual dimensions presented in Table 1, N = 8 for exotyles, N = 10 for other categories)

- Principal spicules – usually straight, slender tylostyles (Figure 34A–C). Length 550–930–1150 μm, diameter of tyle 12.2–14.6–18.2 μm, diameter of shaft 10.4–14.9–18.2 μm.
- Small spicules – stout, more rarely slender tylostyles (Figure 34D–G). Length 120–213–359 μm, diameter of tyle 6.5–8.4–11 μm, diameter of shaft 5.2–8.1–10.1 μm.
- Microxeas in type specimens and specimen MOM 04-1244a smooth, centrotylote (Figure 34H). Length (in type specimens) 65–91–115 μm, diameter of central tyle 3.0–4.4–5.5 μm. Not found in specimen MOM 04-1244b and on slide MNHN D-T 1242.
- Exotyles (cladotylostyles) in type specimens and specimen MOM 04-1244a straight, slightly fusiform (Figure 34I), with well-developed proximal tyles (Figure 34J) and denticulate distal ornamentations comprising numerous acerated jags (Figure 34K).
- Exotyles (cladotylostyles) in specimen MOM 04-1244b much larger than in type specimens (Table 1) and also distinguished by shape – they are usually bent at distal portions (Figure 33C) and possess well-developed proximal tyles (Figure 33D), nearly equidiametric shafts and prominent artichoke- or flowerbud-shaped distal knobs (Figure 33E).
- Exotyles (cladotylostyles) on slide MNHN D-T 1242 straight, stout, with well-developed proximal tyles, two–three ring swellings on shafts directly behind the tyles and denticulate distal ornamentations, occasionally with few extra distal swellings and/or lateral shoots (Figure 33F).

OCCURRENCE

(Figure 12)

Known only from the type locality near Azores, NE Atlantic.

REMARKS

Except for the presence of cladotylostyles, *T. joubini* demonstrates many similarities with *Atergia corticata* Stephens, 1915 – external morphology, a single-layered cortex and choanosomal smooth microxeas (occasionally in *Tylexocladus* and characteristic of *Atergia*). These similarities led Topsent (1928) to suggest that the presence of cladotylostyles was an unstable feature and he synonymized *A. corticata* with *T. joubini*. Among six Azorean specimens described as *T. joubini* in that paper by Topsent, four specimens (including MOM 04-1244a,b and MNHN D-T 1242 described above in the present paper) possessed cladotylostyles while two others lacked this category of spicules. We have examined one of the two sponges without exotyles, MOM 04-1244c. In addition to the principal and small tylostyles it has tylostyles of an extra category, 1700–2720 μm long, forming a marginal fringe which resembles the fringe of MOM 04-1244b made of cladotylostyles. The synonymy of *A. corticata* with *T. joubini* led to a number of misidentifications and confusion – Boury-Esnault *et al.* (1994) recorded *T. joubini* without cladotylostyles from the Mediterranean, and Kelly-Borges & Bergquist (1997) described a new species of *Tylexocladus, T. villosus* which also lacked cladotylostyles, from New Zealand. Evidenly, *Tylexocladus* and *Atergia* are closely affiliated genera, and only phylogenetic analyses based on molecular datasets can reveal the relationships between them. Here we follow Boury-Esnault (2002) who proposed the allocation of all specimens with cladotylostyles to *Tylexocladus* regardless of whether they have microxeas or not, whereas all externally similar sponges possessing microxeas but lacking cladotylostyles are considered as *Atergia*.

Meanwhile, two non-type Azorean specimens with cladotylostyles differ from the type series by several features. Specimen MOM 04-1244b is distinguished by longer cladotylostyles with flowerbud-shaped distal knobs, while specimen MNHN D-T 1242 stands out for its polytylote cladotylostyles

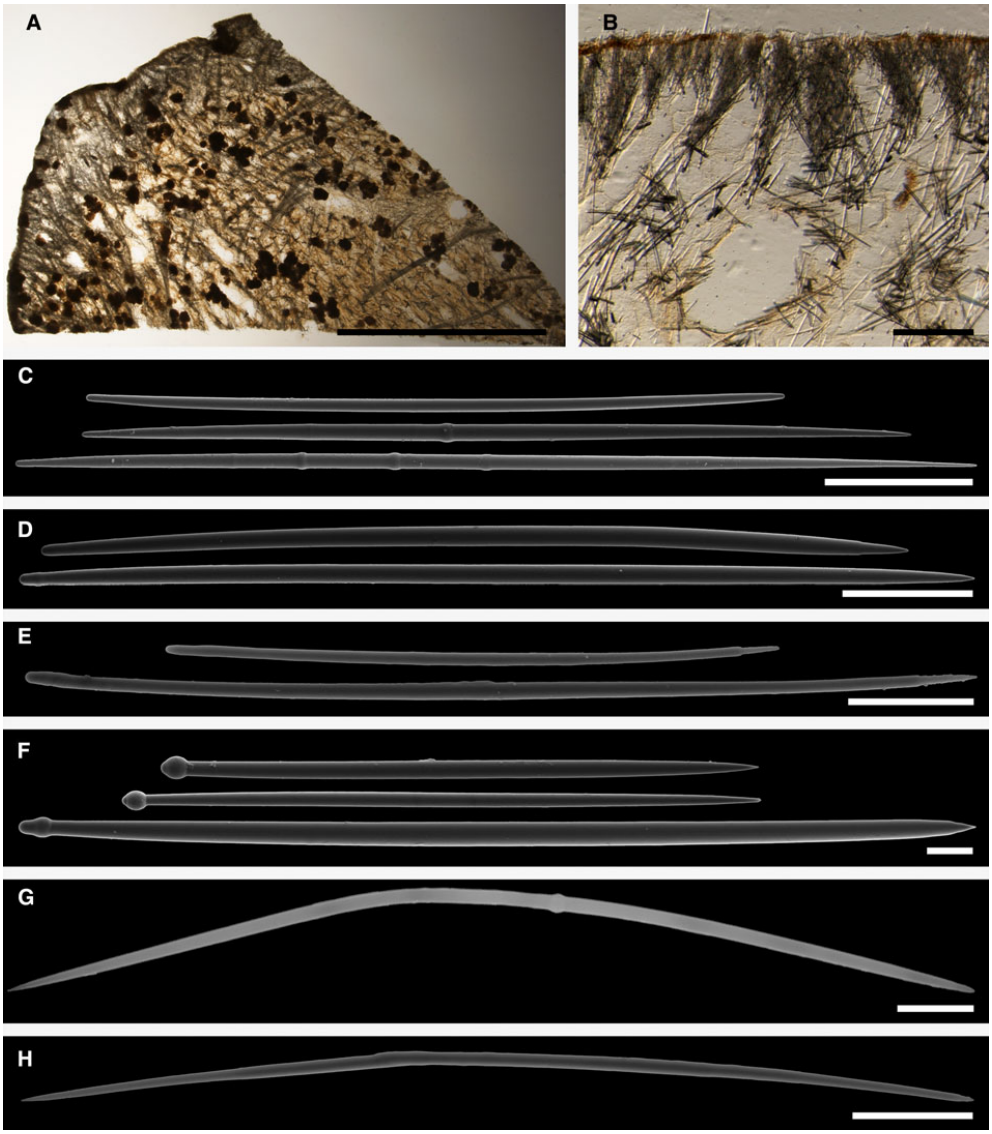

Fig. 35. *Polymastia umbraculum*, holotype NZNM Por 66: (A) longitudinal section through the body, general view; (B) the same section, detail of cortex; (C) principal strongyloxeas; (D) intermediary subtylostyles; (E) slender small styles; (F) stout small tylostyles; (G) centrotylote oxea; (H) oxea lacking tyle. Scale bars: A, 3 mm; B, 0.2 mm; C, 0.1 mm; D, 0.05 mm; E–H, 0.01 mm.

with occasional lateral shoots. Following Topsent (1928) we regard these features as intraspecific variation. However, this assumption should be tested by more accurate molecular approaches on fresh material.

INCERTAE SEDIS

Polymastia umbraculum Kelly-Borges & Bergquist, 1997 (Figures 35 & 36)

Original description: *Polymastia umbraculum* Kelly-Borges & Bergquist, 1997, p. 380, Figure 12.

TYPE MATERIAL

Holotype (specimen in alcohol, a fragment studied): NZNM Por 66, Vivian Bay, Kawau Island, Hauraki Gulf, New Zealand, 36°25′S 174°51′E, 6 m, 10.02.1990. Fragment of holotype (studied): BMNH 1996.2.22.7.

Paratypes (several specimens, not studied): NZNM Por 549, from the same locality as the holotype, 02.01.1990.

DESCRIPTION

External morphology

(according to Kelly-Borges & Bergquist, 1997)

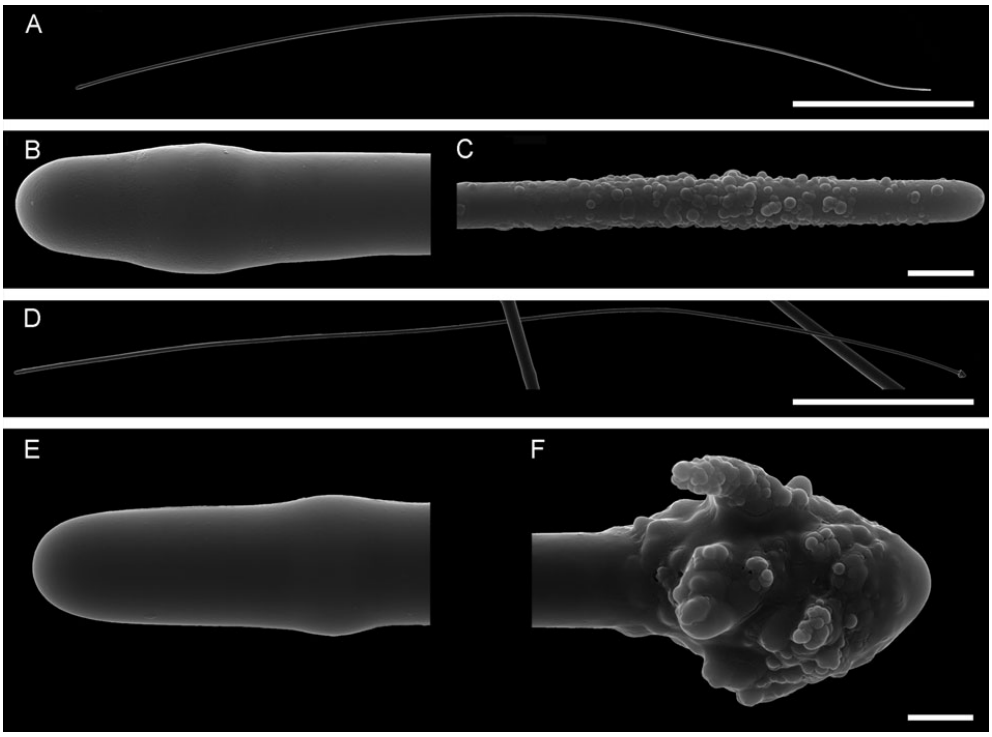

Fig. 36. *Polymastia umbraculum*, holotype NZNM Por 66, exotyles: (A) exotyle without distal knob, general view; (B) proximal tip of the exotyle depicted in A, detailed view; (C) tuberculated distal extremity of the exotyle depicted in A, detailed view; (D) exotyle with distal knob, general view; (E) proximal tip of the exotyle depicted in D, detailed view; (F) umbrelliform distal knob of the exotyle depicted in D, detailed view. Scale bars: A, 0.1 mm; B, 0.001 mm; C, 0.001 mm; D, 0.1 mm; E, 0.001 mm; F, 0.001 mm.

Encrusting sponges growing in oblong patches, 6–7 × 3–4 cm wide and ~ 2 cm thick. Surface granular with foraminiferan symbionts and microhispid with projecting spicules. Papillae considerably reduced. Colouration in life – surface yellowish orange, interior dark orange.

Skeleton
(our observations)
Main choanosomal skeleton composed of tracts of principal spicules. Few thicker tracts (120–250 μm thick) run longitudinally and form numerous thinner meanders in both horizontal and vertical direction, making up a network (Figure 35A). This network reinforced by auxiliary choanosomal skeleton of small and intermediary spicules. Numerous foraminiferans spread over the choanosome. Kelly-Borges & Bergquist (1997) recorded choanosomal stellate crystal formations, but we have not observed such structures. Cortex with a superficial palisade composed of bouquets of small spicules (Figure 35B) reinforced by exotyles forming a thin hispidation above and supported by wide fanned brushes of intermediary spicules from below. Irregularly arranged criss-cross intermediary spicules build an inner cortical layer. Single intermediary spicules and occasional smooth microxeas overlay the superficial palisade and the middle layer of spicule brushes. Symbiotic foraminiferans and crustaceans embedded in the cortex.

Spicules
(our observations, N = 13 for exotyles, N = 10 for other categories)

- Principal spicules – strongyloxeas to fusiform subtylostyles, often polytylote (Figure 35C). Length 573–606–668 μm, maximum diameter of shaft 9.2–10.2–11.3 μm.
- Intermediary spicules – gently curved styles to subtylostyles (Figure 35D). Length 343–428–479 μm, maximum diameter of shaft 4.9–8.1–9.8 μm.
- Small spicules of two subcategories – (1) Slender styles with stepped distal tips (Figure 35E). Length 49–69–103 μm, diameter of shaft 1.0–1.2–1.5 μm. (2) Stouter tylotyles to subtylostyles (Figure 35F). Length 102–145–212 μm, diameter of tyle 3.3–4.5–6.4 μm, proximal diameter of shaft 1.7–3.0–4.7 μm, maximum diameter of shaft 1.9–4.0–6.6 μm.
- Smooth microxeas centrotylote (Figure 35G) or without tyles (Figure 35H). Length 79–176–215 μm, central diameter 1.5–1.9–3.0 μm.
- Exotyles filiform, flexous (Figure 36A, D). Length 167–441–552 μm, diameter of shaft 1.0–2.4–4.1 μm. Proximal extremities rounded, occasionally with weakly developed tyles (Figure 36B, E). Distal extremities of irregular shape, varying from slightly tuberculated tips (Figure 36C) to clubbed knobs, occasionally umbrelliform knobs with

weakly developed protuberances resembling the distal ornamentations on the exotyles in *Proteleia sollasi* (Figure 36F).

OCCURRENCE

(Figure 3)

Known only from the type locality near New Zealand, SW Pacific.

REMARKS

The allocation of this species to a particular genus is difficult as it demonstrates affinities with several genera. The extremely thin exotyles with irregular, clubbed or occasionally umbrelliform distal extremeties resemble those in *Proteleia sollasi*, the reticulated choanosomal skeleton is similar to that in *Weberella* spp. and the smooth centrotylote microxeas recall the microxeas in *Tylexocladus joubini* and *Atergia corticata*. The reduced papillae of *P. umbraculum* are reminiscent of some suberitids rather than polymastiids. As none of these features are present in the type species of *Polymastia, P. mamillaris*, and *P. umbraculum* does not fit well into any other existing genus, awaiting evidence from molecular studies, we propose to keep it as *incertae sedis*.

DISCUSSION

Discrimination between the polymastiid genera and species with exotyles was for years mainly based on the shape of distal ornamentations of these spicules (Ridley & Dendy, 1886, 1887; Swarczewsky, 1906; Topsent, 1898, 1928; Koltun, 1970; Boury-Esnault, 2002). Our study has shown the significance of other characters classified in six groups – (1) number and prominence of papillae, (2) presence of a surface hispidation formed by the protruding tracts of principal spicules, (3) architecture of cortex, (4) density of exotyles in the cortex, (5) size of principal spicules and exotyles, (6) presence of extra spicule categories in addition to the ordinary ones (Table 2). Affinities of the species presented above can therefore be reconsidered in view of these characters.

Six species of *Sphaerotylus* including the type species, *S. capitatus*, along with *S. exotylotus, S. raphidophora, S. sceptrum, S. vanhoeffeni* and *S. verenae* share the presence of weakly developed papillae, relatively short (less than 2 mm) principal spicules, and a delicate but dense surface echination formed by numerous protruding exotyles (Table 2). These exotyles are relatively short (less than 2 mm) and stout, with distal extremities bearing regular ornamentations which vary from weakly developed (*S. raphidophora, S. sceptrum, S. vanhoeffeni* and *S. verenae*) to well-developed spherical or subspherical knobs (*S. capitatus* and *S. exotylotus*). Architecture of the cortex in these six species varies greatly. It may comprise a single palisade of exotyles (*S. exotylotus* and *S. vanhoeffeni*), a single palisade of small tylostyles (*S. raphidophora*) or a superficial palisade of small tylostyles together with an inner layer of criss-cross intermediary monactines delimited by a zone with few spicules (*S. capitatus*). In *S. sceptrum* and *S. verenae* the architecture of the cortex in the areas around papillae and in the periphery is different. Of the six species considered above, five species possess extra spicule categories in addition to ordinary monactines in their auxiliary choanosomal skeleton – exotyles in *S. capitatus, S. exotylotus, S. sceptrum* and *S. vanhoeffeni* and raphides in trichodragmata in *S. raphidophora*.

The type specimens of the type species of *Tylexocladus, T. joubini*, possess at least three affinities with *Sphaerotylus raphidophora* – the presence of weakly developed papillae, a single-layered cortex comprising just a palisade of tylostyles and a delicate but dense superficial echination formed by numerous short and stout exotyles (Table 2). The distinguishing features of these specimens of *T. joubini* are the presence of centrotylote microxeas in the choanosome and the presence of expanded denticulate distal ornamentations on the exotyles. Unlike the type specimens, an aberrant specimen of *T. joubini*, MOM 04-1244b, lacks microxeas and possesses a heterogeneous surface with a central area free of exotyles and a marginal zone echinated by long (more than 2 mm) exotyles bearing artichoke-shaped distal ornamentations (Table 2). The other species of *Tylexocladus, T. hispidus*, is distinguished by a cortex comprising a palisade made of short exotyles of two categories, intermingled with a layer of criss-cross small tylostyles and reinforced by long (more than 2 mm) exotyles of third category forming a sparse surface hispidation (Table 2).

Four species including both species of *Proteleia* known so far, *P. sollasi* and *P. tapetum*, and two species of *Sphaerotylus, S. isidis* and *S. strobilis*, share the presence of well-developed papillae, relatively short (less than 2 mm) principal spicules and a sparse surface echination formed either by both the tracts of principal spicules ascending from the choanosome and the exotyles (*S. isidis* and *S. strobilis*) or only by the exotyles (*Proteleia* spp.) (Table 2). The exotyles are relatively short (less than 2 mm), usually slender (even filiform in *Proteleia* spp.), with well-developed distal ornamentations which may be regularly spherical (*S. isidis*), strobile-shaped (*S. strobilis*), regularly umbrelliform or fungiform (*P. tapetum*) or of irregular, variable shape (*P. sollasi*). The cortex in these four species comprises at least two layers, a superficial palisade of small tylostyles and an inner layer of criss-cross intermediary monactines. In *P. tapetum* these layers are intermingled. In *S. isidis* and *S. strobilis* they are delimited by a zone with few spicules. In *P. sollasi* the superficial palisade and the inner layer are separated by an extra palisade of intermediary monactines.

Six species, namely four *Sphaerotylus* spp. (*S. antarcticus, S. borealis, S. renoufi* and *S. tjalfei*), the only species of *Koltunia* (*K. burtoni*), and the only species of *Trachyteleia* (*T. stephensi*), share the presence of a thick and dense surface hispidation formed by the tracts of principal spicules ascending from the choanosome and reinforced by exotyles (Table 2). A two-layered cortex comprising a superficial palisade of small tylostyles and an inner layer of criss-cross intermediary monactines is recorded in all these species except for *K. burtoni*. Well-developed papillae are shared by *S. antarcticus, S. borealis* and *S. renoufi*. Large principal spicules and exotyles often exceeding 2 mm in length are typical of *K. burtoni, S. antarcticus* and *S. borealis*, while in *S. tjalfei* only few spicules of these categories may reach such a length. Long exotyles are also occasionally present in *S. renoufi*. The shape of distal ornamentations on the exotyles varies greatly. In *S. tjalfei* the ornamentations are usually symmetrical spherical knobs. In *S. antarcticus* and *S. borealis* the ornamentations are variable, often irregularly umbrelliform or fungiform. A similar shape of the distal ornamentations is also observed in some exotyles in *S. renoufi*. In *K. burtoni* the ornamentations are grapnel-shaped, with conspicuous claws. In *T. stephensi* the exotyles are ordinary tylostyles with fine spines on the distal tips, and they are larger than the principal tylostyles.

Table 2. Discriminating characters of polymastiid species with ornamented exotyles.

Characters	Surface			Choanosome		Cortex			Principal monactines	Exotyles		
Species	Texture	Hispidation by ascending choanosomal tracks	Papillae, number and development	Main skeleton	Auxiliary skeleton	Superficial palisade	Intermediary layer	Inner layer of criss-cross spicules	Length	Concentration	Length	Distal ornamentations
Koltunia burtoni	Shaggy	Thick and dense	Absent?	Longitudinal	Monactines	Absent	Absent	Absent	More than 2 mm	Moderate	More than 2 mm	Symmetrical or asymmetrical, grapnel-shaped ornamentations with long claws
Proteleia sollasi	Velvety	Absent	Several, well-developed	Longitudinal	Monactines	Tylostyles	Extra palisade	Present	Less than 2 mm	Low	Less than 2 mm	Irregularly grapnel-shaped or umbrelliform knobs with short claws
Proteleia tapetum	Velvety	Absent	Several, well-developed	Longitudinal	Monactines	Tylostyles	Absent	Present, inter-mingled with palisade	Less than 2 mm	Low	Less than 2 mm	Regularly umbrelliform or fungiform knobs with short protuberances
Sphaerotylus antarcticus	Shaggy	Thick and dense	Several, well-developed	Longitudinal	Monactines	Tylostyles	Absent	Present	More than 2 mm	Moderate	More than 2 mm	Irregularly subspherical, hemispherical, fungiform or umbrelliform knobs
Sphaerotylus borealis	Shaggy	Thick and dense	Several, well-developed	Longitudinal	Monactines	Tylostyles	Absent	Present	More than 2 mm	Moderate	More than 2 mm	Irregularly fungiform, umbrelliform, hemispherical or spherical knobs
Sphaerotylus capitatus	Velvety	Absent	Several, weakly developed	Radial or longi-tudinal	Monactines + exotyles	Tylostyles	Low concen-tration of spicules	Present	Less than 2 mm	High	Less than 2 mm	Regularly spherical or subspherical knobs
Sphaerotylus exospinosus	?	Absent	Absent?	Radial	Monactines	Tylostyles	Absent	Present	Less than 2 mm	High	Less than 2 mm	Cauliflower-shaped knobs
Sphaerotylus exotylotus	Smooth around papilla, velvety in periphery	Absent	Single, weakly developed	Radial	Monactines + exotyles	Exotyles	Absent	Absent	Less than 2 mm	Extremely high (except for area around papilla?)	Less than 2 mm	Regularly bulb- or pear-shaped knobs
Sphaerotylus isidis	Velvety	Thin and sparse	Several, well-developed	Radial or longi-tudinal	Monactines	Tylostyles	Low concen-tration of spicules	Present	Less than 2 mm	Low	Less than 2 mm	Regularly spherical or subspherical knobs
Sphaerotylus raphidophora	Velvety	Absent	Absent?	Longitudinal	Monactines + tricho-dragmata of raphides	Tylostyles	Absent	Absent	Less than 2 mm	High	Less than 2 mm	Rounded, granulated tips, occasionally with weakly developed knobs
Sphaerotylus renoufi	Shaggy	Thick and dense	Several, well-developed	Radial or longi-tudinal	Monactines	Tylostyles	Absent	Present	Less than 2 mm	Moderate	Rarely more than 2 mm	Irregularly fungiform, lobate or subspherical knobs

Sphaerotylus sceptrum	Smooth around papillae, rough in periphery	Absent	Several, weakly developed	Radial	Monactines + exotyles	Areas around papillae – tylostyles, periphery – exotyles	Absent	Present around papillae, absent in periphery	Less than 2 mm	None around papillae, high in periphery	Less than 2 mm	Gradually expanding tuberculated extremities without knobs
Sphaerotylus strobilis	Minutely hispid or velvety	Thin and sparse	Several, well-developed	Radial or longitudinal	Monactines	Tylostyles	Low concentration of spicules	Present	Less than 2 mm	Low	Less than 2 mm	Regular or irregular strobile-shaped knobs
Sphaerotylus tjalfei	Shaggy	Thick and dense	Single, moderately developed	Radial	Monactines	Tylostyles	Absent	Present	Rarely more than 2 mm	Low	Less than 2 mm	Regularly spherical or subspherical knobs
Sphaerotylus vanhoeffeni	Velvety	Absent	Several, weakly developed	Radial	Monactines + exotyles	Exotyles	Absent	Absent	Less than 2 mm	Extremely high	Less than 2 mm	Weakly developed, subspherical knobs
Sphaerotylus verenae	Smooth centre, hispid periphery	Absent	Single, weakly developed	Longitudinal	Unknown	Tylostyles	Absent	Absent in centre, present in periphery	Less than 2 mm	None in centre, high in periphery	Less than 2 mm	Gradually expanding smooth extremities, occasionally with weakly developed knobs
Trachyteleia stephensi	Shaggy	Thick and dense	Absent?	Radial	Monactines	Tylostyles	Absent	Present	Less than 2 mm	Moderate	Less than 2 mm	Acerated, occasionally finely spined tips
Tylexocladus hispidus	Shaggy	Present only in a central spot, thin	Absent?	Radial	Monactines	Exotyles	Absent	Present	Less than 2 mm	High for exotyles I and II, low for exotyles III	I and II – less than 2 mm III – more than 2 mm	I – expanding, denticulate extremities, II and III – acerated, cleft tips
Tylexocladus joubini (type specimens)	Velvety	Absent	Single, weakly developed	Radial	Monactines + centrotylote microxeas	Tylostyles	Absent	Absent	Less than 2 mm	High	Less than 2 mm	Expanding, denticulate extremities
Tylexocladus joubini (specimen MOM 04-1244b)	Velvety, with marginal fringe	Absent	Single, weakly developed	Radial	Monactines	Tylostyles	Absent	Absent	Less than 2 mm	None in centre, high in marginal fringe	More than 2 mm	Symmetrical artichoke-shaped knobs
Incertae sedis: *Polymastia umbraculum*	Minutely hispid	Absent	Several, weakly developed	Reticulated	Monactines	Tylostyles	Brushes of intermediary monactines	Present, intermingled with palisade includes centrotylote microxeas	Less than 2 mm	Low	Less than 2 mm	Tuberculated tips or clubbed knobs, occasionally with short protuberances

Among the species studied, one, *Polymastia umbraculum*, is controversial with respect to its affinities to other genera (Table 2). Whilst its reduced papillae are reminiscent of some suberitids, the cortex, comprising a superficial palisade of small tylostyles underlain by two layers of intermediary monactines and reinforced by sparse filiform exotyles with minutely branching distal ornamentations resembles that of *Proteleia* spp. Finally, the reticulated choanosomal skeleton of *P. umbraculum* is similar to that in *Weberella* spp.

A look at the diversity of the polymastiids with ornamented exotyles from a biogeographic perspective reveals that the known distribution of the 14 species is limited to very small geographic areas. Among these, nine species are endemic to the Pacific. Four species of *Sphaerotylus* are widely distributed, and they comprise two pairs of morphological equivalents distributed in the polar and subpolar zones, each pair containing one species in the northern hemisphere and the other in the southern hemisphere. Substantial morphological and ecological similarities of *S. borealis* and *S. antarcticus* rouse a challenging hypothesis of the existence of a single species with a bipolar distribution (Koltun, 1976). *Sphaerotylus capitatus* and *S. vanhoeffeni* demonstrate more distinctions than revealed in the first pair, but still these species possess many affinities, and a careful re-examination of the Kerguelen and Namibian specimens assigned to *S. capitatus* (Boury-Esnault & Van Beveren, 1982; Uriz, 1988) can probably throw more light on their relationship.

Morphological affinities between the species addressed in the present study should be re-evaluated by an integrative phylogenetic approach based on comprehensive molecular and morphological datasets in order to reveal the natural relationships between all polymastiid species possessing exotyles, both ornamented and non-ornamented.

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Paper III

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III

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ORIGINAL ARTICLE

ORGANISMS DIVERSITY & EVOLUTION

Molecular phylogenies challenge the classification of Polymastiidae (Porifera, Demospongiae) based on morphology

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Abstract Polymastiidae Gray, 1867 is a worldwide distributed sponge family, which has a great significance for understanding of the demosponge deep phylogeny since the former order Hadromerida Topsent, 1894 has been recently split based on the molecular evidence and a new separate order has been established for the polymastiids. However, molecular data obtained from Polymastiidae so far are scarce, while the phylogenetic reconstruction based on morphology has faced a deficit of characters along with the vagueness of their states. The present study is a phylogenetic reconstruction of Polymastiidae based on novel data on two molecular markers, cytochrome oxidase subunit I and large subunit ribosomal DNA, obtained from a broad set of species. Monophyly of the family and nonmonophyly of four polymastiid genera are revealed, suggesting a high level of homoplasy of morphological characters, which are therefore not an appropriate base for the natural classification of Polymastiidae. Although the presented phylogenies cannot yet provide an alternative classification scheme, several strongly supported clades, which may be used as reference points in future classification, are recovered and three taxonomic actions are proposed: transfer of one species from *Radiella* to *Polymastia* Bowerbank, 1862; transfer of three species from *Radiella* Schmidt, 1870 to *Spinularia* Gray, 1867; and the consequent abandonment of *Radiella*.

Keywords Phylogeny · Homoplasy · Polymastiidae · CO1 · 28S rDNA

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Introduction

Polymastiidae Gray (1867), with its 122 species from 15 genera and a worldwide distribution (Van Soest et al. 2015), is one of the key families in Demospongiae Sollas, 1885, the most diverse class of sponges. At the same time, Polymastiidae is one of the problematic taxa with a controversial classification (Plotkin et al. 2012). Classification of the demosponges has traditionally been based on the shape and arrangement of their skeletal elements, i.e., mineral spicules and organic fibers (Hooper and Van Soest 2002). The polymastiids possess a relatively simple spicule assortment providing a rather scant set of taxonomic characters (Plotkin et al. 2012). Polymastiidae comprises sponges of various body shapes, often bearing papillae and possessing a skeleton mainly composed of smooth monactines (Boury-Esnault 2002; see terminology of the sponge morphology in Boury-Esnault and Rützler 1997). Based on the latter feature, this family was until recently affiliated with the demosponge order Hadromerida Topsent, 1894. For the moment, only one morphological feature delimiting Polymastiidae from other demosponges is usually defined, the presence of a superficial cortical palisade made of spicules differing from those composing the

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choanosomal tracts in size and/or in shape (Boury-Esnault 2002; Plotkin and Janussen 2008). However, this feature is in fact also displayed by some taxa from other families, e.g., by *Aaptos* Gray, 1867 belonging to the Suberitidae Schmidt, 1870 (Plotkin et al. 2012).

Discrimination between polymastiid genera is based on the body shape (e.g., radial body in *Radiella* Schmidt, 1870 and columnar body in *Tentorium* Vosmaer, 1887), the architecture of the choanosomal skeleton (diffuse skeleton in *Quasillina* Norman, 1869 and *Ridleia* Dendy, 1888, reticulate skeleton in *Weberella* Vosmaer, 1885 and radial skeleton in the remaining 12 genera) and the presence of spicules other than the ordinary smooth monactines in the choanosome (in four genera), or in the cortex (in five genera) (Boury-Esnault 2002). However, in some cases, these characters are inconsistent. For example, *Polymastia* Bowerbank, 1862 is usually defined as sponges with a radial choanosomal skeleton and smooth monactines constituting both the choanosomal and cortical skeleton (Boury-Esnault 2002) even though several species traditionally affiliated with *Polymastia* display a reticulate skeleton (Plotkin et al. 2012) or extraordinary spicules in the choanosome or in the cortex (Kelly-Borges and Bergquist 1997). Other characters used in the taxonomy of polymastiids include the number of size categories of the ordinary monactines and the minute differences in their shape, the presence and architecture of additional cortical layers, and the anatomy of the papillae (Boury-Esnault 1987, 2002; Kelly-Borges and Bergquist 1997; Morrow and Boury-Esnault 2000; Plotkin and Janussen 2008). These characters are often unstable and provide poor taxonomic information (Plotkin et al. 2012). Particularly, they fail to discriminate between some morphologically similar polymastiids, which inhabit the polar and temperate waters of the northern and southern hemispheres, but do not occur in the tropics, and consequently, a bipolar distribution is presumed for these species (e.g., for *Tentorium semisuberites* (Schmidt 1870) and *Radiella sarsi* (Ridley & Dendy, 1886)).

Phylogenetic reconstruction of Polymastiidae based on 25 binary morphological characters (Plotkin et al. 2012) questioned the monophyly of the family, with *Pseudotrachya hystrix* (Topsent, 1890) not grouping with any other polymastiid and one of the outgroup species, *Aaptos papillata* (Keller, 1880), joining the main polymastiid clade, and demonstrated that *Polymastia* is polyphyletic. At the same time, molecular phylogenies of Polymastiidae have been never properly reconstructed. Until now, common phylogenetic markers as the barcoding regions of cytochrome oxidase subunit I (CO1) and the partial RNA from the large and small ribosomal subunits (28S and 18S) have only been obtained for a small number of polymastiid species, aiming to resolve a deep phylogeny of the class Demospongiae instead of addressing the relationships within Polymastiidae (Nichols 2005; Morrow et al. 2012, 2013; Redmond et al. 2013; Vargas et al. 2015). In all phylogenies resulting from these studies, *Polymastia* was nonmonophyletic, while the family Polymastiidae was monophyletic excluding two species from Nichols (2005). Furthermore, in all molecular phylogenies of Demospongiae, Polymastiidae and other hadromerid families appeared in remote clades that seriously contradicted the traditional classification based on their morphological similarities. Very recently, based on the molecular data, Morrow and Cárdenas (2015) proposed abandoning the order Hadromerida and establishing five new orders for the former hadromerids, with the order Polymastiida including only one family, the Polymastiidae. This proposal highlights the importance of the polymastiids in the context of the deep phylogeny of demosponges.

The purpose of the present study was to reconstruct the phylogeny of the family Polymastiidae based on two broadly used molecular markers, the 5′-end barcoding region of CO1 (Folmer et al. 1994) and a large region of 28S rRNA (helix B10 to helix E19, numeration of the helices according to De Rijk et al. (1999, 2000) and Wuyts et al. (2001)), employing a much larger set of polymastiid species than ever studied before. We also tested the monophyly of the family as well as the monophyly of its genera and traced the evolution of morphological characters along the branches of the consensus molecular tree.

Material and methods

Sampling and taxonomic identification

Eighty-seven polymastiid individuals were collected for our study and deposited in the natural history collections of four museums (see Table 1 for details). Both the individuals in toto assigned for morphological examination and the choanosomal pieces of about 1 cm^3 for DNA extraction were fixed in 95–100 % ethanol. Sponge anatomy was examined under a light microscope on 500–700-μm-thick sections prepared using a precise saw with a diamond wafering blade after embedding of tissue pieces in epoxy resin. Isolated spicules were examined under a light microscope and SEM. Preparations and subsequent taxonomic identification followed well-known routines for polymastiids (Boury-Esnault 1987; Boury-Esnault and Bézac 2007; Plotkin and Janussen 2007, 2008).

Taxonomic scope

In our study, we included genetic data on 24 unambiguously identified species and ten operational taxonomic units (OTUs), of which four were identified to species level with some uncertainty and six could not be referred to any known species and were therefore only identified to genus level (Table 1). These species and OTUs belonged to seven

Table 1 List of specimens used in this study with museum voucher numbers, GenBank accession numbers and localities. New sequences from our study are highlighted in italics. Asterisks before accession numbers indicate the sequences resulting from cloning of the PCR products. When all clones from one product were identical, only one sequence was submitted. A range of accession numbers in one cell indicates a library of nonidentical clones. Abbreviations of the museums: BELUM Ulster Museum, Belfast, UK; GNM Gothenburg Natural History Museum, Sweden; SMF Senckenberg Naturmuseum, Frankfurt am Main, Germany; UCMPWC Museum of Paleontology, University of California, USA; ZMBN Natural History Collections, University Museum of Bergen, Norway

Species	Type species	Museum voucher	28S: B10–C1	28S: D1–D19	28S: D20–E19	CO1	Locality	Type locality
Polymastia andrica de Laubenfels, 1949		ZMBN 98055	*LN873411*	**LN873461*	*LN873424*	*LN606449*	Arctic Ocean, Norway, Svalbard	
Polymastia andrica de Laubenfels, 1949		ZMBN 98057	*HG423736*	*HG423766*	*HG423796*	*HG423707*	North Sea, Norway, Hordaland	
Polymastia andrica de Laubenfels, 1949		ZMBN 98074	*LN873415*	**LN873473–LN873477*	*LN873428*	*LN606453*	Norwegian Sea, Norway, Troms	
Polymastia andrica de Laubenfels, 1949		ZMBN 98102	–	–	–	*LN606454*	Atlantic Ocean, Canada, Newfoundland	
Polymastia andrica de Laubenfels, 1949		ZMBN 98108	–	–	–	*LN606455*	Norwegian Sea, Norway, offshore	
Polymastia arctica (Merejkowsky, 1878)		ZMBN 98060	*LN873412*	**LN873465–LN873468*	*LN873425*	*LN606450*	White Sea, Russia, Karelia	Yes
Polymastia arctica (Merejkowsky, 1878)		ZMBN 98062	*HG423734*	*HG423764*	*HG423794*	*HG423705*	White Sea, Russia, Karelia	Yes
Polymastia arctica (Merejkowsky, 1878)		ZMBN 98063	*LN873413*	**LN873469–LN873470*	*LN873426*	*LN606451*	White Sea, Russia, Karelia	Yes
Polymastia arctica (Merejkowsky, 1878)		ZMBN 98065	*LN873414*	**LN873471–LN873472*	*LN873427*	*LN606452*	Norwegian Sea, Norway, Finnmark	
Polymastia arctica (Merejkowsky, 1878)		ZMBN 98068	*HG423735*	*HG423765*	*HG423795*	*HG423706*	Norwegian Sea, Norway, Finnmark	
Polymastia bartletti de Laubenfels, 1942		ZMBN 98111	*LN606505*	*LN606535*	*LN606565*	*LN606468*	Atlantic Ocean, Canada, Nova Scotia	
Polymastia cf. *bartletti* de Laubenfels, 1942		GNM 904:1	*HG423738*	*HG423768*	*HG423798*	**LN606467*	North Sea, Sweden, Kattegat	
Polymastia boletiformis (Lamarck, 1815)		BELUM: MC5014	HQ379232	HQ379306	HQ379372	–	N/A	
Polymastia boletiformis (Lamarck, 1815)		GNM 901:1	*LN606496*	*LN606526*	*LN606556*	–	North Sea, Sweden, Skagerrak	
Polymastia boletiformis (Lamarck, 1815)		ZMBN 98047	*LN606491*	*LN606521*	*LN606551*	**HG423708*	North Sea, Norway, Hordaland	
Polymastia boletiformis (Lamarck, 1815)		ZMBN 98048	*LN606492*	*LN606522*	*LN606552*	–	North Sea, Norway, Hordaland	
Polymastia boletiformis (Lamarck, 1815)		ZMBN 98081	*LN606493*	*LN606523*	*LN606553*	–	North Sea, Norway, Hordaland	
Polymastia boletiformis (Lamarck, 1815)		ZMBN 98088	*LN606494*	*LN606524*	*LN606554*	–	North Sea, Norway, Vest-Agder	
Polymastia boletiformis (Lamarck, 1815)		ZMBN 98089	*LN606495*	*LN606525*	*LN606555*	**HG423709*	North Sea, Norway, Vest-Agder	
Polymastia cf. *conigera* Bowerbank, 1874		BELUM: MC3722	*HG423828*	*HG423829*	*HG423830*	*HG423827*	Celtic Sea, Ireland, Co. Cork	
Polymastia corticata Ridley & Dendy, 1886		ZMBN 98097	**LN873417*	*LN873445*	*LN873430*	*LN606456*	Atlantic Ocean, Canada, Newfoundland	
Polymastia corticata Ridley & Dendy, 1886		ZMBN 98104	**LN873452*	*HG423825*	*HG423826*	*HG423824*	Atlantic Ocean, Canada, Newfoundland	
Polymastia corticata Ridley & Dendy, 1886		ZMBN 98105	*LN873418*	*LN873446*	*LN873431*	*LN873450*	Atlantic Ocean, Portugal, Azores	
Polymastia euplectella Rezvoj, 1927		ZMBN 98044	*HG423737*	*HG423767*	*HG423797*	*HG423710*	Barents Sea, Norway, Finnmark	
Polymastia euplectella Rezvoj, 1927		ZMBN 98085	*LN606497*	*LN606527*	*LN606557*	*LN606457*	North Sea, Norway, Vest-Agder	
Polymastia euplectella Rezvoj, 1927		ZMBN 98086	*LN606498*	*LN606528*	*LN606558*	*LN606458*	North Sea, Norway, Vest-Agder	
Polymastia euplectella Rezvoj, 1927		ZMBN 98087	*LN606499*	*LN606529*	*LN606559*	–	North Sea, Norway, Vest-Agder	
Polymastia grimaldii (Topsent, 1913)		ZMBN 98064	*LN873419*	**LN873478–LN873482*	*LN873432*	*LN606459*	Barents Sea, Norway, Finnmark	
Polymastia grimaldii (Topsent, 1913)		ZMBN 98110	–	–	–	*LN606460*	Atlantic Ocean, Canada, Newfoundland	
Polymastia grimaldii (Topsent, 1913)		ZMBN 98112	–	–	–	*LN606461*	Barents Sea, Norway, offshore	
Polymastia invaginata Kirkpatrick, 1907		ZMBN 98046	*HG423740*	*HG423770*	*HG423800*	*HG423712*	Bellingshausen Sea, Antarctic, Antarctic Peninsula	
Polymastia invaginata Kirkpatrick, 1907		ZMBN 98093	*LN606500*	*LN606530*	*LN606560*	*LN606462*	Weddell Sea, Antarctic, South Shetland Islands	
Polymastia invaginata Kirkpatrick, 1907		ZMBN 98094	*HG423739*	*HG423769*	*HG423799*	*HG423711*	Weddell Sea, Antarctic, South Shetland Islands	
Polymastia littoralis Stephens, 1915		N/A	–	–	–	KJ129611	N/A	
Polymastia littoralis Stephens, 1915		N/A	–	–	–	NC_023834	N/A	
Polymastia mamillaris (Müller, 1806)	Yes	ZMBN 98078	*HG423741*	*HG423771*	*HG423801*	*HG423713*	North Sea, Norway, Hordaland	
Polymastia mamillaris (Müller, 1806)	Yes	ZMBN 98083	**LN873453*	**LN873462*	*LN873439*	*LN606463*	North Sea, Norway, Vest-Agder	
Polymastia pachymastia de Laubenfels, 1932		UCMPWC 932	AY561924			–	N/A	
Polymastia penicillus (Montagu, 1818)		BELUM: MC5284	HQ393895	HQ393899	HQ393903	–	N/A	
Polymastia penicillus (Montagu, 1818)		BELUM: MC6505	*LN606501*	*LN606531*	*LN606561*	*LN606464*	UK, Northern Ireland	
Polymastia penicillus (Montagu, 1818)		GNM 460:1	*LN606502*	*LN606532*	*LN606562*	–	North Sea, Sweden, Skagerrak	

Table 1 (continued)

Species	Type species	Museum voucher	28S: B10–C1	28S: D1–D19	28S: D20–E19	CO1	Locality	Type locality
Polymastia penicillus (Montagu, 1818)		GNM 460:2	*LN606503*	*LN606533*	*LN606563*	–	North Sea, Sweden, Skagerrak	
Polymastia thielei Koltun, 1964		ZMBN 98052	*LN873420*	**LN873463*	*LN873433*	*LN606469*	Arctic Ocean, Norway, Svalbard	Yes
Polymastia thielei Koltun, 1964		ZMBN 98053	–	–	–	*LN873451*	Arctic Ocean, Norway, Svalbard	Yes
Polymastia thielei Koltun, 1964		ZMBN 98070	–	–	–	*LN606470*	Norwegian Sea, NE Iceland	
Polymastia thielei Koltun, 1964		ZMBN 98107	–	–	–	*LN606471*	Denmark Straight, East Greenland	
Polymastia thielei Koltun, 1964		ZMBN 98109	–	–	–	*LN606472*	Norwegian Sea, Norway, offshore	
Polymastia uberrima (Schmidt, 1870)		ZMBN 98066	HG423744	*LN873483–873484	HG423804	*HG423716*	Norwegian Sea, Norway, Bjørnøya	
Polymastia uberrima (Schmidt, 1870)		ZMBN 98073	**LN873456–873458*	*HG423775*	*HG423805*	*HG423717*	Norwegian Sea, Norway, Troms	
Polymastia sp. 1		ZMBN 98091	*HG423742*	*HG423772*	*HG423802*	*HG423714*	North Sea, Norway, Rogaland	
Polymastia sp. 1		ZMBN 98092	*LN606504*	*LN606534*	*LN606564*	*LN606465*	North Sea, Norway, Rogaland	
Polymastia sp. 2		ZMBN 98080	*HG423743*	*HG423773*	*HG423803*	*HG423715*	North Sea, Norway, Hordaland	
Polymastia sp. 3		ZMBN 98106	–	–	–	*LN606466*	Atlantic Ocean, Canada, Newfoundland	
Quasillina brevis (Bowerbank, 1862)	Yes	BELUM: MC6569	*LN606510*	*LN606540*	*LN606570*	–	Irish Sea, UK, England	
Quasillina brevis (Bowerbank, 1862)	Yes	ZMBN 98049	*LN606506*	*LN606536*	*LN606566*	*LN606473*	North Sea, Norway, Sognefjoden	
Quasillina brevis (Bowerbank, 1862)	Yes	ZMBN 98067	*LN606507*	*LN606537*	*LN606567*	*LN606474*	Denmark Straight, West Iceland	
Quasillina brevis (Bowerbank, 1862)	Yes	ZMBN 98082	*LN606508*	*LN606538*	*LN606568*	*LN606475*	North Sea, Norway, Rogaland	
Quasillina brevis (Bowerbank, 1862)	Yes	ZMBN 98084	*HG423746*	*HG423776*	*HG423806*	*HG423718*	North Sea, Norway, Vest-Agder	
Quasillina brevis (Bowerbank, 1862)	Yes	ZMBN 98090	*LN606509*	*LN606539*	*LN606569*	*LN606476*	North Sea, Norway, Vest-Agder	
Radiella hemisphaerica (Sars, 1872)		ZMBN 98043	*HG423747*	*HG423777*	*HG423807*	*HG423719*	North Sea, Norway, Hordaland	
Radiella hemisphaerica (Sars, 1872)		ZMBN 98056	*HG423748*	*HG423778*	*HG423808*	*HG423720*	North Sea, Norway, Hordaland	
Radiella hemisphaerica (Sars, 1872)		ZMBN 98058	–	–	–	*LN606477*	North Sea, Norway, Hordaland	
Radiella hemisphaerica (Sars, 1872)		ZMBN 98069	–	–	–	*LN606478*	Irminger Basin, South Iceland	
Radiella hemisphaerica (Sars, 1872)		ZMBN 98071	–	–	–	*LN606479*	Barents Sea, Norway, Finnmark	
Radiella hemisphaerica (Sars, 1872)		ZMBN 98077	–	–	–	*LN606480*	North Sea, Norway, Hordaland	
Radiella sarsi (Ridley & Dendy, 1886)		ZMBN 98039	*HG423749*	**HG423779*	*HG423809*	*HG423721*	Norwegian Sea, Norway, offshore	
Radiella sarsi (Ridley & Dendy, 1886)		ZMBN 98098	*LN606512*	*LN606542*	*LN606572*	*LN606481*	Norwegian Sea, Norway, offshore	
Radiella cf. *sarsi* (Ridley & Dendy, 1886)		ZMBN 98103	**LN873459–LN873460*	**HG423780*	**HG423810*	**HG423722*	Indian Ocean, Mozambique, Mozambique Channel	
Radiella sp.		ZMBN 98038	*HG423751*	*HG423781*	*HG423811*	*HG423723*	Norwegian Sea, Norway, offshore	
Radiella sp.		ZMBN 98040	*LN606513*	*LN606543*	*LN606573*	*LN606482*	Norwegian Sea, Norway, offshore	
Radiella sp.		ZMBN 98041	*LN606514*	*LN606544*	*LN606574*	*LN606483*	Norwegian Sea, Norway, offshore	
Sphaerotylus antarcticus Kirkpatrick, 1907		POR 21125	–	–	–	KC869424	N/A	
Sphaerotylus antarcticus Kirkpatrick, 1907		ZMBN 98045	*HG423752*	*HG423782*	*HG423812*	*HG423724*	Bellingshausen Sea, Antarctic, Antarctic Peninsula	
Sphaerotylus borealis (Swarczewsky, 1906)		ZMBN 98036	*HG423753*	**HG423783*	*HG423813*	–	White Sea, Russia, Karelia	Yes
Sphaerotylus borealis (Swarczewsky, 1906)		ZMBN 98059	*HG423754*	*HG423784*	*HG423814*	*HG423725*	White Sea, Russia, Karelia	Yes
Sphaerotylus borealis (Swarczewsky, 1906)		ZMBN 98061	*LN606515*	*LN606545*	*LN606575*	*LN606484*	White Sea, Russia, Karelia	Yes
Sphaerotylus capitatus (Vosmaer, 1885)	Yes	GNM 902	–	–	–	*LN606486*	North Sea, Sweden, Skagerrak	
Sphaerotylus capitatus (Vosmaer, 1885)	Yes	ZMBN 98042	**LN873454*	*LN873448*	*LN873443*	*LN606485*	North Sea, Norway, Hordaland	
Sphaerotylus capitatus (Vosmaer, 1885)	Yes	ZMBN 98075	*HG423755*	*HG423785*	*HG423815*	*HG423726*	Norwegian Sea, Norway, Troms	
Sphaerotylus sp. 1		BELUM: MC4236	HQ379233	HQ379307	HQ379373	HQ379425	Atlantic Ocean, UK, Scotland	
Sphaerotylus sp. 2		BELUM: MC5015	**LN873455*	*HG423831*	*HG423832*	**LN606487*	Celtic Sea, Ireland, Co. Cork	
Spinularia spinularia (Bowerbank, 1866)	Yes	GNM 792:1	*LN606517*	*LN606547*	*LN606577*	–	North Sea, Sweden, Skagerrak	
Spinularia spinularia (Bowerbank, 1866)	Yes	ZMBN 98037	*HG423756*	*HG423786*	*HG423816*	*HG423727*	North Sea, Norway, Hordaland	
Spinularia spinularia (Bowerbank, 1866)	Yes	ZMBN 98050	*LN606516*	*LN606546*	*LN606576*	*LN606488*	North Sea, Norway, Sognefjoden	
Spinularia spinularia (Bowerbank, 1866)	Yes	ZMBN 98076	*LN606518*	*LN606548*	*LN606578*	*LN606489*	North Sea, Norway, Hordaland	
Spinularia spinularia (Bowerbank, 1866)	Yes	ZMBN 98079	*HG423757*	*HG423787*	*HG423817*	*HG423728*	North Sea, Norway, Hordaland	
Tentorium papillatum (Kirkpatrick, 1908)		SMF 10571	*HG423758*	**HG423788*	*HG423818*	*HG423729*	Weddell Sea, Antarctic, Cape Norvegia	
Tentorium papillatum (Kirkpatrick, 1908)		ZMBN 98095	*LN606519*	*LN606549*	*LN606579*	–	Weddell Sea, Antarctic, South Shetland Islands	

Table 1 (continued)

Species	Type species	Museum voucher	28S: B10–C1	28S: D1–D19	28S: D20–E19	CO1	Locality	Type locality
Tentorium papillatum (Kirkpatrick, 1908)		ZMBN 98096	*HG423759*	*HG423789*	*HG423819*	*HG423730*	Weddell Sea, Antarctic, South Shetland Islands	
Tentorium semisuberites (Schmidt, 1870)	Yes	ZMBN 98054	*HG423760*	*HG423790*	*HG423820*	*HG423731*	Arctic Ocean, Norway, Svalbard	
Tentorium semisuberites (Schmidt, 1870)	Yes	ZMBN 98099	*HG423761*	*HG423791*	*HG423821*	*HG423732*	North Sea, Norway, Hordaland	
Tentorium cf. *semisuberites* (Schmidt, 1870)		SMF 10575	*HG423762*	*HG423792*	*HG423822*	–	Weddell Sea, Antarctic, East for Antarctic Peninsula	
Weberella bursa (Müller, 1806)	Yes	ZMBN 98051	*HG423763*	*HG423793*	*HG423823*	*HG423733*	Arctic Ocean, Norway, Svalbard	
Weberella bursa (Müller, 1806)	Yes	ZMBN 98072	*LN606520*	*LN606550*	*LN606580*	*LN606490*	Norwegian Sea, Norway, Troms	
Outgroups								
Family Suberitidae								
Suberites ficus (Johnston, 1842)		BELUM: MC4322	HQ379247	HQ379322	HQ379389	HQ379429	Atlantic Ocean, UK, Scotland	
Family Tethyidae								
Tethya citrina Sarà & Melone, 1965		BELUM: MC5113	HQ379237	HQ379312	HQ379378	HQ379427	N/A	

polymastiid genera. Each genus was represented at least by the type species except for *Radiella* Schmidt, 1870, the type species of which, *Radiella sol* Schmidt, 1870, was unavailable and had an ambiguous status (see "Discussion"). Sequences from 19 species and nine OTUs were novel. Data on two species and one OTU were taken from GenBank and sequences from three species were both obtained by us and taken from GenBank. Two species were chosen as outgroups, the suberitid *Suberites ficus* (Johnston, 1842) and the tethyid *Tethya citrina* Sarà & Melone, 1965. Data on both species were taken from GenBank. This selection was based on the substantial morphological affinities between Suberitidae, Tethyidae Gray, 1848, and Polymastiidae and on the former affiliation of these three families with the order Hadromerida.

DNA extraction, amplification, and sequencing

DNA extractions were made with Qiagen DNeasy Blood and Tissue Minikits or DNeasy Plant Minikits following the manufacturer's protocols (the latter was found to yield DNA of higher quantity and purity).

CO1 barcoding regions were amplified with the Ex Taq polymerase (TaKaRa) and the amplification program from Vargas et al. (2012). For most species and OTUs, we used the primers dgLCO1490/dgHCO2198 (Meyer 2003), which are slight modifications of the universal primers LCO1490/HCO2198 (Folmer et al. 1994). For one species, *Polymastia corticata* Rildey & Dendy, 1886, CO1 sequences of satisfactory quality could be obtained only with the primers jgLCO1490/jgHCO2198 (Geller et al. 2013).

Amplification of the partial 28S ribosomal DNA (rDNA) was performed with three pairs of primers designed by Morrow et al. (2012): Por28S-15F/Por28S-878R for sequencing of the region coding RNA from helix B10 to helix C1, Por28S-830F/Por28S-1520R for the region coding from D1 to D19, and Por28S-1490F/Por28S-2170R for the region coding from D20 to E18–E19. For most species, the amplification of this DNA piece succeeded with the Ex Taq polymerase and a "touchdown" program reported by Morrow et al. (2012), which was optimized by the doubling of the sequence extension time. In the cases of *Polymastia thielei* Koltun, 1964 and *Polymastia uberrima* (Schmidt, 1870), the amplification succeeded only with the LA Taq (TaKaRa) and the following protocol: 94 °C for 3 min (94 °C for 30 s, 50 °C for 45 s, 72 °C for 1 min) × 35 cycles, 72 °C for 7 min. Quality and quantity of the PCR products were estimated by agarose gel electrophoresis. The PCR products were purified with exonuclease 1 and shrimp alkaline phosphatase as described by Eilertsen and Malaquias (2013) and used for sequencing reactions with BigDye terminator 3.1 (Applied Biosystems, Waltham, MA, USA) following

the protocol of the producer. Subsequent sequence reads were performed with an automated ABI 3730XL DNA Analyser (Applied Biosystems) in the University of Bergen.

Contigs were assembled with the application SeqMan of DNASTAR Lasergene 8.0 and manually checked for sequencing errors. The consensus sequences of contigs were trimmed to remove primer residuals and checked by nucleotide BLAST search (Altschul et al. 1990) against GenBank sequences to verify their polymastiid origin. Where BLAST searches revealed epi- or endofaunal contaminations, the PCR products were cloned using StrataClone PCR cloning kit (Agilent Technologies, StrataGene Products Division, Santa Clara, CA, USA) according to the manufacturer's instructions, and 10–20 clones per product were sequenced by LGC Genomics GmbH (Berlin, Germany). If the direct sequence reads displayed double signals, extractions from the respective samples were repeated once again and the PCRs were repeated two or more times in order to exclude eventual cross-contamination and PCR errors. If these repetitive procedures confirmed the double signals in the reads, PCR products were cloned following the same protocol as used for the separation of the native and contaminating DNA fragments. The resulting clones were checked for errors, e.g., as those reported by Speksnijder et al. (2001) and Acinas et al. (2005), against the alignment of the approved direct sequences. Clones with unique nucleotides or gaps in the conservative sites were disregarded. Polymorphism in the remaining clones was regarded as natural. Strict consensuses of the clones with the polymorphic sites encoded with IUPAC symbols were employed in the main phylogenetic analyses. Altogether we submitted 75 CO1 sequences and 236 sequences with the three regions of 28S rDNA including clone libraries (different versions from the same individuals) to GenBank (Table 1).

Alignments

All alignments were performed in SeaView 4.3.4 (Galtier et al. 1996; Gouy et al. 2010). CO1 sequences comprising exactly 658 bp each were aligned manually. The sequences of 28S rDNA varied in length. B10–C1 regions were 799–822 bp long, D1–D19 649–653 bp, and D20–E19 646–648 bp. Sequences of different regions were concatenated with 19 overlapping nucleotides, and their preliminary alignment was performed with the MUSCLE algorithm (Edgar 2004) implemented in SeaView. The initial alignment was further refined manually under consideration of the RNA secondary structure (Erpenbeck et al. 2007a, b, 2008). The GenBank sequence of *Polymastia pachymastia* de Laubenfels, 1932 (accession AY561924), being the longest (3550 bp) polymastiid sequence of 28S rDNA published so far, was used as a template for adapting the secondary structures reconstructed from other families to Polymastiidae. A 90 % consensus of all sequences employed in our study was then adjusted to this template. The resulting alignment was 2155 sites long. Because no secondary structure was proposed for the highly variable C-region (Erpenbeck et al. 2007a, b, 2008) comprising positions 406–813 in our alignment, we treated all sites flanked by the C1-helix strands as single stranded. Main length variation occurred in the C-region (from 366 to 396 bp) and in the terminal loop on helix D5 (alignment positions 940–948, variation from 4 to 8 bp). Search for unambiguously aligned sites was initially performed in GBlocks 0.91b (Castresana 2000) as implemented in SeaView. That excluded 51 sites. However, the resulting set was manually extended to exclude in total only 43 sites, because some obviously homologous sites were neglected by the algorithm. All alignments (CO1 matrix, 28S rDNA complete matrix, and 28S rDNA matrix reduced by 43 sites) were deposited at TreeBase and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S18487. Some descriptive statistics of the alignments was explored with MEGA7 (Nei and Kumar 2000; Kumar et al. 2016).

Selection of substitution models and phylogenetic analyses

For all computing procedures, identical sequences were collapsed into one sequence that is indicated by the sequence labels of the taxa represented in the trees (Figs. 1, 2, and 3). Five datasets, CO1 data alone (35 unique polymastiid sequences), two 28S rDNA matrices (49 sequences), the complete one and the matrix reduced by 43 ambiguously aligned sites, and two corresponding concatenated matrices (47 sequences), CO1 + complete 28S rDNA and CO1 + reduced 28S rDNA, were analyzed. Search for the best fitting substitution model for the CO1 dataset carried out with both hierarchical likelihood ratio tests and Akaike information criterion (AIC) in MrModeltest 2.0 (Nylander 2004) selected GTR+G+I. In all analyses, the CO1 data were split into two partitions, codon positions 1 + 2 and codon position 3. For RNA-specific models for the 28S rDNA datasets, we applied the model selection procedure implemented in PHASE 3.0 (Allen and Whelan 2014), a recent modification of PHASE 2.0 (Gowri-Shankar and Jow 2006), that included running the script "model_selection.pl" (Allen and Whelan 2014). As an input tree topology for this procedure, we used a ML tree calculated in PhyML (Guindon et al. 2010) under the best fitting standard model, GTR+G, determined with AIC in

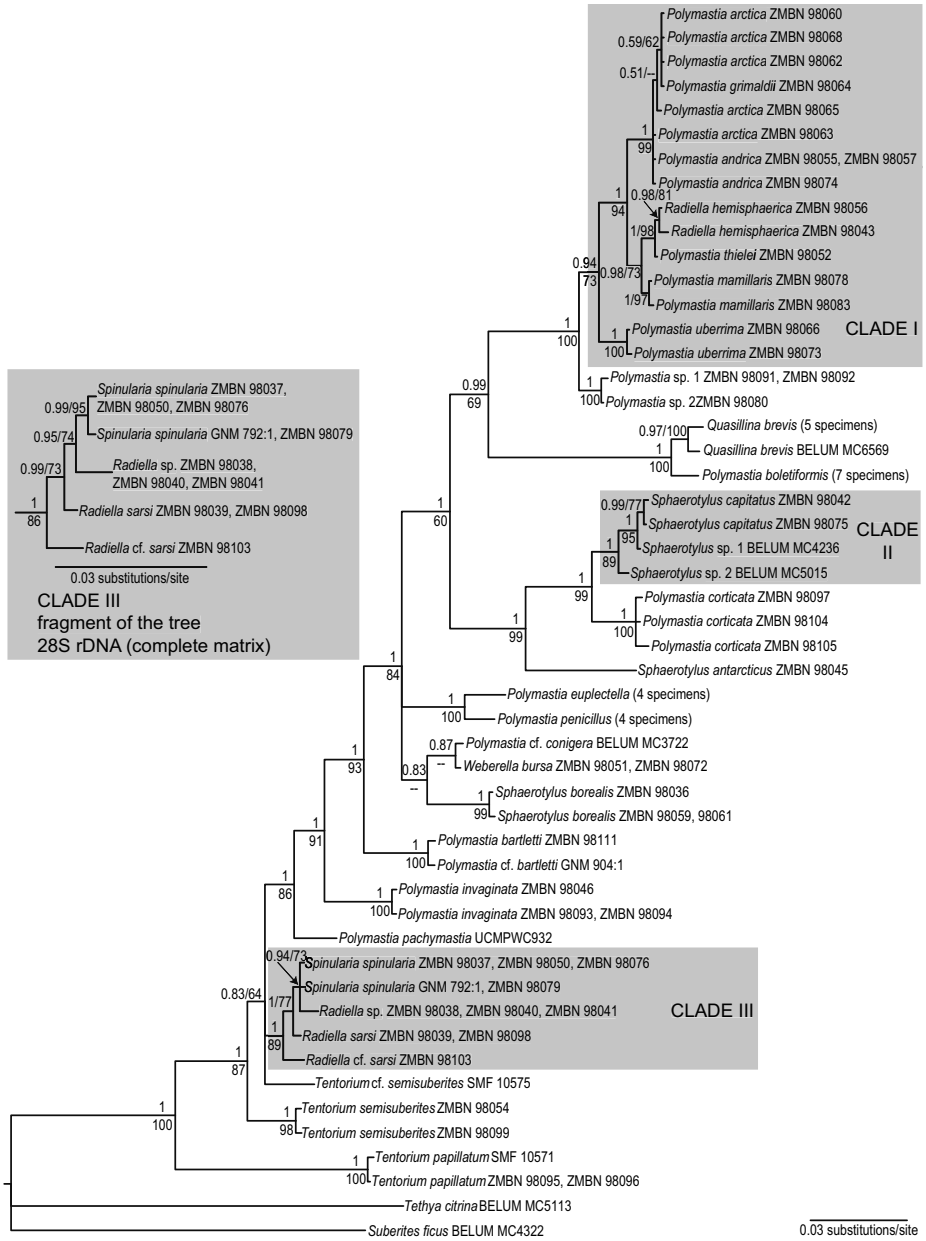
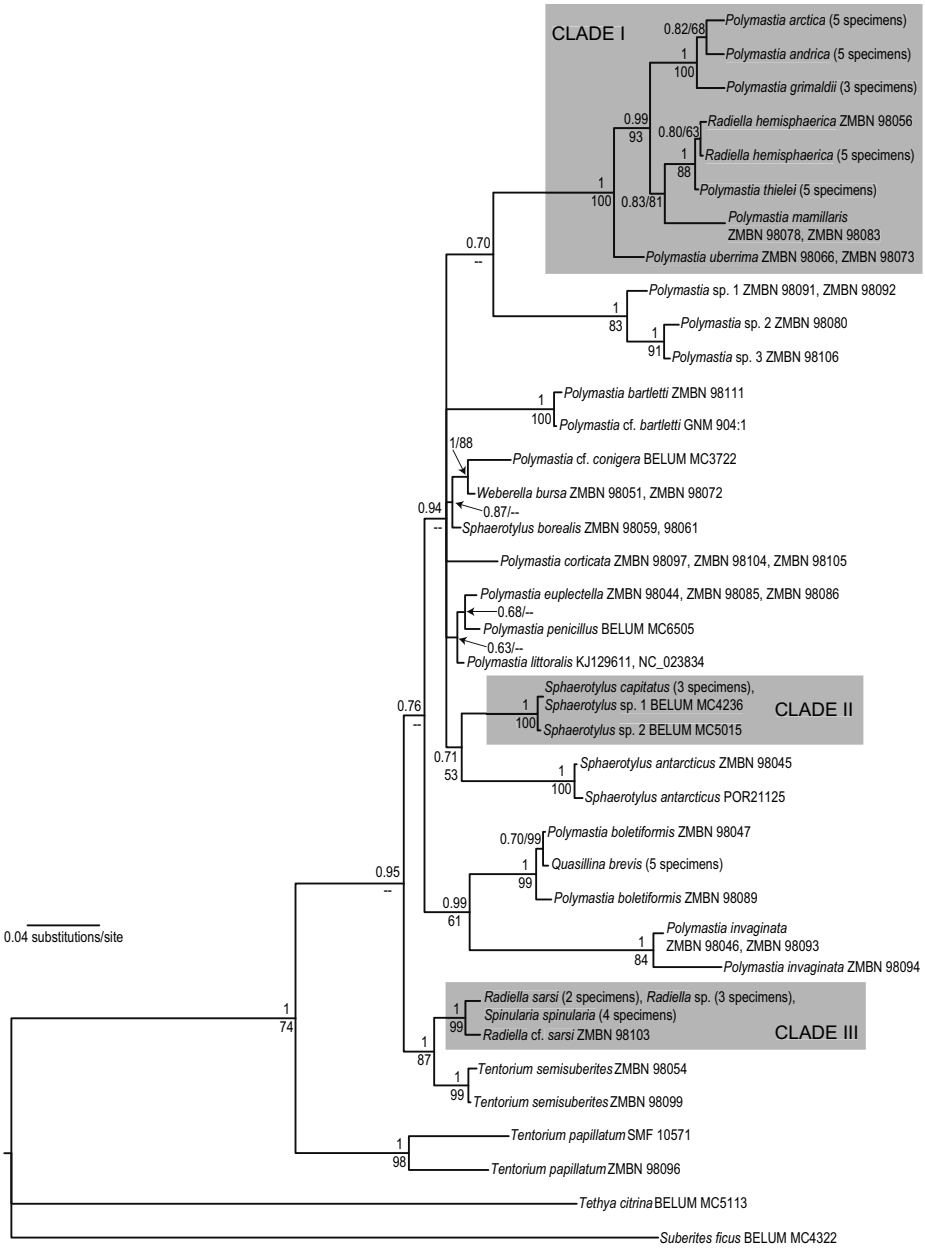


Fig. 1 Bayesian consensus tree reconstructed from the reduced 28S rDNA matrix. *Inset*: a fragment of the comparable Bayesian consensus tree reconstructed from the complete 28S rDNA matrix, displaying better resolution inside Clade III. Nodal supports: upper values—Bayesian posterior probabilities, lower values—ML bootstrap supports in percents. Expansion of the branch labels denoting multiple specimens: *Quasillina brevis* (five specimens)—ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090; *Polymastia boletiformis* (seven specimens)—BELUM: MC5014, GNM 901:1, ZMBN 98047, ZMBN 98048, ZMBN 98081, ZMBN 98088, ZMBN 98089; *Polymastia euplectella* (four specimens)—ZMBN 98044, ZMBN 98085, ZMBN 98086, ZMBN 98087; *Polymastia penicillus* (four specimens)—BELUM: MC5284, BELUM: MC6505, GNM 460:1, GNM 460:2



JModelTest 2.1.6 (Guindon and Gascuel 2003; Darriba et al. 2012). The mixed model RNA16C+G for helix positions and REV+G for loop positions was selected as the best fit. Analyses of the concatenated datasets CO1 + 28S rDNA were run under the mixed model comprising the models selected for the single gene matrices. All datasets were analyzed in a Bayesian inference framework, with MrBayes 3.2 (Ronquist et al. 2011) for the CO1 matrix and with PHASE 3.0 (Allen and Whelan 2014) for the 28S rDNA matrices and the concatenated

◀ **Fig. 2** Bayesian consensus tree reconstructed from the CO1 matrix. Nodal supports: upper values—Bayesian posterior probabilities, lower values—ML bootstrap supports in percents. Expansion of the branch labels denoting multiple specimens: *Polymastia arctica* (five specimens)—ZMBN 98060, ZMBN 98062, ZMBN 98063, ZMBN 98065, ZMBN 98068; *Polymastia andrica* (five specimens)—ZMBN 98055, ZMBN 98057, ZMBN 98074, ZMBN 98102, ZMBN 98108; *Polymastia grimaldii* (three specimens)—ZMBN 98064, ZMBN 98110, ZMBN 98112; *Radiella hemisphaerica* (five specimens)—ZMBN 98043, ZMBN 98058, ZMBN 98069, ZMBN 98071, ZMBN 98077; *Polymastia thielei* (five specimens)—ZMBN 98052, ZMBN 98053, ZMBN 98070, ZMBN 98107, ZMBN 98109; *Sphaerotylus capitatus* (three specimens)—GNM 902, ZMBN 98042, ZMBN 98075; *Quasillina brevis* (five specimens)—ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090; *Radiella sarsi* (two specimens)—ZMBN 98039, ZMBN 98098; *Radiella* sp. (three specimens)—ZMBN 98038, ZMBN 98040, ZMBN 98041; *Spinularia spinularia* (four specimens)—ZMBN 98037, ZMBN 98050, ZMBN 98076, ZMBN 98079

matrices CO1 + 28S rDNA, and in a maximum likelihood framework (ML) with RAxML 8.1.24 (Stamatakis 2014).

MrBayes 3.2 was run on the CIPRES (Cyberinfrastructure for Phylogenetic Research) Science Gateway V. 3.3 (https://www.phylo.org/) and on the Lifeportal at the University of Oslo using the high-performance computing cluster Abel (https://lifeportal.uio.no/). In the MrBayes 3.2 session, the model parameters were optimized independently for each partition. Two runs with eight chains each were launched under the default chain "temperatures" and flat Dirichlet distributions for the model parameter priors. The chains were sampled each 100 generations. The initial 2.5 million of the samples were disregarded in the burn-in. The convergence of the runs was controlled with the average standard deviation of split frequencies in MrBayes 3.2, while the sufficiency of the number of generations was estimated with the effective sample size (ESS) for all parameters in Tracer 1.5 (Rambaut and Drummond 2009). The convergence was reached and the ESSs exceeded 200 after ten million generations had been run.

PHASE analyses were performed on a desktop computer. Ten million iterations with sampling period 200 iterations after a burn-in of one million iterations were initially run. Each analysis was repeated twice, specifying a different random seed. After the output files had been transformed with the Perl script phase2tracer.pl (Voigt et al. 2012, modified from the script of Matt Yoder (https://github.com/mjy/phase-utils/blob/master/phase2tracer.pl)), the stabilization of all parameters was monitored in Tracer 1.5. If stabilization had not been achieved, the computations were repeated under optimized settings and with extra 5–30 million iterations.

RAxML 8.1.24 was run on the CIPRES. Search for the best scoring ML-tree along with rapid bootstrapping (1000 replicates) was performed. Because the model RNA16C is not implemented in RAxML 8.1.24, the more exhaustive model RNA16A was invoked for helix positions of the 28S rDNA data.

Bayesian analyses of the single-gene datasets revealed some incongruence between the CO1 and 28S rDNA phylogenies. To illustrate the conflicts, we repeated the analyses on the matrices with the identical set of 47 taxa for both CO1 and 28S rDNA (reduced matrix) and, based on the resulting consensus trees, computed a rooted galled network (Huson et al. 2009) with Dendroscope 3 (Huson and Scornavacca 2012) (Fig. 4). To explore these conflicts, we performed an incongruence length difference test (ILD, Farris et al. 1994) on the concatenated dataset CO1 + reduced 28S rDNA with all parsimony uninformative sites excluded running 500 replicates in PAUP* 4.0b10 (Swofford 2002). Furthermore, we used Bayes factor comparisons of the model likelihoods to test the conflicting topological hypotheses on the single-gene datasets following Kass and Raftery (1995). To obtain more accurate model likelihoods, stepping-stone samplings were performed in MrBayes 3.2. The monophyly of the congruent clades was constrained as recommended by Bergsten et al. (2013). Two runs with two chains each were launched. Four million generations on the CO1 data and ten million generations on the 28S rDNA were run to reach the convergence of the runs.

Additionally, in order to examine the intragenomic polymorphism of the D1–D19 region of 28S rDNA, a dataset comprising all versions of this region in three species of *Polymastia*, *Polymastia andrica* de Laubenfels, 1949, *Polymastia arctica* (Merejkowsky, 1878), and *Polymastia grimaldii* (Topsent, 1913), with *Polymastia mamillaris* (Müller, 1806) as the outgroup taxon, was analyzed with Minimum-spanning network algorithm (Bandelt et al. 1999) implemented in PopArt 1.7 (http://popart.otago.ac.nz) and in a ML framework with PhyML (Guindon et al. 2010). Consensus trees resulting from the Bayesian analyses along with the ML-tree illustrating the intragenomic polymorphism were deposited at TreeBase and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S18487.

Tracing of the evolution of morphological characters

The consensus tree resulting from the Bayesian analysis of the concatenated dataset CO1 + reduced 28S rDNA was chosen for tracing of the morphological evolution. Branches corresponding to different individuals of the same species or OTU were collapsed. A matrix with 21 morphological characters of the respective 30 polymastiid taxa and two outgroup taxa was built based on the dataset for phylogenetic scenario 3 from Plotkin et al. (2012), but with two emendations: the multistate character "Growth pattern" was assigned by the amalgamation of four binary characters and the character "Longitudinal tracts of principal monactines in the

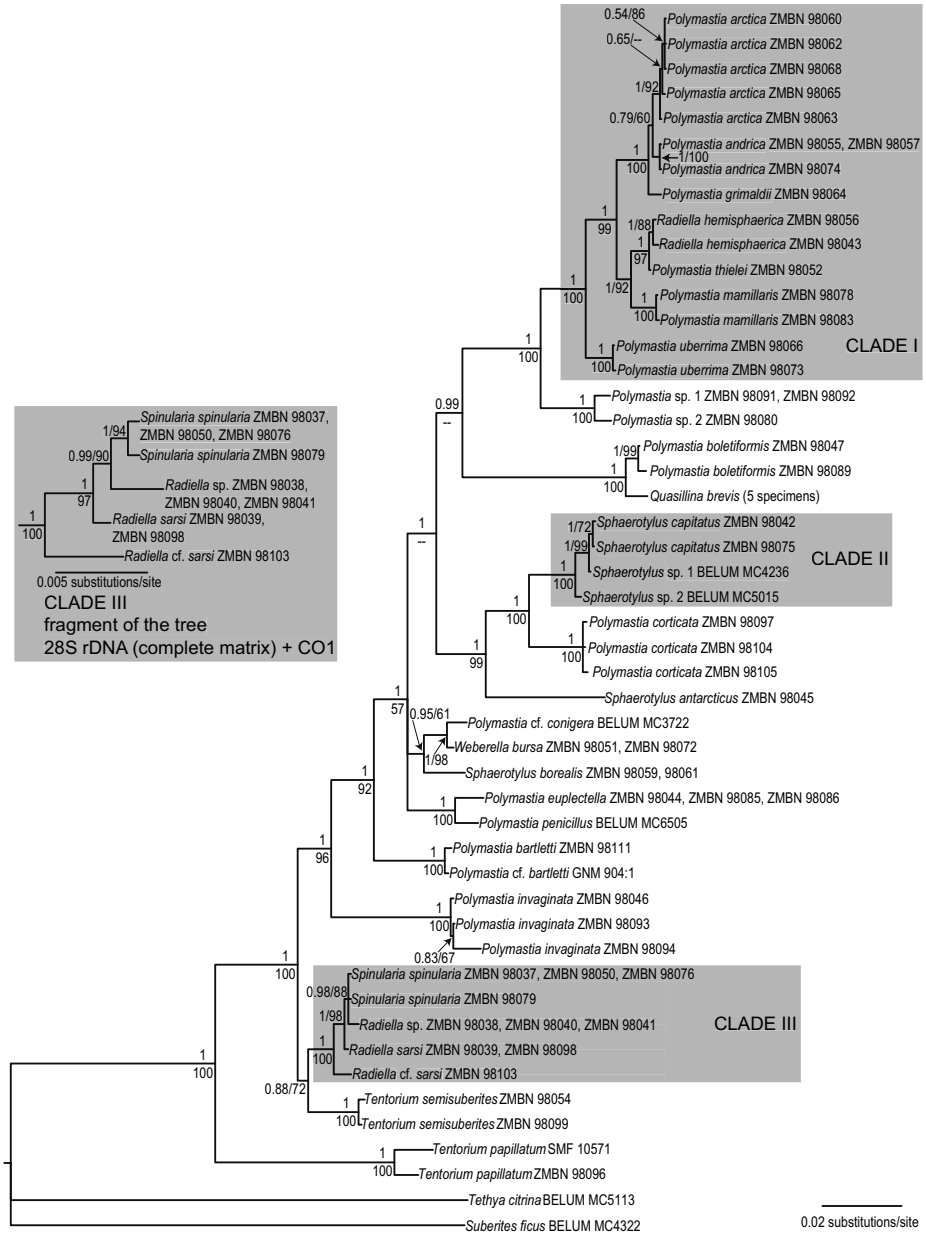


Fig. 3 Bayesian consensus tree reconstructed from the concatenated dataset CO1 + reduced 28S rDNA of Polymastiidae. *Inset*: a fragment of the comparable Bayesian consensus tree reconstructed from the concatenated dataset CO1 + complete 28S rDNA, displaying better resolution inside Clade III. Nodal supports: upper values—Bayesian posterior probabilities, lower values—ML bootstrap supports in percents. Expansion of the branch label denoting five specimens of *Quasillina brevis*—ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090

cortex" (presence/absence) was excluded since in the matrix used in this study the state "present" was autapomorphy of *Quasillina brevis* (Bowerbank, 1866) (Online Resources 1–3). The ancestral state

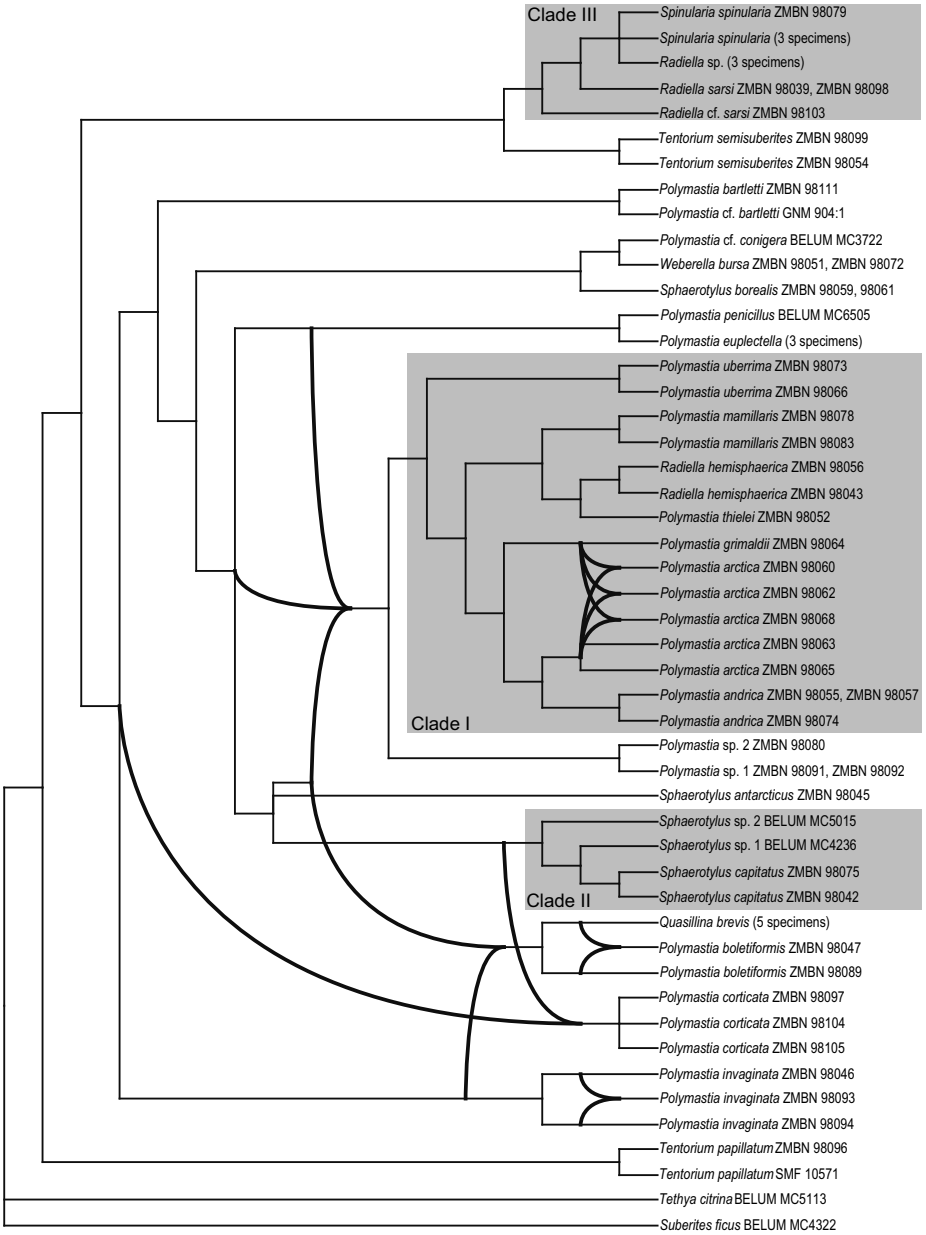


Fig. 4 Rooted galled network compiled from the Bayesian consensus trees reconstructed from CO1 alone and 28S rDNA alone with identical sets of taxa. *Bold curves* indicate discrepancies in the topology. Expansion of the branch labels denoting multiple specimens: *Spinularia spinularia* (three specimens)—ZMBN 98037, ZMBN 98050, ZMBN 98076; *Radiella* sp. (three specimens)—ZMBN 98038, ZMBN 98040, ZMBN 98041; *Polymastia euplectella* (three specimens)—ZMBN 98044, ZMBN 98085, ZMBN 98086; *Quasillina brevis* (five specimens)—ZMBN 98049, ZMBN 98067, ZMBN 98082, ZMBN 98084, ZMBN 98090

reconstruction with parsimony criterion for each character was performed in Mesquite 3.04 (Maddison and Maddison 2015), while the consistency indices were computed in PAUP* 4.0b10 (Swofford 2002).

Results

Statistics of alignments

Parsimony-informative sites comprised almost 25 % of all sites in the CO1 matrix and about 15 % of all sites in the 28S rDNA matrices. Sites with intragenomic polymorphisms comprised ca. 1 and 0.7 % of all sites in the 28S rDNA complete matrix and reduced matrix, respectively (Table 2).

Congruent aspects of the CO1 and 28S rDNA phylogenies

Separate analyses of CO1 and 28S rDNA resulted in similar overall phylogenies (Figs. 1 and 2) except for few conflicts (see the section "Incongruence between the CO1 and 28S rDNA phylogenies" below). The phylogenies were not affected by intraspecific or intragenomic polymorphisms except for the relationships within three small terminal subclades (see the section "Intraspecific and intragenomic polymorphism" below). All analyses supported the monophyly of the polymastiids against the two outgroups (Figs. 1, 2, and 3). At the same time, the polymastiid genera *Polymastia*, *Radiella*, *Sphaerotylus* Topsent, 1898, and *Tentorium* Vosmaer, 1887 were nonmonophyletic. *Polymastia* spp. were scattered over different clades, *Radiella hemisphaerica* (Sars, 1872) fell distantly from other *Radiella* spp., *Sphaerotylus borealis* (Swarczewsky, 1906) lay remotely from its congeners, and *Tentorium papillatum* (Kirkpatrick, 1908) fell on a long branch as the sister group to a clade of the remaining polymastiids. Moreover, in the 28S rDNA tree, the type species of *Tentorium*, *Ten. semisuberites*, and *Ten.* cf. *semisuberites* did not group together, although the support for their nonmonophyly was very weak (Fig. 1). Unfortunately, no CO1 data from *Ten.* cf. *semisuberites* were obtained. Three clades of species (highlighted in Figs. 1, 2, 3, and 4) were recovered by all analyses.

Clade I comprised *Polymastia andrica*, *P. arctica*, *P. grimaldii*, *P. mamillaris* (type species of *Polymastia*), *P. thielei*, *P. uberrima*, and *Radiella hemisphaerica*. The support for this clade in the 28S rDNA tree was slightly weaker (Fig. 1) than in the CO1 tree (Fig. 2). Analyses of the 28S rDNA alone and the concatenated datasets CO1 + 28S rDNA supported the sister relationships between the pair *Polymastia* sp. 1 + *Polymastia* sp. 2 and Clade I (Figs. 1 and 3). In the CO1 tree, *Polymastia* sp. 1 and *Polymastia* sp. 2 grouped with *Polymastia* sp. 3, and the position of this trio as the sister to Clade I had very weak support (Fig. 2). Unfortunately, no 28S rDNA was obtained from *Polymastia* sp. 3. Inside Clade I, *P. uberrima* was the sister to the subclade of the remaining six species strongly supported by all analyses (Figs. 1, 2, and 3). In its turn, this subclade split up into two groupings, *P. mamillaris* + *P. thielei* + *R. hemisphaerica* and *P. andrica* + *P. arctica* + *P. grimaldii*. The pair *P. thielei* + *R. hemisphaerica* was supported by all analyses (Figs. 1, 2, and 3). But the grouping of *P. mamillaris* as the sister to this pair was strongly supported only by the analyses of 28S rDNA alone (Fig. 1) and the concatenated datasets (Fig. 3), while the analysis of CO1 alone demonstrated just a very weak support for this relationship (Fig. 2). The grouping *P. andrica* + *P. arctica* + *P. grimaldii* was supported in all analyses (Figs. 1, 2, and 3), but the relationships between these three species were unresolved in the 28S rDNA tree (Fig. 1) because of the intraspecific and intragenomic polymorphism (see the respective section below) and resolved with just a low support for *P. andrica* + *P. arctica* in the CO1 tree (Fig. 2).

Clade II comprised *Sphaerotylus capitatus* (Vosmaer, 1885) (type species of *Sphaerotylus*), *Sphaerotylus* sp. 1 and *Sphaerotylus* sp. 2. This clade was strongly supported in all trees (Figs. 1, 2, and 3). *Sph. capitatus* and *Sphaerotylus* sp. 1 had identical CO1 and were sisters in the 28S rDNA tree with strong support (Fig. 1).

Clade III comprised *Radiella sarsi*, *Radiella* cf. *sarsi*, *Radiella* sp., and *Spinularia spinularia* (Bowerbank, 1866) (type species of *Spinularia* Gray, 1867). This clade was strongly supported in all trees (Figs. 1, 2, and 3). In the CO1 tree, Clade III and *Tentorium semisuberites* were sisters with strong support (Fig. 2), but this was not confirmed by the analyses of 28S rDNA alone (Fig. 1). *R. sarsi*, *Radiella* sp., and *Spi. spinularia* had identical CO1 and formed a strongly supported subclade in the 28S rDNA tree (Fig. 1). In the same tree, there was also some support for the sister relationships between *Radiella* sp. and *Spi. spinularia*. *Spi. spinularia* was represented by two groups of individuals that differed from each other by two nucleotides in 28S rDNA and each of them differed from *Radiella* sp. (identical sequences from three individuals) by 11 nucleotides. Three of these 11 nucleotides were located in the 43 sites excluded as ambiguously aligned in the matrix as a whole. The exclusion of these sites from the

Table 2 Basic statistics of the analyzed alignments

Alignment	Length	Variable sites	Parsimony-informative sites	Sites with intragenomic polymorphism	Empirical nucleotide frequencies, % for T, C, A, G	Average p-distance (SE)
CO1	658	201	163	0	36.3, 15.8, 25.7, 22.2	0.080 (0.006)
LSU, complete matrix	2155	363	326	21	20.7, 23.7, 23.4, 32.2	0.047 (0.003)
LSU, reduced matrix	2112	347	310	14	20.7, 23.6, 23.6, 32.2	0.045 (0.003)

analyses led to a polytomy formed by *Radiella* sp. and the two groups of individuals of *Spi. spinularia* (main trees in Figs. 1 and 3). However, within Clade III, these excluded sites could be aligned unambiguously and provided a sufficient phylogenetic signal to resolve the polytomy (insets in Figs. 1 and 3).

Furthermore, all analyses strongly supported the pair *Polymastia boletiformis* (Lamarck, 1815) + *Q. brevis* (type species of *Quasillina*) (Figs. 1, 2, and 3) and also revealed the grouping *Sphaerotylus borealis* + *Polymastia* cf. *conigera* Bowerbank, 1874 + *Weberella bursa* (Müller, 1806), although the latter was strongly supported only by the analyses of the concatenated data CO1 + 28S rDNA (Fig. 3), while its support in the single-gene trees was very weak (Figs. 1 and 2). Within this grouping, *P.* cf. *conigera* and *W. bursa* were sisters with a strong support in the CO1 tree (Fig. 2), but a much weaker support in the 28S rDNA tree (Fig. 1). At the same time, the analyses of the 28S rDNA alone strongly supported the pair *Polymastia euplectella* Rezvoj, 1927 + *Polymastia penicillus* (Montagu, 1818) (Fig. 1), while the support for this pair in the CO1 tree was negligible (Fig. 2).

Incongruence between the CO1 and 28S rDNA phylogenies

ILD test of the concatenated dataset CO1 + 28S rDNA rejected the hypothesis of congruent data with a p value of 0.002. The conflicts between the single gene phylogenies are visualized as reticulations in the galled network (Fig. 4). One conflict concerned dissimilarity in the relationships between *Polymastia boletiformis* + *Quasillina brevis* and other taxa. In the CO1 tree, this pair was the sister to *Polymastia invaginata* Kirkpatrick, 1907 (Fig. 2), whereas in the 28S rDNA tree, it was the sister to the grouping Clade I + *Polymastia* sp. 1 + *Polymastia* sp. 2 (Fig. 1). Bayesian support for the indicated relationships was strong in each gene tree, and they were not affected by polymorphism in any of the species. Bayes factor tests revealed no support for the alternative hypothesis in either of the two topologies (Table 3). Two conflicts were due to the low resolution of the CO1 tree. In this tree, five clades formed an unresolved polytomy with *Polymastia corticata*, while *Sphaerotylus antarcticus* Kirkpatrick, 1907 was the sister to Clade II, although with very weak Bayesian support (Fig. 2). Conversely, in the 28S rDNA tree, *P. corticata* was the sister to Clade II, and *Sph. antarcticus* in its turn was the sister to *P. corticata* + Clade II with strong support (Fig. 1). The conflicts between the CO1 and 28S rDNA phylogenies caused by the gene polymorphism were revealed within three small terminal subclades, the trio *Polymastia andrica* + *P. arctica* + *P. grimaldii* in Clade I, the pair *P. boletiformis* + *Q. brevis*, and the group of three individuals of *P. invaginata* (Fig. 4). These conflicts are considered below.

Intraspecific and intragenomic polymorphism

The most conspicuous intraspecific polymorphism was revealed in four sites of the B10–C1 region (positions 578–580 and 583 in the complete matrix) and in seven sites of the D1–D19 region (positions 941–943, 947–948, and 1294–1295) of 28S rDNA in *Polymastia andrica*, *P. arctica*, and *P. grimaldii*. The variation within B10–C1 was estimated on the direct sequences. The sequences of this region from three *P. andrica* were identical, while *P. arctica* displayed a polymorphism—individual ZMBN 98063 differed from *P. andrica* just by one ambiguity, individual ZMBN 98068 by three nucleotides, and two individuals, ZMBN 98060 and ZMBN 98062, by four nucleotides. *P. grimaldii* ZMBN 98064 differed from the latter two individuals of *P. arctica* just by one ambiguity. The variation within D1–D19 was estimated on four direct sequences (two from *P. andrica* and two from *P. arctica*) and 18 clones from five individuals (one of *P. andrica*, three of *P. arctica*, and one of *P. grimaldii*). Of the seven polymorphic sites, five were parsimony-informative when the sequences were aligned with the corresponding DNA region of *P. mamillaris* as the outgroup (see the respective alignment at http://purl.org/phylo/treebase/phylows/study/TB2:S18487). Here an intragenomic polymorphism was discovered. Seven versions of D1–D19 were spread among the individuals of different species (Fig. 5):

Version 1 (in three *P. andrica*, three *P. arctica*, and the only *P. grimaldii* employed in this analysis)
Version 2 (in one *P. andrica* and one *P. arctica*)
Version 3 (in one *P. arctica* and *P. grimaldii)*
Version 4 (only in *P. grimaldii*)
Version 5 (in one *P. andrica*, two *P. arctica*, and *P. grimaldii*)
Version 6 (in one *P. andrica*, two *P. arctica*, and *P. grimaldii*)
Version 7 (in one *P. andrica* and one *P. arctica*)

While the 28S rDNA sequences of *P. andrica*, *P. arctica*, and *P. grimaldii* displayed intraspecific and intragenomic polymorphism, the CO1 data from these species were consistent, i.e., the sequences from the individuals of the same species were identical. Based on these data, *P. andrica* and *P. arctica* were sisters, although the support for this relationship was weak (Fig. 2).

Among other species, intraspecific polymorphism was revealed in *Polymastia boletiformis* and *P. invaginata*. All seven *P. boletiformis* studied had identical 28S rDNA and grouped with *Quasillina brevis* (Fig. 1). CO1 was obtained only from two individuals of *P. boletiformis*, of which one, ZMBN 98047, differed from *Q. brevis* just by one nucleotide in this gene, while the other, ZMBN 98089, differed from *Q. brevis* by six nucleotides, that resulted in a grouping of ZMBN 98047 with *Q. brevis* instead of the conspecific ZMBN 98089 (Fig. 2).

Table 3 Results of the testing of two conflicting topological hypotheses about the relationships of *Polymastia boletiformis* + *Quasillina brevis* with other taxa

Phylogenetic marker	Topological hypothesis	Mean marginal likelihood, natural log units	Compared hypotheses	2 × log Bayes factor
CO1	H0: *P. boletiformis* + *Q. brevis* is sister to Clade I + *Polymastia* sp. 1 + *Polymastia* sp. 2	−4082.3	H1 vs. H0	−32.66
	H1: *P. boletiformis* + *Q. brevis* is sister to *P. invaginata*	−4065.97		
28S rDNA	H0: *P. boletiformis* + *Q. brevis* is sister to Clade I + *Polymastia* sp. 1 + *Polymastia* sp. 2	−7609.05	H1 vs. H0	34.96
	H1: *P. boletiformis* + *Q. brevis* is sister to *P. invaginata*	−7626.53		

Cloning of the PCR products confirmed these relationships. Two individuals of *P. invaginata*, ZMBN 98093 and ZMBN 98094, had identical 28S rDNA, whereas individual ZMBN 98046 differed from them by two nucleotides (Fig. 1). Conversely, CO1 of ZMBN 98046 and ZMBN 98093 were identical, while ZMBN 98094 differed from them by 19 nucleotides (Fig. 2).

Homoplasy of the morphological characters

Only two characters were fully consistent—character 5, "Exhalant papillae", and character 9, "Oscula on the body surface" (Online Resources 1–3). All polymastiid taxa studied possess exhalant papillae and lack oscula on the surface, i.e.,

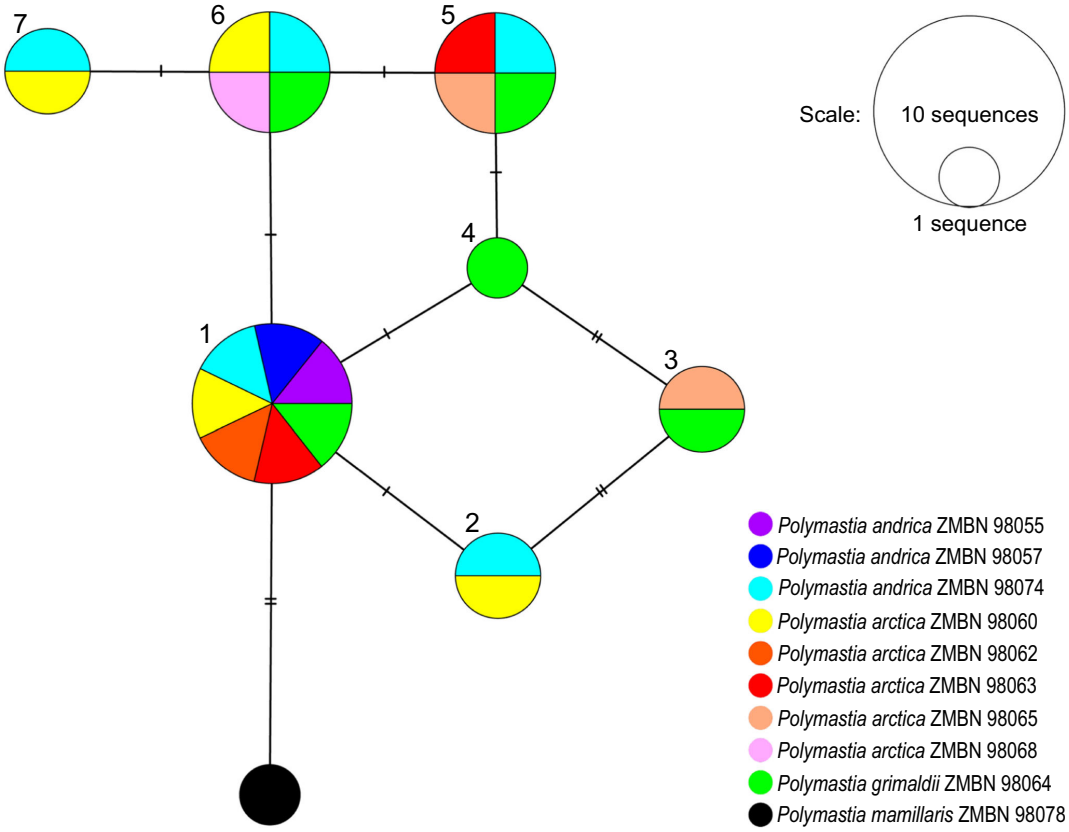


Fig. 5 Minimum-spanning network reconstructed from the dataset of the clones and direct sequences of the D1–D19 region, 28S rDNA from *Polymastia andrica*, *P. arctica*, and *P. grimaldii* with *P. mamillaris* as outgroup. *Numbers* denote gene versions described in the text. *Hatch marks* on the branches indicate mutations

these features are the synapomorphies of the polymastiid clade compared with the given outgroups. All other characters were more or less homoplastic (Online Resources 1–3).

Most polymastiids possess one or several well-developed exhalant papillae (characters 6 and 7 in Online Resources 1–3, Fig. 6a–c). Weakly developed papillae (Fig. 6d–e) are present in the taxa from three remote groupings (Fig. 6b)—in all species from Clade III + *Tentorium semisuberites*, in *Sphaerotylus capitatus* from Clade II and in *Quasillina brevis* from the pair *Polymastia boletiformis* + *Q. brevis*. Among these taxa, only *Q. brevis* and three species of *Radiella*, *R. sarsi*, *R.* cf. *sarsi*, and *Radiella* sp., always possess just single papillae (Fig. 6d), whereas *Spinularia spinularia*, *Ten. semisuberites*, and *Sph. capitatus* may have several exhalant papillae as all other polymastiids studied (Fig. 6e).

Exotyles, i.e., extraordinary spicules protruding above the sponge surface (character 14 in Online Resources 1–3), are present in three remote groupings (Fig. 6f). Within the *Sphaerotylus borealis* + *Polymastia* cf. *conigera* + *Weberella bursa* group, the first two species possess exotyles, whereas *W. bursa* does not. All three species of Clade II have exotyles (Fig. 6h–i). Of the two species grouping with Clade II in the 28S rDNA tree, one species, *Sphaerotylus antarcticus*, possesses exotyles, while the other, *Polymastia corticata*, has no exotyles. Among the species of Clade I, *P. andrica* possesses exotyles.

The presence of a marginal fringe of extra-long spicules (character 16 in Online Resources 1–3, Fig. 6g) is shared by all species of Clade III, but also recorded in two species falling into two different subclades of Clade I, *Radiella hemisphaerica* (Fig. 6j) and *Polymastia grimaldii*.

Most polymastiids share the presence of a well-developed and regular choanosomal skeleton with one of the outgroup species, *Tethya citrina* (character 17 in Online Resources 1–3, Fig. 6k). The only exception is *Quasillina brevis* (Fig. 6m), which shares the presence of an irregular and reduced choanosomal skeleton with the other outgroup taxon, *Suberites ficus*.

The main choanosomal skeleton in most polymastiids as well as in *Tet. citrina* is radial (character 18 in Online Resources 1–3, Fig. 6l, n). Reticulate choanosomal skeleton (Fig. 6o) is recorded in taxa from four remote groupings—in *Weberella bursa* (*Sphaerotylus borealis* + *Polymastia* cf. *conigera* + *W. bursa*), in *Polymastia corticata* (*P. corticata* + Clade II), in *Polymastia boletiformis* (*P. boletiformis* + *Quasillina brevis*), and in *Polymastia thielei* (Clade I).

Discussion

Monophyly of Polymastiidae and taxa of uncertain family affiliation

Our analyses demonstrated the monophyly of the clade formed by all polymastiid taxa studied when using a suberitid and a tethyid as outgroups, and these results are congruent with most previous studies (Morrow et al. 2012, 2013; Redmond et al. 2013; Vargas et al. 2015). However, it should be noted that due to the lack of reliable molecular data, our analyses did not consider the two taxa with uncertain family affiliation, the genus *Pseudotrachya* Hallmann, 1914 and the species *Aaptos papillata*, which seem to be important for understanding of the polymastiid early evolution. *A. papillata* is usually regarded as a suberitid (Van Soest et al. 2015), despite that it displays strong morphological similarities with Polymastiidae, and in the trees reconstructed from a morphological dataset by Plotkin et al. (2012), it formed a polytomy with *Tentorium semisuberites* and the clade of other polymastiids. *Pseudotrachya* was commonly regarded as a polymastiid genus (Boury-Esnault 2002). But the type species of this genus, *P. hystrix*, did not group with other polymastiids in the phylogenies based on morphological data (Plotkin et al. 2012), and similarly *Pseudotrachya* sp. fell outside the main polymastiid clade in the tree reconstructed from a 28S rDNA dataset (Nichols 2005). Meanwhile, the taxonomic identification in the latter study raised some doubts because in the same 28S rDNA tree, another polymastiid, *Polymastia* sp. 1, also did not group with the main polymastiid clade, while in the CO1 trees, *Pseudotrachya* sp., *Polymastia* sp. 1, and *Polymastia* sp. 2 appeared in different clades. Additionally, the 28S rDNA sequences of these three species recovered by Nichols (2005) were much shorter than those used in our study, and therefore, we did not include Nichols' sequences in the analyses.

Molecular phylogenies contradict the morphology-based classification of polymastiids

Our most important outcome is the inapplicability of morphological characters, the majority of which has appeared to be highly homoplastic, for the phylogenetic reconstruction and hence for the natural classification of Polymastiidae. Homoplasy is a general problem in morphological taxonomy of the demosponges (e.g., Cárdenas et al. 2011; Morrow et al. 2013). Meanwhile, inside Polymastiidae, our study has recovered three clades strongly supported by the data from both genes studied. Each clade includes the type species of the certain genus, Clade I—the type species of *Polymastia*, *P. mamillaris*; Clade II—the type species of *Sphaerotylus*, *Sph. capitatus*; and Clade III—the type species of *Spinularia*, *Spi. spinularia*, and hence, these clades may be used as reference points in future classification of the family. However, no morphological synapomorphies can at present be defined for the clades revealed. Moreover, about 58 % of the species studied do not fall into any of these clades. Thus, for the time being, we cannot propose a satisfactory classification of Polymastiidae.

A
Character 6: Number of exhalant papillae
always a single
may be several
not applicable
B
Character 7: Development of exhalant papillae
weak
normal
not applicable
C
D
E
F
Character 14: Exotyles
present
absent
G
Character 16: Marginal spicule fringe
present
absent
H
I
J
K
Character 17: Main choanosomal skeleton
irregular and reduced
regular and well-developed
equivocal
L
Character 18: Arrangement of the regular choanosomal skeleton
reticulate
radial
not applicable
M
N
O
CLADE 1
CLADE 2
CLADE 3
CLADE 4
Polymastia arctica
Polymastia andrica
Polymastia grimaldii
Radiella hemisphaerica
Polymastia thielei
Polymastia inflatipilaris
Polymastia ubemma
Polymastia sp. 1
Polymastia sp. 2
Polymastia boletiformis
Quasilina brevis
Sphaerotylus capitatus
Sphaerotylus sp.
Sphaerotylus rendufi
Polymastia corticata
Sclerilytus antarcticus
Polymastia cf. congera
Weberella bursa
Sphaerotylus borealis
Polymastia euplectella
Polymastia penicillus
Polymastia barfletti
Polymastia cf. barfletti
Polymastia invaginata
Spinularia spinularia
Radiella sp.
Radiella sarsi
Radiella cf. sarsi
Tentorium semisuberites
Tentorium papillatum
Tethya citrina
Suberites ficus

◀ **Fig. 6** Key morphological characters of Polymastiidae: depiction of states and tracing of evolution along the Bayesian consensus tree reconstructed from the concatenated dataset CO1 + reduced 28S rDNA (the same as in Fig. 3, but with the branches corresponding to different individuals of the same species collapsed). **a** Evolution of the character "Number of exhalant papillae" (N 6 in Online Resources 1–3); **b** evolution of the character "Development of exhalant papillae" (N 7 in Online Resources 1–3); **c** numerous normally developed papillae in *Polymastia bartletti* ZMBN 98111 (University Museum of Bergen); **d** single weakly developed papilla of *Radiella* sp. ZMBN 98040 (sampled from the Norwegian Sea); **e** three weakly developed papillae in *Spinularia spinularia* (not deposited); **f** evolution of the character "Exotyles" (N 14 in Online Resources 1–3); **g** evolution of the character "Marginal spicule fringe" (N 16 in Online Resources 1–3); **h** exotyles projecting above the cortex, histological section through the body of *Sphaerotylus capitatus* BMNH 10.1.1.1199 (paralectotype, Natural History Museum, London); **i** distal ornamentation of an exotyle, SEM image, preparation from *Sphaerotylus capitatus* ZMB 10855 (Museum für Naturkunde, Berlin); **j** prominent marginal spicule fringe bordering the body of *Radiella hemisphaerica* NHMO-B862 (holotype, Natural History Museum, University of Oslo); **k** evolution of the character "Main choanosomal skeleton" (N 17 in Online Resources 1–3); **l** evolution of the character "Arrangement of the regular choanosomal skeleton" (N 18 in Online Resources 1–3); **m** irregular and reduced choanosomal skeleton, histological section through the body of *Quasillina brevis* (not deposited); **n** regular radial choanosomal skeleton, histological section through the body of *Polymastia arctica* ZMBN 98068 (University Museum of Bergen); **o** regular reticulate choanosomal skeleton, histological section through the body of *Weberella bursa* (not deposited). *Scale bars* **c**–**e**: 1 cm, **h**: 0.1 mm; **i**: 0.01 mm; **j**: 1 cm; **m**–**o**: 1 mm

Abandonment of *Radiella*

We can, however, propose to transfer *Radiella hemisphaerica* to *Polymastia* since this species groups with the type species of *Polymastia* and five other *Polymastia* spp. forming Clade I. Likewise, *Radiella sarsi*, *Radiella* cf. *sarsi*, and *Radiella* sp. can be allocated to *Spinularia* since these three species and the type species of *Spinularia* form a monophyletic group, Clade II. The status of the genus *Radiella* is controversial (Boury-Esnault 2002; Plotkin and Janussen 2008; Plotkin et al. 2012), and the affinities of its type species, *R. sol* Schmidt, 1870, still remain ambiguous. Type material is lost, fresh material is not available, and the age-old nontype specimen identified as *R. sol* by Schmidt (1880) and redescribed by Boury-Esnault (2002) displays similarity to *R. hemisphaerica*, that does not match the drawing in the original description (Schmidt, 1870), which rather shows similarity between *R. sol* and *R. sarsi* (Plotkin and Janussen 2008; Plotkin et al. 2012). Nevertheless, no matter whether *R. sol* is related to *R. hemisphaerica* or *R. sarsi*, we propose to abandon *Radiella* since *R. hemisphaerica* is placed in *Polymastia*, *R. sarsi* is placed in *Spinularia*, and both *Polymastia* Bowerbank, 1862 and *Spinularia* Gray, 1867 are older names than *Radiella* Schmidt, 1870.

Disjunct distributions of polymastiid species are questioned

Our study questions the bipolar distribution of two polymastiid species, *Tentorium semisuberites* and *Radiella sarsi*. In the 28S rDNA tree, morphologically very similar *Ten. semisuberites* from the North Atlantic and *Ten.* cf. *semisuberites* from the Antarctic (see Table 1 for details on geography) did not group together. The type locality of *Ten. semisuberites* is Greenland (Schmidt 1870), and hence, we assume that the Antarctic sponges may be a separate species. Likewise, the pair of morphologically similar *R. sarsi* from the Norwegian Sea and *Radiella* cf. *sarsi* from Mozambiquean Coast was nonmonophyletic in both CO1 and 28S rDNA trees that questioned the allocation of these two to the same species.

Another example calling for reflection on disjunct distributions of sponge species is the pair *Polymastia euplectella* and *Polymastia bartletti* de Laubenfels, 1942, which display strong morphological similarities. The former species was recorded from the Barents Sea and adjacent areas (Rezvoj 1927; Plotkin 2004), whereas the latter was, before our study, known only from the type locality in the Baffin Sea (de Laubenfels 1942). We got genetic data from four Norwegian individuals identified as *P. euplectella*, one Canadian individual identified as *P. bartletti*, and a juvenile sponge from Sweden considered as *P.* cf. *bartletti*. These sponges were very similar in morphology, but in all phylogenetic trees, the Canadian individual and the Swedish sponge fell quite distantly from the Norwegian *P. euplectella*. All *P. euplectella* had identical CO1 and 28S rDNA except for one individual, for which no CO1 was obtained. *P. bartletti* and *P.* cf. *bartletti* slightly differed in both genes, but still were sisters. These results argue for that *P. euplectella* and *P. bartletti* are valid species. However, without studying more material, we cannot judge whether the small genetic differences between the Canadian *P. bartletti* and the Swedish *P.* cf. *bartletti* is just an intraspecific polymorphism, or these two are different, recently diverged species. Consequently, we cannot conclude whether *P. bartletti* is geographically isolated from *P. euplectella* or not.

Possible reasons for inconsistence between the single-gene phylogenies

Our analyses and tests supported dissimilar relationships of *Polymastia boletiformis* + *Quasillina brevis* with other clades and taxa in the single-gene trees. In the CO1 tree, this pair was the sister to *Polymastia invaginata*, whereas in the 28S rDNA tree, it was the sister to Clade I + *Polymastia* sp. 1 + *Polymastia* sp. 2. This may be due to real differences in genealogical histories of the mitochondrial and nuclear genes. However, on the other hand, the position of *P. boletiformis* + *Q. brevis* in the CO1 tree may be affected by very low resolution leading to Clade I. Likewise, unresolved

relationships of *Polymastia corticata* along with weakly supported grouping of *Sphaerotylus antarcticus* with Clade II in the CO1 tree are obviously due to low resolution and hence to weak phylogenetic signal provided by our CO1 data.

Intraspecific polymorphism indicates incomplete lineage sorting?

Several inconsistencies between the CO1 and 28S rDNA phylogenies within three small terminate subclades were caused by intraspecific polymorphism. Our study could not resolve the relationships between three sibling species of *Polymastia*, *P. andrica*, *P. arctica*, and *P. grimaldii*. The CO1 data on these species were congruent with the morphological differences between them—individuals of the same species possessed identical CO1 and morphologically similar *P. andrica* and *P. arctica* grouped together against morphologically more distinct *P. grimaldii*, although the Bayesian and ML bootstrap support for this relationship was rather weak. At the same time, the analysis of 28S rDNA failed to resolve the relationships between these three species because of the intraspecific and intragenomic polymorphism. Intraspecific polymorphism was also revealed in *P. boletiformis* and *P. invaginata*. Two individuals of *P. boletiformis* possessed identical 28S rDNA, but fairly different CO1. In three individuals of *P. invaginata*, the identity by the mitochondrial gene mismatched that by the nuclear gene.

The ascertained cases of intraspecific polymorphism may indicate incomplete lineage sorting in the closely related polymastiid species and their populations. For example, each lineage may carry one unique version of CO1, but several versions of 28S rDNA, if its ancestor was polymorphic by this gene, and vice versa. When further divergence of the lineages takes place, some gene versions inherited from the polymorphic ancestor may be lost owing to genetic drift or selection (Rogers and Gibbs 2014). Another explanation of the revealed polymorphism may be a gene flow through hybridization between different species, but this assumption requires more thorough studies.

Insufficient variability in the 5′-end barcoding region of CO1

To reconstruct the CO1 phylogeny of Polymastiidae we used "Folmer's" barcoding region, which was successfully applied to recover phylogenies of many invertebrate taxa (Folmer et al. 1994), in particular two large sponge families, Geodiidae (Cárdenas et al. 2010) and Halichondriidae (Erpenbeck et al. 2012). Our results revealed the insufficiency of variation of this region in Polymastiidae that might cause some inconsistencies between the CO1 and 28S rDNA phylogenies (see above) and also hindered the separation of the species in Clades II and III based on CO1 alone, while these species were otherwise successfully separated by the 28S rDNA data. The similar problem with the "Folmer's" region was reported for some other sponge families, e.g., Lubomirskiidae (Schröder et al. 2002), Clionaidae (Ferrario et al. 2010), and Irciniidae (Pöppe et al. 2010). To solve this problem, sequencing of an additional downstream region of the CO1 gene providing more variability was recommended (Erpenbeck et al. 2006, Sponge Barcoding Project at http://www.palaeontologie.geo.uni-muenchen.de/SBP/). The analyses of the extended CO1 barcoding region may probably reduce the inconsistence between the CO1 and 28S rDNA phylogenies of Polymastiidae and resolve the relationships between sibling polymastiid species.

Conclusions

Our study presents the first comprehensive phylogenetic reconstruction of the family Polymastiidae based on molecular data. Our results show that its classification based on morphology is in a strong conflict with molecular phylogenies. Accordingly, the majority of previously assumed morphological synapomorphies appear to be highly homoplastic, and a natural classification of Polymastiidae will require a thorough and comprehensive taxonomic revision. Here we have set up a sound molecular framework for this task and recovered several strongly supported clades. In order to determine the morphological synapomorphies of these clades, a reinterpretation of the currently used characters and a selection of additional characters are needed. Furthermore, we have reported evidence for that sorting of lineages of different genes may follow different ways under the evolutionary divergence of sponge species and that the gene flow between populations of recently diverged species may also take place. Finally, we have demonstrated that the standard 5′-end barcoding region of CO1 provides insufficient data that may result in some inconsistence between the CO1 and 28S rDNA phylogenies and failure to reconstruct the relationships between some polymastiid species, which are otherwise recovered with 28S rDNA data. Hence, we argue once again for the advantages of multigene datasets and extended barcoding regions for reconstructing of phylogenies at the family and generic level.

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Authors' contributions Conceiving the study: AP and HTR. Sampling the material: AP and HTR. Primary treatment and taxonomic identification of the samples: AP. DNA extraction, amplification and sequencing: AP. Contributing reagents/consumables/tools: HTR. Reconstruction of the secondary structure, 28S rDNA: OV. Alignments and phylogenetic analyses: OV, AP and EW. Tracing morphological characters along the molecular tree: EW and AP. Writing the manuscript: AP. Revising the manuscript: OV, EW and HTR.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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