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The complex case of the calcareous sponge *Leucosolenia complicata* (Porifera: Calcarea): hidden diversity in Boreal and Arctic regions with description of a new species

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Abstract:	In this study, we present the first integrative revision of the Boreal and Arctic calcareous sponges of the genus <i>Leucosolenia</i> with a specific focus on its biodiversity in the White Sea. The material for this work included a combination of newly collected specimens from different regions of the North-East Atlantic and the White Sea and historical museum collections. An integrative analysis was implemented based on vast morphological data (light microscopy, scanning and transmission electron microscopy), microbiome observations, ecological data, accompanied by molecular phylogenetic and species delimitation analyses based on three nuclear markers (28S rRNA, 18S rRNA and histone H3). We demonstrate that <i>Leucosolenia complicata</i> , previously reported from Arctic waters, is restricted to the North-East Atlantic, while in the Arctic, the <i>Leucosolenia</i> diversity is represented by at least four species: <i>Leucosolenia corallorrhiza</i> , <i>Leucosolenia variabilis</i> and two new species, one of which is described herein under the name <i>Leucosolenia creepae</i> sp. nov. The molecular phylogeny analysis supports the species identity of these species. In addition to conventional morphological characters, new informative fine morphological characters (skeleton and oscular crown organisation; cytological structure including morphotypes of symbiotic bacteria) were found, providing a baseline for further revision of this group in other regions.

1 **Abstract**

2 In this study, we present the first integrative revision of **the Boreal** and Arctic calcareous sponges
3 of the genus *Leucosolenia* with **a** specific focus on its biodiversity in the White Sea. The material
4 for this work included a combination of newly collected specimens **from** different regions of the
5 North-East Atlantic and the White Sea **and historical** museum collections. An integrative analysis
6 was implemented based on vast morphological data (light microscopy, scanning and transmission
7 electron microscopy), microbiome observations, ecological data, accompanied by molecular
8 phylogenetic and species delimitation analyses based on three nuclear markers (28S rRNA, 18S
9 rRNA and histone H3). We demonstrate that *Leucosolenia complicata*, previously reported from
10 Arctic waters, is restricted to the North-East Atlantic, while in the Arctic, the *Leucosolenia*
11 diversity is represented by at least four species: *Leucosolenia corallorrhiza*, *Leucosolenia*
12 *variabilis* and two new species, one of which is described herein under the name *Leucosolenia*
13 *creepae* sp. nov. The molecular **phylogeny** analysis supports the species identity of these species.
14 **In addition to conventional morphological characters, new** informative fine morphological
15 characters (**skeleton and oscular crown organisation; cytological structure including morphotypes**
16 **of symbiotic bacteria**) **were found**, providing a baseline for further revision of this group in other
17 regions.

18
19 **Keywords:** biogeography, molecular phylogeny, phylogenetic systematics, species delineation,
20 species boundaries, North Atlantic, ultrastructure, scanning electron microscopy

23 Introduction

24 The calcareous sponges (Class Calcarea) represent rather a small group in terms of
25 biodiversity across all sponge taxa (<5% of diversity) (Manuel *et al.*, 2004), but are characterized
26 by a unique mineral skeleton of calcium carbonate spicules and display great diversity in their
27 body organisation (Borojevic *et al.*, 1990). The classification of this group is currently facing many
28 challenges and rearrangements at all taxonomical levels due to high levels of homoplasy and
29 convergent evolution (Manuel *et al.*, 2004; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012; Voigt &
30 Wörheide, 2016; Alvizu *et al.*, 2018). In the course of the last two decades, the integrative
31 taxonomy has become the most popular approach to define and describe taxa on different
32 taxonomic levels and produce a reliable phylogeny-based classification of living organisms
33 (Dayrat, 2005; Padial *et al.*, 2010; Schlich-Steiner *et al.*, 2010). In many cases, such studies can
34 resolve existing taxonomic disputes strictly and definitively (Goulding & Dayrat, 2016).
35 Molecular phylogenetics augments the ability of a researcher to find species boundaries and thus
36 taxonomically important morphological characters. The integrative studies of calcareous sponges
37 gave a new insight into understanding the actual diversity and evolutionary history of this group,
38 with numerous new taxa being described during the last years (Azevedo *et al.*, 2017; Riesgo *et al.*,
39 2018; van Soest & de Voogd 2018; Alvizu *et al.*, 2019; Córdor-Luján *et al.*, 2019; Sanamyan *et*
40 *al.*, 2019; Chu *et al.*, 2020; Klautau *et al.*, 2020 and many others).

41 The Russian Arctic represents a poorly studied region with an unknown diversity of
42 calcareous sponges. At the same time, extensive studies of Calcarea in the western part of the
43 Arctic were carried out, thanks to which new species were described and a deep revision of the
44 species described by that time was carried out (Rapp *et al.*, 2001; Rapp, 2006, 2015; Alvizu *et al.*,
45 2019). In other regions, like the Mediterranean, Caribbean and some others, the diversity of
46 calcareous sponges has been extensively studied using molecular methods (Cavalcanti *et al.*, 2014;
47 Klautau *et al.*, 2016, 2021; Fontana *et al.*, 2018; van Soest & de Voogd, 2018). However,
48 contemporary studies of the Russian Arctic tend to lend heavily to traditional morphology with

1
2 49 limited regard for newer methods and trends in taxonomy and biogeography (Breitfuss, 1898a,b,c;
3
4 50 Koltun, 1952; Ereskovsky, 1994a). Despite limited taxonomic **studies**, calcareous sponges from
5
6 51 the White **and Barents Seas** are widely involved in various ecological (Ereskovsky, 1994b,c;
7
8 52 1995a,b), physiological and embryological (Anakina, 1997; Anakina & Korotkova, 1989; Anakina
9
10 53 & Drozdov, 2000, 2001) researches. Two of **the** focus-sponges, *Leucosolenia complicata*
11
12 54 (Montagu, 1814) and *Sycon ciliatum* (Fabricius, 1780) have **become rising model species** in
13
14 55 evolutionary developmental studies (Leininger *et al.*, 2014; Fortunato *et al.*, 2014, 2015, 2016;
15
16 56 Ereskovsky *et al.*, 2017a; Lavrov *et al.*, 2018, 2022; Lavrov & Ereskovsky, 2022; Melnikov *et al.*,
17
18 57 2022).

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22
23 58 The genus *Leucosolenia* Bowerbank, 1864 (phylum Porifera, class Calcarea, subclass
24
25 59 Calcaronea) **includes more than 40 distinct species distributed worldwide, being more specious in**
26
27 60 **the northern regions** (Burton, 1963; Borojevic *et al.*, 2000). The history of **systematics** of this group
28
29 61 is complicated **by the** different species conceptions proposed by various researchers since
30
31 62 Haeckel's monograph on calcareous sponges (Haeckel, 1872). Haeckel **established** 21 new genera,
32
33 63 seven of which represent an asconoid type of **organisation**. However, he did not consider any
34
35 64 generic names for calcareous sponges used before him; hence, this system has undergone various
36
37 65 modifications (Minchin, 1904). At the species-level, many of his names and his 'connection
38
39 66 varieties' for leucosolenoid sponges were further considered synonyms of three widely distributed
40
41 67 species: *Leucosolenia botryoides* (Ellis & **Solander**, 1786), *L. complicata*, *Leucosolenia variabilis*
42
43 68 **Haeckel, 1870** (Minchin, 1904, 1905). Further research highlighted the complications of
44
45 69 taxonomical studies due to **the** absence of informative characters. As a result, some North Atlantic
46
47 70 species, *i.e.*, *L. complicata*, *L. variabilis* and *L. botryoides* were believed to have a wide
48
49 71 cosmopolitan distribution ranging from the Arctic to South Africa, New Zealand and **the** Antarctic
50
51 72 (Burton, 1963). Sarà (1956) found a connection between spicular characters in these species and
52
53 73 **suggested** they may **hybridise**, which caused even more simplification of the system with a single
54
55 74 species, *L. botryoides* represented by different 'forms' of uncertain taxonomical status (Burton,

1
2 75 1963). With the advent of new microscopic techniques and molecular studies, the systematics of
3
4 76 the genus *Leucosolenia* has received much attention during the last twenty years. The valid status
5
6 77 of some species was reconsidered, e.g., North-eastern Atlantic *Leucosolenia corallorrhiza*
7
8 78 (Haeckel, 1872) (Rapp, 2015) and *Leucosolenia somesii* (Bowerbank, 1874) (van Soest *et al.*,
9
10 79 2007). Also, several new species were described based on studies using scanning electron
11
12 80 microscopy that allowed the identification of fine spines on spicules (van Soest, 2017; Chu *et al.*,
13
14 81 2020). Recent molecular phylogenetic analysis of North Atlantic *Leucosolenia* species showed the
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16 82 paraphyly of the genus (Alvizu *et al.*, 2018) and the high rate of hidden biodiversity within it.
17
18 83 Nevertheless, no integrative revision has been conducted yet, and the taxonomical status of most
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20 84 North-Atlantic species remains unverified.
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25 85 The main goal of our study is to revise the diversity and taxonomy of the genus *Leucosolenia*
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27 86 from the White Sea using an integrative approach, which includes vast molecular, morphological
28
29 87 and cytological data. Additionally, the specific aims of the study are to identify phylogenetically
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31 88 significant morphological characters and to propose optimal sets of molecular markers for further
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33 89 taxonomical and phylogenetic research on calcareous sponges.
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91 **Material and methods**

92 *Material*

93 The representatives of **the** genus *Leucosolenia* were collected in the White Sea at N.A.
94 Pertsov White Sea Biological Station MSU (66°34'N, 33°08'E) during 2016–2018 at the upper
95 subtidal zone and by SCUBA diving (Table S1). The identification of the specimens was based on
96 external **and internal** (skeletal) characteristics, such as spicule types and their **spatial** arrangement
97 in different regions of the sponge body. Additionally, we studied specimens of *Leucosolenia*
98 *complicata* collected in the English Channel (Roscoff, France) and **a** specimen of *Leucosolenia*
99 *somesii* from the collection of **the** Zoological Museum of Amsterdam (ZMA). Several **spicule**
100 slides from the collection of **the British** Museum of Natural History (BMNH) of *L. complicata*, *L.*
101 *variabilis* and *L. somesii*, including the type material for the latter two species, were also examined
102 for comparison with species from the White Sea (Table S1). Collection data, voucher numbers and
103 GB accession numbers are summarised in Table S1. All **new** samples were fixed in 96% ethanol.
104 Voucher specimens are deposited in the collections of the Zoological Museum of Moscow State
105 University, White Sea Branch (WS).

106 *Taxon sampling*

107 Our molecular sampling included 279 individuals from the White Sea, **the** Netherlands and
108 Roscoff (Table S1). For species delimitation, the 28S C-region was sequenced for all specimens
109 available for study (to make sure that the morphological identification is correct). In several cases,
110 28S did not give enough resolution to undoubtedly support the genetic distinctness of species;
111 therefore, for a large number of samples from the White Sea, we additionally obtained **a** novel
112 histone H3 marker (see below for details). Finally, 18S was obtained only for several specimens
113 for the concatenated phylogenetic analysis to test the monophyly of the genus *Leucosolenia*.

114 **Seventeen *Leucosolenia*** specimens from the White Sea, Greenland, Norway and **the North-**
115 **East** Atlantic (unspecified), for which only GenBank sequences were available, were also included
116 in the analyses (Table S1). **The final datasets for each gene included as many specimens from**

1
2 117 different localities as possible to improve the resolution of phylogenetic reconstructions.
3
4 118 *Plectroninia novaecaledoniense* Vacelet, 1981 and *Clathrina arnesenae* (Rapp, 2006) were
5
6 119 chosen as outgroups based on recent papers on the calcaronean phylogeny (Voigt & Wörheide,
7
8 120 2016; Alvizu *et al.*, 2018). To test the identity of high-level taxonomic groups, specimens included
9
10 121 in the most recent Calcaronea phylogenetic study (Alvizu *et al.*, 2018) were used in the
11
12 122 concatenated analysis (Table S1).
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15 123 **DNA extraction, amplification and sequencing**

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18 124 DNA was extracted from small pieces of tissue using PALL AcroPrep 96-well plates (PALL
19
20 125 Corp., USA) (Ivanova *et al.*, 2006) and the Diatom DNA Prep100 kit (Isogen Lab, Russia)
21
22 126 according to manufacturer protocol with minor modifications: an increased lysis stage time for
23
24 127 better dissolving of tissues and a reduced volume of extraction reagents at the final step for
25
26 128 increasing the final DNA concentration. Extracted DNA was used as a template for the
27
28 129 amplification of partial 28S rRNA (C-region, LSU) and 18S rRNA (SSU). To support the results
29
30 130 obtained with ribosomal markers, we also included the nuclear protein-coding gene histone *H3*
31
32 131 (*H3*) in the analysis. Although this marker is commonly used in systematic and phylogenetic
33
34 132 studies in other invertebrate groups, sequences for the calcarean sponges are absent in GenBank.
35
36 133 Primers were modified from standard *H3* metazoan primers (*H3AF*: 5'-ATG GCT CGT ACC
37
38 134 AAG CAG ACV GC-3' *H3AR*: 5'-ATA TCC TTR GGC ATR ATR GTG AC-3' see Colgan,
39
40 135 1998), the annotated transcriptome of *S. ciliatum* (Fortunato *et al.*, 2014) was used for a primer
41
42 136 design (Table 1). Polymerase chain reactions were carried out in a 25- μ L reaction volume, which
43
44 137 included 5 μ L of 5x Taq Red Buffer (Eurogen Lab, Russia), 0.5 μ L of HS-Taq Polymerase
45
46 138 (Eurogen Lab, Russia), 0.5 μ L of dNTP (50 μ M stock), 0.3 μ L of each primer (10 μ M stock), 1
47
48 139 μ L of genomic DNA and 17.7 μ L of sterile water. The PCR conditions for the corresponding
49
50 140 primers are given in Table 1. Sequencing for both strands proceeded with the Big Dye Terminator
51
52 141 v3.1 sequencing kit (Applied Biosystems, USA), the same primers as for PCR were used.
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2 142 Sequencing reactions were analysed using the ABI 3500 Genetic Analyzer (Applied Biosystems,
3
4 143 USA). All new sequences were deposited in GenBank (Table S1).

6 144 ***Phylogenetic reconstruction***

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9 145 Raw reads for each gene were assembled and checked for improper base-calling using
10
11 146 GeneiousPro 4.8.5 (Biomatters, New Zealand). Original data and publicly available sequences
12
13 147 were aligned with the MUSCLE (Edgar, 2004) algorithm in MEGA7 (Kumar *et al.*, 2016). Protein-
14
15 148 coding sequences were translated into amino acids to verify the coding sequences. The resulting
16
17 149 alignments were 353 bp for 28S, 1493 bp for 18S and 359 bp for H3. Phylogenetic analyses were
18
19 150 conducted for each marker individually and for the concatenated dataset. Sequences were
20
21 151 concatenated by a simple biopython script (Chaban *et al.*, 2019). The best-fitting nucleotide
22
23 152 evolution model was tested in MEGA7 based on the Bayesian information criterion (BIC) for each
24
25 153 partition. The best-fitting model for the 28S partition was HKY+G, for the 18S partition – K2, and
26
27 154 for the H3 partition – K2P+G. Phylogenetic reconstruction of the concatenated dataset was
28
29 155 performed applying evolutionary models for partitions separately. The Bayesian estimation of
30
31 156 posterior probability for all datasets was performed in MrBayes 3.2 (Ronquist & Huelsenbeck,
32
33 157 2003). Markov chains were sampled at intervals of 500 generations. The analysis was started with
34
35 158 random starting trees and 10⁷ generations. Maximum likelihood-based phylogeny inference for all
36
37 159 datasets was performed in the HPC-PTHREADS-AVX version of RaxML (Stamatakis, 2014) with
38
39 160 ultrafast bootstrapping (UFBoot approximation approach) (Minh *et al.*, 2013) in 1000
40
41 161 pseudoreplicates under the GTRCAT model of nucleotide evolution. Bootstrap values were placed
42
43 162 on the best tree found with SumTrees 3.3.1 from DendroPy Phylogenetic Computing Library
44
45 163 Version 3.12.0 (Sukumaran & Holder, 2010). The final phylogenetic tree images were rendered in
46
47 164 FigTree 1.4.0.

54 165 ***Species delimitation***

56
57 166 Uncorrected inter- and intra-specific *p*-distances were calculated in MEGA7 (Kumar *et al.*,
58
59 167 2016). Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al.*, 2021), calculation

1
2 168 of uncorrected *p*-distances and single-gene trees were applied to assist in the species delimitation
3
4 169 analysis. 28S and H3 sequences of *Leucosolenia* species (excluding outgroups) used in the
5
6 170 phylogenetic analysis, were aligned for ASAP analysis with the MUSCLE algorithm (Edgar,
7
8 171 2004) in MEGA7 (Kumar *et al.*, 2016). 18S sequences were not used in the analysis due to low
9
10 172 substitution rates. The analysis was run on the online version of the programme
11
12 173 (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) with the default setting and three proposed
13
14 174 models: Jukes-Cantor (JC), Kimura (K80) and Simple distance. Uncorrected *p*-distances were
15
16 175 calculated for the same alignments used for ASAP in MEGA7 (Kumar *et al.*, 2016). Single-gene
17
18 176 trees were calculated using the Maximum Likelihood approach in HPC-PHTHREADS-AVX
19
20 177 version of RaxML (Stamatakis, 2014) with ultrafast bootstrapping (UFBoot approximation
21
22 178 approach) (Minh *et al.*, 2013) with 1000 pseudoreplicates under the GTRCAT model of nucleotide
23
24 179 evolution.

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28
29 180 A visualisation of character heterogeneity was assessed using PopART 1.7
30
31 181 (<http://popart.otago.ac.nz>) (Leigh & Bryant, 2015) with the TCS network algorithm (Clement *et*
32
33 182 *al.*, 2002) and a connection limit of 5%. The resulting networks were edited in Adobe Illustrator
34
35 183 CS 2015 to highlight certain features.

184 ***Morphological studies***

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40 185 The external morphology of each species was studied under Leica M165FC
41
42 186 stereomicroscope (Leica, Germany) equipped with a digital camera Leica DFC420 (Leica,
43
44 187 Germany). Extraction of spicules and slide preparation for a total of 34 specimens were made
45
46 188 according to standard protocols (Klautau & Valentine, 2008). The general skeleton morphology
47
48 189 of both the oscular rim and cormus tubes was studied under Leica M165C stereomicroscope
49
50 190 (Leica, Germany). For detailed studies of skeleton morphology, parts of the oscular rim and
51
52 191 cormus tubes were treated with Murray's Clear according to the standard protocol to clarify soft
53
54 192 tissues (Miller *et al.*, 2005). Slides of dissociated spicules and skeletons were studied under Zeiss
55
56 193 Axioplan 2 (Carl Zeiss, Germany) and Leica DM2500 (Leica, Germany) with the digital cameras

1
2 194 AxioCam HRm (Carl Zeiss, Germany) and Leica DFC420C (Leica, Germany), respectively.
3
4 195 Scanning electron microscopy (SEM) **analysis of the spicules** was performed under Carl Zeiss
5
6 196 EVO-40 (Carl Zeiss, Germany), Hitachi S-405A (Hitachi, Japan) and CamScan S2 (Clinton
7
8 197 Electronics Corp., UK) scanning electron microscopes. For this purpose, isolated spicules in 96%
9
10 198 ethanol were transferred to cover slips, which were mounted onto stubs with nail polish, dried and
11
12 199 sputter-coated. **Figures of spicules under SEM are montages of the most typical spicule**
13
14 200 **morphology obtained from a number of specimens of each studied species.**

15
16
17
18 201 **The measurements of the spicules** (length and **basal** width at the base of the actines) were
19
20 202 made for every spicule category on SEM images using ImageJ **v. 1.48** software (National Institute
21
22 203 of Health, USA). Strait Line tool was used for **straight** spicules and rays, Segmented Line tool **was**
23
24 204 **used** for curved and undulating spicules and rays.

25 26 27 205 ***Cytological studies***

28
29 206 For semithin sections and transmission electron microscopy (TEM), sponges were fixed **with**
30
31 207 2.5% glutaraldehyde (Ted Pella, USA) on 0.2M Millonig's phosphate buffer (Millonig, 1964), and
32
33 208 postfixated with 1% OsO₄ (Spi Supplies, USA) according to standard protocol (Lavrov *et al.*, 2016a,
34
35 209 2018; Lavrov & Ereskovsky, 2022). Specimens were embedded in SPI-Pon 812/Araldite 6005
36
37 210 epoxy embedding media (Spi Supplies, USA) according to **the** manufacturer's instructions. Semi-
38
39 211 thin sections (1 µm in thickness) were cut on a Reichert Jung ultramicrotome (Reichert, USA)
40
41 212 equipped with a "Micro Star" 45° diamond knife before being stained with toluidine blue and
42
43 213 observed under a WILD M20 microscope (Wild-Leitz, Germany). Digital photographs were taken
44
45 214 with a Leica DMLB microscope (Leica Microsystems, Germany) using the Evolution LC colour
46
47 215 photo-capture system (Media Cybernetics, USA). Ultrathin sections (60–80 nm) were cut with a
48
49 216 Leica UCT6 (Leica, Germany) and PowerTome XL ultramicrotomes equipped with a Drukkert
50
51 217 45° diamond knife. Ultrathin sections, contrasted with uranyl acetate, were observed under Zeiss-
52
53 218 1000 TEM (Carl Zeiss, Germany) and Tecnai G2 20 TWIN (FEI Company, USA). The detailed
54
55 219 cytological studies were conducted only for *Leucosolenia corallorrhiza*, as it represents the most

1
2 220 common species in the White Sea. For other species, only the general morphology of the cell types
3
4 221 was described. Figures of cell types are montages of the most typical cell morphology obtained
5
6 222 from a number of specimens of each studied species.
7

8
9 223 *Nomenclatural acts*

10
11 224 The electronic edition of this article conforms to the requirements of the amended
12
13 225 International Code of Zoological Nomenclature (ICZN), and hence the new name contained herein
14
15 226 is available under that Code from the electronic edition of this article. The LSID for this
16
17 227 publication is: <urn:lsid:zoobank.org:pub:BA13614B-2884-4E02-9D45-0EE44CBD01A5>.
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For Review Only

229 **Results**

230 *Phylogenetic reconstruction*

231 We obtained 486 new sequences of different *Leucosolenia* species (Table S1). The sequence
232 alignment of concatenated 28S, 18S and H3 loci includes 2160 positions. Single-gene trees based
233 on 28S and H3 loci give good resolution at the species level while, 18S single-gene tree is
234 unresolved due to low substitution rates (Data S1).

235 The topology of the resulting concatenated trees from Bayesian Inference (BI) and
236 Maximum Likelihood (ML) analyses are congruent and well-supported in most cases (Figs 1, S1,
237 S2). The genus *Leucosolenia* is recovered monophyletic with moderate support (posterior
238 probabilities from BI (PP) = 0.9; bootstrap support from Maximum Likelihood (ML) = 92). Within
239 *Leucosolenia* clade three species groups are monophyletic and highly supported: Clade I, including
240 most specimens from the White Sea (*Leucosolenia* sp. 1, *Leucosolenia* sp. 3 and *Leucosolenia* sp.
241 4) and several specimens from GenBank initially identified as *Leucosolenia* cf. *variabilis*,
242 *Leucosolenia* cf. *corallorrhiza* and *Leucosolenia* sp. 1 (PP = 1; ML = 99); Clade II with specimens
243 from the White Sea (*Leucosolenias* sp. 2), *Leucosolenia* *somesii* from Netherlands, *Leucosolenia*
244 sp. from GenBank (accession number AF100945) and *Leucosolenia* *botryoides* from GenBank
245 (voucher number SA60) (PP = 1; ML = 93); and Clade III, which comprised only *Leucosolenia*
246 *complicata* specimens from Roscoff (ws11881-11883) and from GenBank (PP = 1; ML = 100).
247 The Clade III (*L. complicata*) is sister to the Clade II (PP = 1; ML = 93). Within the Clade I at
248 least six monophyletic units are found: (1) *Leucosolenia* sp. 1, clustering with *Leucosolenia* cf.
249 *variabilis* FB33 and *Leucosolenia* cf. *variabilis* FB12 from GenBank (PP = 0.99; ML = 96), (2)
250 *Leucosolenia* cf. *variabilis* FB58 and *Leucosolenia* cf. *variabilis* FB60 (PP = 1; ML = 100), (3)
251 *Leucosolenia* sp. 3 and *Leucosolenia* cf. *variabilis* SA62 (PP = 1; ML = 98), (4) *Leucosolenia* sp.
252 4 and *Leucosolenia* cf. *corallorrhiza* FB14, FB20, SA43 (PP = 0.81; ML = 100), (5) *Leucosolenia*
253 cf. *corallorrhiza* FB59 and SA44 (PP = 1; ML = 100) and (6) *Leucosolenia* sp. 1 FB73 and FB81
254 from GenBank (PP = 0.61; ML = 100). Representatives of (1), (2), (4), (5) clades form a compact

255 monophyletic group (PP = 0.99; ML = 96) with very low genetic distances within it (Table 2).
256 *Leucosolenia* sp. 3 is recovered sister to it (PP = 0.92; ML = 98), and *Leucosolenia* sp. 1 from
257 GenBank shows sister relationships to other representatives of Clade I.

258 Within Clade II, *L. somesii* specimens from the Netherlands form a single clade with
259 *Leucosolenia* sp. from GenBank. *Leucosolenia* sp. 2 also forms a well-supported clade (PP = 1;
260 ML = 93). It represents a monophyletic group with *L. botryoides* and *L. somesii* (PP = 1; ML =
261 93), but relationships within this clade are unsupported.

262 *Species delimitation*

263 ASAP analysis of 271 LSU sequences recovers a different number of operational
264 taxonomical units (OTUs) depending on the ASAP score (Data S2). The lowest ASAP score is
265 found for threshold distances of 2.16%, 2.29%, 4.30% or 6.68% in this case five OTUs are
266 recovered: four of them correspond to species from Clades II and III (*Leucosolenia complicata*, *L.*
267 *botryoides*, *L. somesii* and *Leucosolenia* sp. 2) and the fifth group includes all specimens of Clade
268 I. A scenario revealing the same 10 species-level units as in the phylogenetic analysis receives a
269 relatively high ASAP score (5.00) with a threshold distance of 0.69%. ASAP analysis of the H3
270 dataset (170 sequences) contains only five candidate species (*Leucosolenia* sp. 1, *Leucosolenia* sp.
271 2, *Leucosolenia* sp. 3, *Leucosolenia* sp. 4, *L. complicata*) due to the absence of H3 sequences for
272 calcareous sponges in GenBank. The lowest ASAP score (2.00) receives a scenario with all five
273 candidate species as distinct (the threshold distance is 1.38%). Scenarios with an ASAP score of
274 2.5–4.0 reveal 2–4 candidate species; the identities of *Leucosolenia* sp. 1, *Leucosolenia* sp. 3, and
275 *Leucosolenia* sp. 4 are not supported in this case.

276 A visualisation of character heterogeneity using the Medium Parsimony Network (TCS
277 algorithm) reveals similar results in the 28S and H3 datasets (Fig. 2). On the 28S network, each
278 candidate species either forms a distinct group (*Leucosolenia* sp. 1, *Leucosolenia* sp. 2,
279 *Leucosolenia* sp. 3, *L. complicata*) or has a unique genotype (*Leucosolenia* sp. 4, *L. somesii*, *L.*
280 *botryoides* and three candidate species from GenBank) (Fig. 2A). These groups, or genotypes,

1
2 281 differ from each other by **three** substitutions among species of **Clade I** and by 8–11 substitutions
3
4 282 among species of **Clade II**. The intraspecific differences do not exceed **two** substitutions. There
5
6 283 are 15–22 substitutions between representatives of **Clades I, II, III**.

7
8
9 284 **The same results are observed** in the H3 dataset, but the overall nucleotide diversity is higher
10
11 285 (Fig. 2B). There are 1–2 substitutions within each candidate species except *Leucosolenia* sp. 3,
12
13 286 where genotypes differ **by** 1–4 substitutions. **Differences of** 6–23 substitutions are found between
14
15 287 candidate species.

16
17
18 288 Uncorrected *p*-distance values of 28S and H3 markers are presented in Tables 2–3 (data from
19
20 289 GenBank are not included). Overall, intraspecific *p*-distances of 28S **within Clade I** show **an**
21
22 290 overlapping range with interspecific distances (0–0.4% intraspecific, 0.4–1.3% intraspecific
23
24 291 distances). **The distances between the** three large clades vary from 5.1 to 10.5%. **The** H3 marker
25
26 292 shows a higher diversity: intraspecific distances vary from 0 to 1.7%, while interspecific distances
27
28 293 vary from 2.5 to 5% within larger clades and are of 7.9–10.9% between the clades.

29 30 31 32 33 294 ***Comparison of morphological and molecular data***

34
35 295 Three recovered monophyletic lineages corresponding to the genus *Leucosolenia*, have
36
37 296 distinct morphological traits. They differ in the external appearance, the general skeleton
38
39 297 composition, the spicular set and the cellular composition (Figs 3–24; Table 4–9). Definitions and
40
41 298 technical terms used in this study can be found elsewhere (Boury-Esnault & Rützler, 1997;
42
43 299 Ereskovsky & Lavrov, 2021; Łukowiak *et al.*, 2022).

44
45
46 300 **Clade III** is represented by a single species, *Leucosolenia complicata*, which was found
47
48 301 only in European waters (**Roscoff and Norway in our material and public data**). **Although the type**
49
50 302 **material for this species is not known if it ever existed, morphologically our specimens perfectly**
51
52 303 **fit** the descriptions made by previous authors with few exceptions (Haeckel, 1872; Minchin, 1904;
53
54 304 Rapp, 2015). The main diagnostic traits in spicular characters are (Fig. 4): (1) two populations of
55
56 305 diactines, large lanceolate smooth diactines and small trichoxeas with irregular spines; (2)

1
2 306 parasagittal tri- and tetractines, with the unpaired actine is commonly longer than the paired ones;
3
4 307 (3) tetractines are commonly found in both the oscular region and the cormus.

5
6 308 The studied representatives of Clade II (*Leucosolenia* sp. 2 and *Leucosolenia somesii*) have
7
8 309 an echinate external appearance due to the high number of diactines protruding through the
9
10 310 external surface (Fig. 19). Diactines are non-lanceolate, with rows of spines on the outer tip
11
12 311 (commonly only two rows are visible, but there are more) or smooth (Fig. 20A, B). In these
13
14 312 species, tri- and tetractines are thinner than in representatives of Clade C (see below), and
15
16 313 abnormal spicules are common (Fig. 20C-E; Table 8). At the same time, *Leucosolenia* sp. 2 differs
17
18 314 from *Leucosolenia somesii* by having only a single population of spined diactines, while
19
20 315 *Leucosolenia somesii* possesses a second type of smooth non-lanceolate diactines (Fig. 24A-C).

21
22 316 Species of Clade I have several common traits: all of them have unique cells with inclusions
23
24 317 (see below) (Figs 10C-E, 15C, E-F; Table S2); tri- and tetractines are predominantly T-shaped, the
25
26 318 angle between paired actines is commonly 130-150° (Figs 8C-D, 12B, D, 18D, F; Table 5-7);
27
28 319 lanceolate diactines are always present, with or without spines (Figs 8A-B, 12A, 13, 18A-C).

29
30 320 Within this clade, *Leucosolenia* sp. 1 has the largest angle between the paired actines (mean
31
32 321 140°) and the unpaired actine is much shorter than the paired ones in tri- and tetractines (Fig. 8C-
33
34 322 D; Table 5). Also, it has only lanceolate diactines bearing short spines on the lanceolate tip in some
35
36 323 cases (Fig. 8A-B). These characters perfectly fit the description and illustrations of *L.*
37
38 324 *corallorrhiza* (Haeckel, 1872), but not *L. variabilis*.

39
40 325 *Leucosolenia* sp. 3 commonly forms a large voluminous rounded cormus with a very large
41
42 326 oscular tube (there may be more than one oscular tube, but one of them is always enlarged) (Fig.
43
44 327 11A). Other representatives of Clade A commonly have cormus spreading along substrate with
45
46 328 numerous oscular tubes of more or less equal size (Figs 7A, 17A). In spicular morphology, this
47
48 329 species has a unique type of diactines: extremely long and thin trichoxeas, covered with irregularly
49
50 330 placed spines (Fig. 13). Lanceolate diactines lack spines (Fig. 12A). The length of unpaired actines
51
52 331 in tri- and tetractines is commonly the same as that of paired ones (Fig. 12B-D; Table 6). The
53
54
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60

332 shape and measurements of spicules, **as well as** overall body shape mostly resemble those states
333 described for *L. variabilis* (Haeckel, 1872).

334 *Leucosolenia* sp. 4 is very **similar** to *Leucosolenia* sp. 1 in external appearance. In spicular
335 characters, this species contains very few tetractines in both the oscular region and the cornus
336 (Fig. 17B-D). Triactines are usually with undulated paired actines (Fig. 18F). We also detected a
337 high amount of abnormal tri- and tetractines (Fig. 18E), while in other species their amount is
338 lower. Although these differences seem to be valuable to support the species distinctness, the
339 limited studied material (only three specimens available) **does** not allow us to test **for** possible
340 intraspecific variation. Also, this species demonstrates low genetic divergence from *Leucosolenia*
341 sp. 1.

342 To sum up, our integrative approach indicates that the species *Leucosolenia complicata* is
343 restricted to European waters, while in the White Sea, the genus *Leucosolenia* is represented by a
344 complex of four species: *Leucosolenia corallorrhiza* (= *Leucosolenia* sp. 1), *Leucosolenia*
345 *variabilis* (= *Leucosolenia* sp. 3), and two undescribed species. *Leucosolenia* sp. 2 is described
346 herein under the name *Leucosolenia creepae* sp. nov., while in the case of *Leucosolenia* sp. 4
347 (further named *Leucosolenia* sp. A), more material is needed to confirm or reject its identity as a
348 separate species from the closely related *Leucosolenia corallorrhiza*.

350 Systematic descriptions

351 **Subclass Calcaronea Bidder, 1898**

352 **Order Leucosolenida Hartman, 1958**

353 **Family Leucosoleniidae Minchin, 1990**

354 ***Leucosolenia* Bowerbank, 1864**

355 *Type species: Spongia botryoides* (Ellis & Solander, 1786) (by original designation).

356 *Type locality: Harbour near Emsworth, between Sussex and Hampshire, the English Channel.*

1
2 357 *Diagnosis* (based on Hooper et al., 2002): Leucosoleniidae, in which the skeleton can consist of
3
4 358 diactines, triactines and/or tetractines. There is no reinforced external layer on the tubes.
5

6
7 359

8
9 360 ***Leucosolenia complicata* (Montagu, 1814)**

10
11 361 (Figs 3-6; Table 4)

12
13 362 *Type material*: Not known, probably lost.

14
15 363 *Type locality*: British Isles, Devon coast (Montagu, 1818).

16
17 364 *Material studied*: 3 specimens. Molecular data – 3 specimens (WS11661, WS11662, WS11663),
18
19 365 external morphology – 3 specimens (WS11661, WS11662, WS11663), skeleton organisation – 1
20
21 366 specimen (WS11662), spicules (SEM) – 3 specimens (WS11661, WS11662, WS11663), cytology
22
23 367 (TEM) – 3 specimens (WS11661, WS11662, WS11663) (Table S1).
24

25
26 368 *External morphology*: Cormus more or less spherical, bearing multiple erect oscular tubes with
27
28 369 short lateral diverticula in basal part (Fig. 3A). Prominent perioscular spicular crown absent (Fig.
29
30 370 3B). Surface minutely hispid. Colouration of living and preserved specimens grayish white (Fig.
31
32 371 3A).
33

34
35 372 *Spicules*: Diactines (Fig. 4A-C). Two populations: (1) Curved lanceolate diactines (Fig. 4A), mean
36
37 373 length 263.7 μm , mean width 9.5 μm (Table 4). Slightly curved, smooth, with lanceolate outer tip,
38
39 374 variable in length. (2) Trichoxeas (Fig. 4B), mean length 127.3 μm , mean width 2.4 μm (Table 4).
40
41 375 Thin, straight, narrowing toward outer end, both ends pointed, not lance-shaped. Numerous
42
43 376 irregularly distributed spines, number and size of spines decrease toward inner end (Fig. 4C).
44

45
46 377 Triactines (Fig. 4D). Predominantly parasagittal V-shaped (mean angle 125.7°), with
47
48 378 unpaired actine usually longer than paired (mean length: 113.5 μm – unpaired, 94.9 μm – paired)
49
50 379 (Table 4), but equal and shorter unpaired actines also occur. Unpaired actine usually slightly
51
52 380 slender than paired (mean width: 6.3 μm – unpaired, 6.8 μm – paired) (Table 4). T-shaped sagittal
53
54 381 triactines absent.
55
56
57
58
59
60

1
2 382 Tetractines (Fig. 4E). Predominantly parasagittal V-shaped (mean angle 123.5°). Unpaired
3
4 383 actine usually longer than paired, rarely equal (mean length: 109.3 μm – unpaired, 93.9 μm –
5
6 384 paired, 23.8 μm – apical) (Table 4). Paired and unpaired actines equal in width, apical actine more
7
8 385 slender (mean width: 6.7 μm – unpaired, 6.9 μm – paired, 5.3 μm – apical) (Table 4). Apical actine
9
10 386 curved and smooth.

11
12
13 387 *Skeleton*: Skeleton of both oscular and cormus tubes predominantly formed by tetractines,
14
15 388 triactines quite rare (Fig. 3C, D). In oscular tubes spicules constitute organized array with their
16
17 389 unpaired actines directed toward cormus and oriented more or less in parallel to proximo-distal
18
19 390 axis of oscular tube (Fig. 3C). In cormus tubes spicule array less organized (Fig. 3D). Lanceolate
20
21 391 diactines cover tubes surface, orienting in different directions and extending outside by lance-
22
23 392 shaped tip. Trichoxeas sparsely distributed on outer surface. No prominent spicular crown on
24
25 393 oscular rim (Fig. 3B).

26
27 394 *Cytology*: Body wall, 6-9 μm thick, three layers: exopinacoderm, loose mesohyl, choanoderm
28
29 395 (Fig. 5A, B; Table S2). Flat endopinacocytes located in only distal part of oscular tube (oscular
30
31 396 ring) replacing choanocytes. Inhalant pores scattered throughout exopinacoderm, except the
32
33 397 oscular ring area.

34
35 398 Exopinacocytes non-flagellated T-shaped, rarely flat (Fig. 5C). External surface covered by
36
37 399 glycocalyx. Cell body (height 4.8 μm , width 2.8 μm), containing nucleus (diameter 2.2 μm),
38
39 400 submersed in mesohyl (Fig. 5C). Cytoplasm with specific spherical electron-dense inclusions (0.3-
40
41 401 0.4 μm diameter) (Fig. 5C).

42
43 402 Endopinacocytes non-flagellated flat cells, size 20-30 μm \times 2-2.5 μm (Fig. 5D). External
44
45 403 surface covered by glycocalyx. Nucleus (2.4x1.8 μm) oval with or without nucleolus. Cytoplasm
46
47 404 without specific inclusions (Fig. 5D).

48
49 405 Choanocytes flagellated trapeziform or prismatic (height 6 μm , width 3.7 μm) (Fig. 5E).
50
51 406 Flagellum surrounded by collar of microvilli. Characteristic pyriform nucleus (diameter 2.3 μm)
52
53 407 in apical position. Cytoplasm with phagosomes and small vacuoles (Fig. 5E).

1
2 408 Porocytes tubular cylindrical (height 4.5 μm , width 2 μm), connecting external milieu with
3
4 409 choanocyte tube (Fig. 5B, F). Nucleus oval to spherical, diameter 1.8 μm , some time with
5
6 410 nucleolus. Cytoplasm with phagosomes and small vacuoles (Fig. 5F).
7

8
9 411 Sclerocytes amoeboid, size 4 μm \times 2 μm (Fig. 6A). Nucleus usually oval or pear-shaped
10
11 412 (diameter 1.6 μm), containing single nucleolus. Well-developed Golgi apparatus and rough
12
13 413 endoplasmic reticulum. Cytoplasm usually with phagosomes and/or lysosomes (Fig. 6A).
14

15
16 414 Amoebocytes of different shape (from oval to amoeboid) without special inclusions, size 5.8
17
18 415 μm \times 3.4 μm (Fig. 6B). Nucleus spherical (diameter 2.2 μm), sometimes with nucleolus.
19

20
21 416 Two morphotypes of bacterial symbionts in mesohyl. Morphotype 1 most abundant. Bacteria
22
23 417 large rod-shaped slightly curved, diameter 0.3 μm , length 2.2 μm (Fig. 6C). Double cell wall,
24
25 418 cytoplasm transparent, nucleoid region filamentous.
26

27
28 419 Morphotype 2 rare. Bacteria rod-shaped, diameter 0.18 μm , length 1.2 μm (Fig. 6D). Double
29
30 420 cell wall, cytoplasm transparent, nucleoid region filamentous.
31

32 421 *Distribution:* Boreal species. Molecular species identity confirmed for specimens from France
33
34 422 (Roscoff). Live in low intertidal and subtidal zones up to 20 m depth, on rocks and kelps (Borojevic
35
36 423 et al., 1968).
37

38
39 424 *Reproduction:* The specimens collected in February 2017 in Roscoff contain oocytes at the early
40
41 425 stages of development.
42

43 426 *Remarks:* *Leucosolenia complicata* was one of the most **undoubted** species described in **the** XIX
44
45 427 century. According to our data, it shows stable internal characters and easily diagnosable external
46
47 428 features, *i.e.*, erect multiply oscular **tubes extending** from **the** small cormus. **The** species identity
48
49 429 and validity of *L. complicata* **are** strongly supported by our molecular data as well. It represents a
50
51 430 distinct monophyletic lineage on all phylogenetic trees, and *p*-distance values to other
52
53 431 *Leucosolenia* species are very high (more than 5% in LSU and 3.8% in SSU). Extensively studied
54
55 432 morphology allows clarifying the species diagnosis, which **varied** from author to author (Haeckel,
56
57 433 1872; Minchin, 1904; Jones, 1954; Rapp, 2015): small cormus, erect multiple oscular tubes, two
58
59
60

1
2 434 populations of diactines (curved lanceolate diactines and small trichoxea), parasagittal tri- and
3
4 435 tetractines with predominately longer unpaired **actines**, skeleton of tubes predominately formed
5
6 436 by tetractines. *Leucosolenia complicata* is easily differentiated from other *Leucosolenia* species
7
8 437 (*Leucosolenia variabilis*, *L. somesii* and others) in these traits. In addition, **the** mesohyl cell
9
10 438 composition of *L. complicata* is very poor **compared** to other studied *Leucosolenia* species: the
11
12 439 mesohyl contains only sclerocytes and amoebocytes (Table S2). **The** composition of symbiotic
13
14 440 bacteria (two morphotypes of rod-shaped bacteria) differs **in** *L. complicata* from *L. corallorrhiza*
15
16 441 and *L. variabilis* (Table S2).

17
18
19
20 442 Although the type material of this species is not available, if **it** ever existed, we studied
21
22 443 spicule slides from Minchin type collections (BMNH 1910.1.1.415a and BNMH
23
24 444 1910.1.1.435.Aa). They are listed as the type material of *L. complicata* in **the** BMNH collection.
25
26 445 These slides contain hand-**written** information on the corresponding paragraphs in Minchin (1904)
27
28 446 work with relevant collection information (slides nos. 1, 2; Minchin, 1904:372). Accordingly, both
29
30 447 slides appeared from Canon Normans's Collection. The specimen BMNH 1910.1.1.415a was
31
32 448 collected at Scarborough (**the North Sea**) by Bean and sent to Haeckel for examination. The
33
34 449 specimen BNMH 1910.1.1.435.Aa was collected at Guernsey **Islands (the English Channel)** by J.
35
36 450 Bowerbank and likely represents a syntype of *Ascandra contorta* (Bowerbank, 1866). According
37
38 451 to Minchin (1904), this slide contains an admixture of *L. complicata* spicules with *A. contorta*. All
39
40 452 this indicates that slides BMNH 1910.1.1.415a and BNMH 1910.1.1.435.Aa are not the type
41
42 453 material of *L. complicata*, and the label 'type' likely refers to the Minchin's type collection, **which**
43
44 454 **contained** most typical specimens. Since no type material exists, the designation of neotype is
45
46 455 needed once the material from the type locality (British Isles, **Devon coast**) becomes available for
47
48 456 the molecular study.

49
50
51
52
53
54 457 *Ascandra pinus* Haeckel, 1872 and *Leucosolenia fabricii* Schmidt, 1869 are regarded herein
55
56 458 as minor synonyms. *Ascandra pinus* lacks small trichoxeas, which **were most probably** overlooked
57
58 459 by Haeckel (Minchin, 1904), and in *Leucosolenia fabricii*, the skeleton is formed mostly by

1
2 460 triactines, which was considered intraspecific variation by many authors (Minchin, 1904; Burton,
3
4 461 1963; Rapp, 2015). However, our data shows that these characters may be regarded as diagnostic
5
6 462 interspecific features, **as shown for the** *L. variabilis* species complex (see below); therefore, both
7
8 463 **of** these species names should be taken into account for future research **on** European *Leucosolenia*.

10
11 464 Our data also **suggest the** absence of *L. complicata* in the White Sea. In works by Breitfuss
12
13 465 (1898a), three *Leucosolenia* species were found **at** different localities in the White Sea and
14
15 466 described under the names *Ascandra variabilis* **Haeckel, 1872**, *Ascandra contorta* (**Bowerbank,**
16
17 467 **1866**) and *Ascandra fabricii* (Schmidt, 1869). Minchin (1904) later considered the latter two
18
19 468 species *sensu* Breitfuss (1898a) as minor synonyms of *Leucosolenia complicata* due to external
20
21 469 morphological characters, while spicular characters were ignored in most cases. However,
22
23 470 *Ascandra contorta sensu* Breitfuss (1898a) possesses tri- and tetractines with short unpaired
24
25 471 actines, which **is most likely a** diagnostic feature for *L. variabilis*. Due to **the** absence of *L.*
26
27 472 *complicata* in our material from the White Sea and uncertainties in previous **research**, more
28
29 473 material is required from different localities in the White **and Barents** Seas to clarify the
30
31 474 distribution ranges of this species in Arctic waters.
32
33
34
35

475

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37
38 476 ***Leucosolenia corallorrhiza* (Haeckel, 1872)**

39
40 477 (Figs 7-10; Table 5)

41
42 478 **=*Ascortis corallorrhiza* Haeckel, 1872**

43
44 479 **=*Sycorrhiza corallorrhiza*, Haeckel, 1870**

45
46 480 **=*Auloplegma corallorrhiza* Haeckel, 1872**

47
48 481 **=*Leucosolenia* cf. *variabilis* (Alvizu *et al.*, 2018; Lavrov *et al.*, 2018).**

49
50 482 **=*Leucosolenia variabilis* (Lavrov & Ereskovsky, 2022; Lavrov *et al.*, 2022; Melnikov *et al.*,
51
52 483 2022).**

53
54 484 **=*Leucosolenia complicata* (Ereskovsky *et al.*, 2017a).**

55
56 485 ***Type material:*** Type material is not known.
57
58
59
60

1
2 486 *Type locality*: Haeckel based his description on one specimen from Norway and one from
3
4 487 Greenland, without designating the type material (Rapp, 2015).

5
6 488 *Material studied*: 177 specimens. Molecular data – 177 specimens, external morphology – 177
7
8 489 specimens, skeleton organisation – 2 specimens (WS11650, WS11653), spicules (SEM) – 5
9
10 490 specimens (WS11649, WS116450, WS11653, WS11657, WS11658), cytology (TEM) – 6
11
12 491 specimens (WS11631, WS11632, WS11634, WS11635, WS11636, WS11637) (Table S1).

13
14
15 492 *External morphology*: Cormus formed by basal reticulation of tubes, from which erect oscular
16
17 493 tubes and long diverticula arising. Sponge bear from one to multiple slightly curved oscular tubes
18
19 494 with or without short lateral diverticula in basal part. Oscular tubes gradually narrows to oscular
20
21 495 rim, possessing short spicular crown (Fig. 7A, B). Surface minutely hispid or echinate. Colouration
22
23 496 of living and preserved specimens grayish white (Fig. 7A).

24
25
26 497 *Spicules*: Diactines (Fig. 8A, B). Curved lanceolate diactines, mean length 179 μm , mean width 6
27
28 498 μm (Table 5). Slightly curved with lanceolate outer tip, variable in size, smooth or with few small
29
30 499 spines at lanceolate tip (Fig. 8B).

31
32
33 500 Triactines (Fig. 8D). T-shaped sagittal (mean angle 142.9°), unpaired actine usually shorter
34
35 501 than paired (mean length: 70.5 μm – unpaired, 82.7 μm – paired) (Table 5), rarely equal. Actines
36
37 502 equal in width (mean width: 6.5 μm – unpaired, 6.5 μm – paired) (Table 5).

38
39
40 503 Tetractines (Fig. 8C). T-shaped sagittal (mean angle 151.4°), unpaired actine shorter than
41
42 504 paired or equal (mean length: 68.8 μm – unpaired, 80.7 μm – paired, 22.9 μm – apical) (Table 5).
43
44 505 All actines equal in width (mean width: 5.6 μm – unpaired, 5.8 μm – paired, 5.5 μm – apical)
45
46 506 (Table 5). Apical actine curved and smooth.

47
48
49 507 *Skeleton*: Skeleton of oscular tubes predominantly formed by both tri- and tetractines, while in
50
51 508 cormus tubes tetractines rare (Fig. 7C, D). In oscular tubes, spicules constitute organized array
52
53 509 with their unpaired actines directed toward cormus and oriented more or less in parallel to
54
55 510 proximo-distal axis of oscular tube (Fig. 7C). In cormus tubes, spicule network appears completely
56
57 511 disordered (Fig. 7D). Both populations of diactines forming small oscular crown up to 60 μm and

1
2 512 cover tubes surface, orienting in different directions and extending outside by lance-shaped tip
3
4 513 (Fig. 7B).

5
6 514 *Cytology*: Body wall, 8.4-12 μm thick, three layers: exopinacoderm, loose mesohyl, choanoderm
7
8 515 (Fig. 9A, B; Table S2). Flat endopinacocytes located only in the distal part of oscular tube (oscular
9
10 516 ring) replacing choanocytes. Inhalant pores scattered throughout exopinacoderm, except the
11
12 517 oscular ring area.

13
14
15 518 Exopinacocytes non-flagellated T-shaped, rarely flat (Fig. 9C). External surface covered by
16
17 519 glycocalyx. Cell body (height 7-10.5 μm , width 4.3-5.5 μm), containing spherical to oval nucleus
18
19 520 (diameter 3.1 μm), submersed in mesohyl. Cytoplasm with specific spherical electron-dense
20
21 521 inclusions (0.2-0.4 μm diameter) (Fig. 9C).

22
23
24 522 Endopinacocytes non-flagellated flat cells, size 16.8 $\mu\text{m} \times 2.2 \mu\text{m}$ (Fig. 9D). External surface
25
26 523 covered by glycocalyx. Nucleus (2.1 $\mu\text{m} \times 1.6 \mu\text{m}$) spherical to oval with nucleolus. Cytoplasm
27
28 524 with specific spherical electron-dense inclusions (0.2-0.5 μm diameter) (Fig. 9D).

29
30
31 525 Choanocytes flagellated trapeziform or prismatic (height 8.2 μm , width 4.1 μm) (Fig. 9E).
32
33 526 Flagellum surrounded by collar of microvilli. Characteristic pyriform nucleus (2.6 $\mu\text{m} \times 4.1 \mu\text{m}$)
34
35 527 in apical position. Cytoplasm with phagosomes and small vacuoles. Choanocytes united by
36
37 528 specialized intercellular contacts similar to septate junctions, but has no basal membrane (Fig. 9E).

38
39
40 529 Porocytes tubular cylindrical (height 5.5 μm , width 4.2 μm), connecting external milieu with
41
42 530 choanocyte tube (Fig. 9F). Nucleus pyriform (diameter 3.1 μm), containing nucleolus. Cytoplasm
43
44 531 with phagosomes, small vacuoles and spherical electron-dense inclusions identical with inclusions
45
46 532 of exopinacocytes.

47
48
49 533 Sclerocytes amoeboid, size 8.7 $\mu\text{m} \times 3.5 \mu\text{m}$ (Fig. 10A). Nucleus usually oval or pear-shaped
50
51 534 (diameter 2.5 μm), containing single nucleolus. Well-developed Golgi apparatus and rough
52
53 535 endoplasmic reticulum. Cytoplasm usually with phagosomes and/or lysosomes. During spicules
54
55 536 secretion, sclerocytes form groups of three to six cells, connected by septate junctions (Fig. 10A).

56
57
58 537 Amoebocytes of different shape (from oval to amoeboid) without special inclusions, size 5.7
59
60

1
2 538 $\mu\text{m} \times 4.7 \mu\text{m}$ (Fig. 10B). Nucleus spherical (diameter $2.9 \mu\text{m}$), sometimes with nucleolus.

3
4 539 Granular cells oval, size $9 \mu\text{m} \times 5.5 \mu\text{m}$. Regularly distributed numerous cells, usually
5
6 540 located under choanocytes (Fig. 10C-E). Nucleus in peripheral position, spherical (diameter 2.5
7
8 541 μm). Cytoplasm with oval electron dense inclusions (size $0.9\text{-}2.7 \mu\text{m} \times 1.1\text{-}3.7 \mu\text{m}$) (Fig. 10E).
9
10 542 Inclusion content homogenous or granulated. Often found in stage of degradation, cytoplasm
11
12 543 completely filled with 2-4 large oval inclusions with highly osmiophilic granulated content (Fig.
13
14 544 10F).
15
16

17
18 545 Myocytes are fusiform cells, size $22 \mu\text{m} \times 2.7 \mu\text{m}$; distributes in the mesohyl mostly in the
19
20 546 oscular ring. Nucleus usually oval ($2.9 \mu\text{m} \times 1.6 \mu\text{m}$), without nucleolus (Fig. 10G). Cytoplasm
21
22 547 includes mitochondria, ribosomes, small vesicles and, most importantly, the presence of
23
24 548 cytoplasmic myofilaments of $19\text{-}12 \text{ nm}$ in diameter (Fig. 10G). Myofilaments form bundles (0.37-
25
26 549 $0.16 \mu\text{m}$ diameter) that located along the long axis of the cell.
27
28

29
30 550 One **morphotype** of bacterial symbionts in mesohyl. Bacteria numerous, rod-shaped with
31
32 551 double cell wall, diameter $0.3\text{-}0.33 \mu\text{m}$, length $3.0\text{-}5.6 \mu\text{m}$ (Fig. 10H). Nucleoid region electron-
33
34 552 dense with irregular network of filaments.
35

36
37 553 *Distribution*: Boreal-Arctic species. Molecular identity confirmed for Greenland and the White
38
39 554 Sea (Alvizu *et al.*, 2018). In the White Sea it is the most abundant species, inhabiting kelps and
40
41 555 hard substrates in low intertidal and subtidal zones up to $15\text{-}20 \text{ m}$ depth.
42

43
44 556 *Reproduction*: In the White Sea specimens collected in late October contained early oocytes,
45
46 557 specimens collected in January-February contained fully-developed larvae.
47

48
49 558 *Remarks*: In the White Sea, this species was initially identified as *Leucosolenia variabilis*, based
50
51 559 on its external morphology (Lavrov *et al.*, 2018). In addition, most of our sequences for this species
52
53 560 were identical to LSU and SSU sequences downloaded from the GenBank under the name *L.*
54
55 561 *variabilis*. Regarding morphology, **the** spicular characters of our specimens were different from
56
57 562 the original description of *L. variabilis* (Haeckel, 1872), but partly fit the description given in
58
59 563 Minchin (1904). The main differences relate to **diactine** morphology: in our specimens, there is a

1
2 564 single type of curved lanceolate diactines. In *L. variabilis sensu* Haeckel (1872), two diactine
3
4 565 populations were found: the first is small straight trichoxea, and the second is normal curved
5
6 566 lanceolate diactines. Minchin (1904) found connectivity in size among small and long diactines
7
8
9 567 and suggested they represented a single type of diactines, which was overlooked by Haeckel. Since
10
11 568 our specimens possess only a single diactine population, it might support Minchin's conclusions.
12
13 569 However, *Leucosolenia variabilis sensu* Minchin (1904) is a species complex since he designated
14
15 570 *Leucosolenia somesii* a junior synonym of *L. variabilis*, while morphological and molecular data
16
17 571 supported its identity as a distinct species (see below, see also van Soest *et al.*, 2007). Therefore,
18
19 572 the diagnosis provided by Minchin (1904) should not be taken into consideration.

20
21
22 573 To address the possible ontogenetic variation of diactines, we studied the type material *L.*
23
24 574 *variabilis* from the collection of BMNH (syntype BMNH-1910.1.1.421). The spicular characters
25
26 575 of this specimen perfectly fit the original description made by Haeckel (1872), with two diactine
27
28 576 types, tri- and tetractines of equal abundance and unpaired actine in tri- and tetractines always
29
30 577 shorter than paired ones. On the other hand, specimens in our material possess only a single
31
32 578 diactine type, and tetractines are rare. Therefore, the species from the White Sea is not *L. variabilis*,
33
34 579 despite its molecular similarity to specimens, placed in the GenBank under the name *L. variabilis*.

35
36 580 Another species, that is characterised by a single diactine type and short unpaired actines in
37
38 581 tri- and tetractines is *Leucosolenia corallorrhiza*, which was designated a valid species in the most
39
40 582 recent morphology-based revision of Greenland calcareous sponges (Rapp, 2015). Haeckel (1872)
41
42 583 described this species under the name *Ascortis corallorrhiza*, addressing a small proportion or
43
44 584 absence of tetractines, small and thick triactines with short unpaired actine. Diactines are curved
45
46 585 lance-shaped (Haeckel, 1872:74). This feature is characteristic of samples from the White Sea,
47
48 586 although in our specimens, some diactines bear small spines on their lance-shaped tips. These
49
50 587 spines are hardly visible with light microscopy and may be overlooked even during SEM studies.
51
52 588 Since we could not study the morphology of specimens whose sequences were obtained from
53
54 589 GenBank, and morphological data for those specimens are absent in the respective paper (Alvizu

1
2 590 *et al.*, 2018), we designate our specimens from the White Sea as *Leucosolenia corallorrhiza*, until
3
4 591 both morphological and molecular conformation for specimens from the type localities would be
5
6 592 available. Also, neotype designation for this species is necessary to establish the type material,
7
8 593 specimens for this purpose should be collected in the type locality. It should be mentioned, that
9
10 594 our specimens demonstrate minor differences in colouration from the original description (*L.*
11
12 595 *corallorrhiza* is brown according to Haeckel, 1872). Also, actines in tri- and tetractines are thicker
13
14 596 in initial description (widths ~ 15 µm in Haeckel, 1872; up to 12.5 µm in our material (Table 5);
15
16 597 up to 10.7 µm in Rapp, 2015), but this difference may be associated either with ontogenetic or
17
18 598 intraspecific variation, or different measurement procedures and equipment.

19
20 599 From *Leucosolenia variabilis* this species differs by spicular characters: in *L. variabilis*,
21
22 600 there are two types of diactine, while there is only one type of diactines in *L. corallorrhiza*.
23
24 601 *Leucosolenia corallorrhiza* never forms a large, massive cormus. *Leucosolenia corallorrhiza* also
25
26 602 differs from other species in cytological characteristics (Table S2): in contrast to *L. complicata*,
27
28 603 the mesohyl of *L. corallorrhiza* includes not only amoeboid cells but also numerous granular cells,
29
30 604 regularly distributed in the body wall; in contrast to *L. variabilis*, *L. corallorrhiza* has larger
31
32 605 granular cells, no spherulous cells and only one morphotype of rod-shaped symbiotic bacteria.

33
34 606 ***Leucosolenia variabilis* Haeckel, 1870**

35
36 607 (Figs 11-16; Table 6)

37
38 608 *Type material*: Syntype BMNH-1910.1.1.421. Other type material is not known.

39
40 609 *Type locality*: Norway, Bergen.

41
42 610 *Material studied*: 40 specimens. Molecular data – 40 specimens, external morphology – 40
43
44 611 specimens, skeleton organisation – 3 specimens (WS11643, WS11708, WS11735), spicules (light
45
46 612 microscopy, SEM) – 7 specimens (WS11707, WS11714, WS11731, WS11732, WS14637,
47
48 613 WS14671, WS14681), cytology (TEM) – 3 specimens (WS11643, WS11644, WS11645) (Table
49
50 614 S1).

1
2 615 *External morphology:* Length of cormus up to 5 cm. Cormus massive, often spherical, otherwise
3
4 616 formed by basal reticulation of tubes. Cormus built as reticulation around one or several largest
5
6 617 central tubes. Outline of cormus formed by numerous short diverticula. Largest tubes of cormus
7
8 618 always end with oscula. Main oscular tubes large prominent erect, bearing many small diverticula,
9
10 619 spreading to 2/3 of tubes length. Oscular tube gradually narrows to oscular rim, possessing short
11
12 620 spicular crown (Fig. 11A, B). In addition to main oscula on largest tubes of cormus, smaller oscular
13
14 621 tubes usually scattered all over the cormus. Surface minutely hispid. Colouration of living
15
16 622 specimens grayish white. Colouration of preserved specimens from grayish white to ochre (Fig.
17
18 623 11A).

19
20
21
22 624 *Spicules:* Diactines (Figs 12A, 13, 16A). Two populations: (1) Curved smooth lanceolate diactines
23
24 625 (Fig. 12A), mean length 306.7 μm , mean width 9.8 μm , (Table 6). Slightly curved, smooth, with
25
26 626 lanceolate outer tip, variable in length. (2) Trichoxeas (Fig. 13). Thin (mean width 0.9 μm) (Table
27
28 627 6), with numerous irregularly distributed spines (Fig. 13C), long, but usually represented by
29
30 628 fragments of variable length (up to 362.4 μm long) (Table 6).

31
32
33
34 629 Triactines (Figs 12B, C, 16A). Predominantly T-shaped sagittal (mean angle 138.5°),
35
36 630 unpaired actine variable in length: most frequently equal to paired actines, commonly shorter or
37
38 631 rarely longer than paired (mean length: 122.3 μm – unpaired, 128.0 μm – paired) (Table 6).
39
40 632 Abnormal triactines with one of paired actines undulated also common (Fig. 12C). Actines equal
41
42 633 in width (mean width: 8.1 μm – unpaired, 8.5 μm – paired) (Table 6).

43
44
45 634 Tetractines (Fig. 12D). Predominantly T-shaped sagittal (mean angle 142.2°), unpaired
46
47 635 actine variable in size: equal to, shorter or longer than paired actines (mean length: 147.6 μm –
48
49 636 unpaired, 143.0 μm – paired, 22.8 μm – apical) (Table 6). Unpaired actine usually slightly slender
50
51 637 than paired (mean width: 8.5 μm – unpaired, 9.1 μm – paired) (Table 6). Apical actine curved,
52
53 638 smooth and slender (mean width 5.9 μm) (Table 6).

54
55
56
57 639 *Skeleton:* Skeleton of both oscular and cormus tubes formed by dense net of tetractines and
58
59 640 triactines (Fig. 11C, D). In oscular tubes spicules constitute organized array with their unpaired

1
2 641 actines directed toward cormus and oriented more or less in parallel to proximo-distal axis of
3
4 642 oscular tube (Fig. 11C). In cormus tubes spicule array completely disordered (Fig. 11D). Diactines
5
6 643 form small oscular crown up to 100 μm (Fig. 11B) and cover tubes surface, orienting in different
7
8 644 directions and extending outside by lance-shaped tip.

9
10 645 *Cytology*: Body wall, 9-13.8 μm thick, three layers: exopinacoderm, loose mesohyl, choanoderm
11
12 646 (Fig. 14A, B; Table S2). Flat endopinacocytes located only in distal part of oscular tube (oscular
13
14 647 ring) replacing choanocytes. Inhalant pores scattered throughout exopinacoderm, except the
15
16 648 oscular ring area.

17
18 649 Exopinacocytes non-flagellated T-shaped, rarely flat (Fig. 14C). External surface covered
19
20 650 by glycocalyx. Cell body (height 6.3 μm , width 3.7 μm), containing spherical to oval nucleus
21
22 651 (diameter 2.7 μm), submersed in mesohyl. Cytoplasm with specific spherical electron-dense
23
24 652 inclusions (0.2-0.35 μm diameter) (Fig. 14C).

25
26 653 Endopinacocytes non-flagellated flat cells, size 16 $\mu\text{m} \times 2.8 \mu\text{m}$. External surface covered
27
28 654 by glycocalyx. Nucleus (3.2 $\mu\text{m} \times 2.3 \mu\text{m}$) spherical to oval with nucleolus. Cytoplasm without
29
30 655 specific inclusions (Fig. 14F).

31
32 656 Choanocytes flagellated trapeziform or prismatic (height 10.7 μm , width 4.1 μm) (Fig. 14D).
33
34 657 Flagellum surrounded by collar of microvilli. Characteristic pyriform nucleus (diameter 2.5 μm)
35
36 658 in apical position. Cytoplasm with phagosomes and small vacuoles (Fig. 14D).

37
38 659 Porocytes tubular cylindrical (height 2.5-4.7 μm , width 4.3-5 μm), connecting external
39
40 660 milieu with choanocyte tube (Fig. 14E). Nucleus spherical (diameter 2.7 μm), containing
41
42 661 nucleolus. Cytoplasm with spherical electron-dense inclusions identical with inclusions of
43
44 662 exopinacocytes (Fig. 14E).

45
46 663 Sclerocytes amoeboid, size 6 $\mu\text{m} \times 3.1 \mu\text{m}$ (Fig. 15G). Nucleus usually oval or pear-shaped
47
48 664 (diameter 2.2 μm), containing single nucleolus. Well-developed Golgi apparatus and rough
49
50 665 endoplasmic reticulum. Cytoplasm usually with phagosomes and/or lysosomes (Fig. 15G).

51
52 666 Amoebocytes of different shape (from oval to amoeboid) without special inclusions, size 3

1
2 667 $\mu\text{m} \times 4\text{-}7.5 \mu\text{m}$ (Fig. 15A). Nucleus spherical (diameter $2.7 \mu\text{m}$), sometimes with nucleolus.

3
4 668 Large amoeboid cells of different shape (from elongate to amoeboid), size $20 \mu\text{m} \times 4.2 \mu\text{m}$
5
6 669 (Fig. 15B). Rare cells located under choanoderm. Nucleus oval (size $4.8 \mu\text{m} \times 1.7 \mu\text{m}$). Cytoplasm
7
8 670 with numerous large heterophagosomes (diameter $1.1\text{-}3.2 \mu\text{m}$), well-developed Golgi apparatus
9
10 671 (Fig. 15B).

11
12 672 Granular cells small oval, size $4 \mu\text{m} \times 3.3 \mu\text{m}$ (Fig. 15C). Rare cell type, located under the
13
14 673 choanoderm. Nucleus in peripheral position, spherical (diameter $1.7 \mu\text{m}$) with large amounts of
15
16 674 heterochromatin, associated with nucleus membrane Cytoplasm with electron dense oval
17
18 675 inclusions (size $0.7\text{-}6 \mu\text{m} \times 0.4\text{-}1.1 \mu\text{m}$) and rare spherical electron-transparent vacuoles (diameter
19
20 676 $1.2 \mu\text{m}$) (Fig. 15C).

21
22 677 Spherulous cells with irregular shape from amoeboid to crescent, size $2.7\text{-}9.2 \mu\text{m} \times 4.7\text{-}5.3$
23
24 678 μm (Fig. 15E-G). Regularly distributed numerous cells, usually located under choanocytes.
25
26 679 Distance between cells $2\text{-}9 \mu\text{m}$ (Fig. 15F). Nucleus deformed (size $2.4 \mu\text{m} \times 1.7 \mu\text{m}$). Cytoplasm
27
28 680 mostly occupied by large crescent or irregular electron-dense homogenous inclusions (diameter
29
30 681 $1.8\text{-}4.5 \mu\text{m}$) and less electron-dense fine-granular inclusions (diameter $0.7\text{-}2.6 \mu\text{m}$). Granular or
31
32 682 foamy material fills cytoplasm spaces between inclusions (Fig. 15E, G).

33
34 683 Myocytes rare fusiform cells, size $18 \mu\text{m} \times 2.7 \mu\text{m}$, located in mesohyl (Fig. 15D). Nucleus
35
36 684 oval ($3.5 \mu\text{m} \times 2.7 \mu\text{m}$), with nucleolus. Cytoplasm with mitochondria, ribosomes, small vesicles
37
38 685 and cytoplasmic myofilaments. Myofilaments grouped in bundles (diameter $0.07\text{-}0.2 \mu\text{m}$) located
39
40 686 along long axis of myocyte (Fig. 15D).

41
42 687 Three morphotypes of bacterial symbionts in mesohyl (Fig. 15H-J). Morphotype 1 numerous
43
44 688 (Fig. 15H). Bacteria large spiral-shaped, diameter $0.2 \mu\text{m}$, length $2.5\text{-}3.9 \mu\text{m}$. Spiral turns regular
45
46 689 and compact. Single membrane cell wall, cytoplasm granular, nucleoid region tubular (Fig. 15H).

47
48 690 Morphotype 2 rare (Fig. 15I). Bacteria small spiral-shaped, diameter $0.3 \mu\text{m}$, length $1.5\text{-}1.8$
49
50 691 μm . Spiral turns irregular and sparse. Cytoplasm transparent, nucleoid region tubular (Fig. 15I).

51
52 692 Morphotype 3 rare (Fig. 15J). Bacteria small rod-shaped bacteria, diameter $0.23 \mu\text{m}$, length

1
2 693 0.8 μm . Double membrane cell wall, cytoplasm with dark filamentous materials, no distinction
3
4 694 between cytoplasm and nucleoid region (Fig. 15J).

5
6 695 *Distribution*: Boreal-Arctic species, described from Norway. Molecular identity confirmed for the
7
8 696 White Sea and Greenland (Alvizu *et al.*, 2018). In the White Sea occurs in low intertidal and
9
10 697 subtidal zones up to 40-45 m depth, on rocks and kelps.

11
12
13 698 *Reproduction*: No data about reproduction time for this species.

14
15 699 *Remarks*: We studied three type specimens (slides with spicules) of *Leucosolenia variabilis* from
16
17 700 the British Museum of Natural History (BMNH): BMNH-1910.1.1.421, BMNH-1906.12.1.40 and
18
19 701 BMNH-1906.12.1.50. Spicules are similar morphologically across these specimens (Fig. 16B-D),
20
21 702 which supports the idea that they belong to the same species. At the same time, their type status
22
23 703 should be reconsidered due to the data represented in the revision by Minchin (1904). Slide labels
24
25 704 contain specific information (exact page and number), allowing an unambiguous comparison with
26
27 705 the collection data of these samples given in Minchin (1904). Accordingly, BMNH-1906.12.1.50
28
29 706 was collected from Bantry Bay, Ireland, by C. Norman and identified by him as *Leucosolenia*
30
31 707 *botryoides*; this label was endorsed by Haeckel '*Ascandra variabilis*' (slide no. 1; Minchin,
32
33 708 1904:385). BMNH-1906.12.1.40 was received by Haeckel for reexamination from Bowerbank and
34
35 709 collected from Guernsey (slide no. 4; Minchin, 1904:385). Finally, BMNH-1910.1.1.421 was
36
37 710 collected by Haeckel in Bergen, Norway, the type locality of this species, and contained a printed
38
39 711 label '*Ascandra variabilis* H.' (slide no. 3; Minchin, 1904:385). Therefore, the slide BMNH-
40
41 712 1910.1.1.421 could be designated as a syntype.

42
43 713 The analysis of *L. variabilis* syntype BMNH-1910.1.1.421 indicated two diactine types
44
45 714 (lanceolate diactines and trichoxeas), and V- and T-shaped tri- and tetractines with shorter
46
47 715 unpaired actines (Fig. 16D). Although Haeckel's description lacks long trichoxeas, it should be
48
49 716 mentioned that such spicules are easily broken during preparation. It may also be suggested that
50
51 717 the second type of diactines without lanceolate tips described by Haeckel (1872) is in fact broken
52
53
54
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59
60

1
2 718 long trichoxeas. Direct comparison of spicule slides of specimens from the White Sea with *L.*
3
4 719 *variabilis* syntype BMNH-1910.1.1.421 shows strong correspondence between them.

5
6 720 *Leucosolenia variabilis* has a large, massive, sometimes spherical cormus, which could be a
7
8 721 good distinctive trait, since all other sympatrically living species (*Leucosolenia complicata*, *L.*
9 722 *corallorrhiza*, *Leucosolenia* sp. A) are represented by basal reticulation of tubes with extended
10
11 723 oscular tubes. In spicular characters, *L. variabilis* differs from *L. somesii* by the presence of
12
13 724 lanceolate spined diactines; and from *L. complicata* and *Leucosolenia* sp. A by the presence of
14
15 725 extremely long and highly spined trichoxeas. *Leucosolenia variabilis* also has the highest diversity
16
17 726 of mesohyl cells and symbiotic bacteria among the studied *Leucosolenia* species (Table S2). In
18
19 727 addition to the usual amoebocytes, *L. variabilis* also has rare large amoebocytes and small granular
20
21 728 cells, as well as numerous unusual spherulous cells of different shapes regularly distributed in the
22
23 729 body wall. The composition of symbiotic bacteria of *L. variabilis* includes three morphotypes: one
24
25 730 typical rod-shaped and two unusual spiral-shaped.

26
27
28
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32 731

33 732 *Leucosolenia* sp. A

34
35 733 (Figs 17-18; Table 7)

36
37 734 *Material studied:* Three specimens. Molecular data – 3 specimens (WS11692, WS11752,
38
39 735 WS11770), external morphology – 3 specimens (WS11692, WS11752, WS11770), skeleton
40
41 736 organisation – 2 specimens (WS11752, WS11770), spicules (SEM) – 2 specimens (WS11692,
42
43 737 WS11770) (Table S1).

44
45 738 *External morphology:* Studied specimens small. Length of cormus up to 1 cm. Cormus represented
46
47 739 by compact reticulation of tubes, from which several oscular tubes arising. Ocular tubes erect and
48
49 740 almost straight. Surface minutely hispid. Colouration of living and preserved specimens grayish
50
51 741 white (Fig. 17A).

52
53 742 *Spicules:* Diactines (Fig. 18A-C). Two populations: (1) Curved spiny lanceolate diactines (Fig.
54
55 743 18B), mean length 189.1 μm , mean width 7.2 μm (Table 7). Small, from almost straight to slightly

1
2 744 curved and undulating, with lanceolate and spiny outer tip, spines in distinct rows (Fig. 18C). (2)
3
4 745 Curved smooth diactines (Fig. 18A), mean length 515.0 μm , mean width 11.6 μm (Table 7). **Rare,**
5
6 746 **long,** slightly curved, without spines and lanceolate tips (Fig. 18C).
7
8
9 747 Triactines (Fig. 18D, E). Predominantly T-shaped sagittal (mean angle 146.5°) (Table 7).
10
11 748 Unpaired actine variable in size: equal to, shorter or longer than paired actines, but shorter unpaired
12
13 749 actine most common (mean length: 118.5 μm – unpaired, 125.1 μm – paired) (Table 7). Both
14
15 750 straight and bent paired actines common. Abnormal triactines in high number (Fig. 18E),
16
17 751 sometimes with undulated actines. Unpaired actine usually slightly slender than paired (mean
18
19 752 width: 11.1 μm – unpaired, 11.6 μm – paired) (Table 7).
20
21
22 753 Tetractines (Fig. 18F). Quite rare. Predominantly T-shaped (mean angle 140.8°) (Table 7),
23
24 754 variable in size. Unpaired actine equal to paired ones (mean length: 114.3 μm – unpaired, 113.2
25
26 755 μm – paired, 30.0 μm – apical) (Table 7). Unpaired actine straight, paired actines straight or
27
28 756 undulating, apical actine curved or undulating, smooth. Paired and unpaired actines equal in width,
29
30 757 apical actine more slender (mean width: 8.6 μm – unpaired, 8.5 μm – paired, 7.1 μm – apical)
31
32 758 (Table 7).
33
34
35 759 *Skeleton:* Skeleton of both oscular rim and cormus tubes predominantly formed by triactines,
36
37 760 tetractines rare (Fig. 17C, D). In oscular tubes spicules constitute organized array with their
38
39 761 unpaired actines directed toward cormus and oriented more or less in parallel to proximo-distal
40
41 762 axis of oscular tube (Fig. 17C). In cormus tubes spicule array completely disordered (Fig. 17D).
42
43 763 Prominent oscular crown absent (Fig. 17B). Both populations of diactines cover tubes surface,
44
45 764 orienting in different directions and extending outside.
46
47 765 *Cytology:* No material was available for cytological studies.
48
49
50 766 *Distribution:* Arctic species. Molecular identity confirmed only for the White Sea and Greenland.
51
52 767 Found subtidal up to 15 m on rocks and red algae.
53
54
55 768 *Reproduction:* No data about reproduction time for this species.
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1
2 769 *Remarks:* Although both our species delimitation analysis based on the H3 dataset and
3
4 770 morphological data suggest that this species represents a distinct species-level unit, we avoid
5
6 771 **describing** a new species as this case requires additional studies for several reasons. *Leucosolenia*
7
8 772 sp. shares some features with *Leucosolenia corallorrhiza*: (1) **the** external appearance is similar,
9
10 773 (2) **the** angle between unpaired actines in tri- and tetractines is similar (**the** mean angle is 142.9°
11
12 774 in *L. corallorrhiza* and 146.5° in *Leucosolenia* sp. A), (3) **the** unpaired actine in tri- and tetractines
13
14 775 is commonly shorter **than** the unpaired ones. However, these two species show several differences.
15
16 776 Firstly, *Leucosolenia* sp. A has two populations of diactines, the smaller with lanceolate tips and
17
18 777 **the rare** large curved smooth non-lanceolate diactines, whereas in *L. corallorrhiza* only the first
19
20 778 type is present. Also, the tetractines are rare in both **the** cormus and oscular **regions of** *Leucosolenia*
21
22 779 sp. A, while they are commonly present in **the** osculum of *L. corallorrhiza*. *Leucosolenia* sp. A
23
24 780 commonly has triactines with bent unpaired actines, which are straight in *L. corallorrhiza*. Finally,
25
26 781 the mean length of actines in tri- and tetractines of *L. corallorrhiza* is shorter than those of
27
28 782 *Leucosolenia* sp. A (*L. corallorrhiza*: 70.5 µm – unpaired actine mean length, 82.7 µm – paired
29
30 783 actine mean length; *Leucosolenia* sp. A: 118.5 µm – unpaired mean length, 125.1 µm – paired
31
32 784 mean length). At the same time, **the** limited material of *Leucosolenia* sp. A (only three specimens
33
34 785 were collected and studied) does not allow us to study the possible interspecific variation and
35
36 786 ontogenetic variation. Therefore, we avoid **the** designation of this species as a distinct one, until
37
38 787 more material would be available for study.
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48 789 ***Leucosolenia creepae* sp. nov.**

49 790 (Figs 19-22; Table 8)

50 791 ZooBank LSID: [urn:lsid:zoobank.org:act:D60461BE-F215-4BE2-AFB5-25AF542FC4B9](https://zoobank.org/urn:lsid:zoobank.org:act:D60461BE-F215-4BE2-AFB5-25AF542FC4B9)

51 792 *Type material:* *Holotype:* WS11702, White Sea, Kandalaksha Bay, Velikaya Salma Strait, vicinity
52
53 793 of the N.A. Pertsov White Sea Biological Station, 0-2 m depth, 28.viii.2018, coll. A.I. Lavrov.

54 794 *Paratypes:* WS11703, 1 specimen, White Sea, Kandalaksha Bay, Velikaya Salma Strait, vicinity

1
2 795 of the N.A. Pertsov White Sea Biological Station, 0-2 m depth, 28.viii.2018, coll. A.I. Lavrov.
3
4 796 **WS11728 paratype agrees in locality, date and collector with holotype WS11702 and paratype**
5
6 797 **WS11703. WS11655 was collected in 30.viii.2017, and WS11725, WS11726, WS11771 were**
7
8 798 **collected in 24.viii.2018, but all agree in locality and collector with holotype WS11702 and**
9
10
11 799 **paratype WS11703**

12
13 800 *Type locality:* White Sea, Kandalaksha Bay, Velikaya Salma Strait, vicinity of the N.A. Pertsov
14
15 801 White Sea Biological Station (66°34'N, 33°08'E).

16
17 802 *Material studied:* 54 specimens. **Molecular data – 54 specimens, external morphology – 54**
18
19 803 **specimens, skeleton organisation – 3 specimens (WS11655, WS11728, WS11762), spicules**
20
21 804 **(SEM) – 5 specimens (WS11579, WS11605, WS11704, WS11729, WS11775), cytology (TEM)**
22
23 805 **– 3 specimens (WS11579, WS11600, WS11698) (Table S1).**

24
25 806 *Etymology:* From English “creep”, referring to specific decumbent cormus and unusual **growth**
26
27 807 **form** of this species in contrast to sympatrically living *Leucosolenia corallorrhiza*.

28
29 808 *External morphology:* Length of cormus up to 5 cm. Cormus formed by basal reticulations of
30
31 809 creepy tubes with one or several oscular tubes (Fig. 19A). Tubes brittle. Ocular tubes creepy
32
33 810 slightly curved and erect at distal end, sometimes with few diverticula. Ocular rim gradually
34
35 811 narrows, possessing prominent spicular crown (Fig. 19A). Surface echinate. Colouration of living
36
37 812 and preserved specimens grayish white (Fig. 19A).

38
39 813 *Spicules:* Diactines (Fig. 20A, B). Spiny diactines, mean length 194.9 µm, mean width 5.1 µm
40
41 814 (Table 8). Extremely variable in length, without lanceolate tips, spiny. Largest diactines slightly
42
43 815 curved, intermediate and short diactines straight. Spines in distinct rows at one end of diactines,
44
45 816 more or less reduced in large ones (Fig. 20B).

46
47 817 Triactines (Fig. 20C, D). Sagittal, T-shaped and V-shaped (mean angle 131.1°) (Table 8),
48
49 818 usually recurved, unpaired actine variable in length: most frequently shorter than paired actines,
50
51 819 but equal and longer unpaired actines rarely occur (mean length: 80.7 µm – unpaired, 94.9 µm –
52
53 820 paired) (Table 8). Aberrant T- and V-shaped triactines present, sometimes with undulated rays

821 (Fig. 20D). Unpaired actine often more slender than paired actines (mean width: 5.4 μm –
822 unpaired, 5.9 μm – paired) (Table 8).

823 Tetractines (Fig. 20E). Quite rare. Sagittal, T-shaped and V-shaped (mean angle 139.5°)
824 (Table 8), variable in size and proportions. Unpaired actine variable in length: longer, shorter and
825 equal to unpaired actines (mean length: 85.1 μm – unpaired, 95.3 μm – paired, 25.6 μm – apical)
826 (Table 8). Paired and unpaired actines equal in width (mean width: 6.2 μm – unpaired, 6.3 μm –
827 paired) (Table 8). Apical actine curved, smooth and slender (mean width 5.2 μm) (Table 8).

828 *Skeleton*: Skeleton of oscular rim predominantly formed by both tri- and tetractines, while in other
829 body parts tetractines absent (Fig. 19C, D). In oscular tubes spicules constitute organized array
830 with their unpaired actines directed toward cormus and oriented more or less in parallel to
831 proximo-distal axis of oscular tube (Fig. 19C). In cormus tubes spicule array completely
832 disordered (Fig. 19D). Diactines forms extending oscular crown up to 500 μm (Fig. 19B) and
833 cover tubes surface in large number, orienting in different directions and making it hispid.

834 *Cytology*: Body wall, 9-14 μm thick, three layers: exopinacoderm, loose mesohyl, choanoderm
835 (Fig. 21A, B; Table S2). Flat endopinacocytes located only in distal part of oscular tube (oscular
836 ring) replacing choanocytes. Inhalant pores scattered throughout exopinacoderm, except the
837 oscular ring area.

838 Exopinacocytes non-flagellated T-shaped, rarely flat (Fig. 21C). External surface covered
839 by glycocalyx. Cell body (height 5.8 μm , width 2.8 μm), containing spherical to oval nucleus
840 (diameter 2.7 μm), submersed in mesohyl. Cytoplasm with specific spherical electron-dense
841 inclusions (0.25-0.35 μm diameter) (Fig. 21C).

842 Endopinacocytes non-flagellated flat cells, size 16.2 $\mu\text{m} \times 2.7 \mu\text{m}$ (Fig. 21D). External
843 surface covered by glycocalyx. Nucleus (diameter 2.7 μm) spherical without nucleolus. Cytoplasm
844 without specific inclusions (Fig. 21D).

845 Choanocytes flagellated trapeziform or prismatic (height 11.4 μm , width 3.6 μm) (Fig. 21E).
846 Flagellum surrounded by collar of microvilli. Characteristic pyriform nucleus (diameter 2.3 μm)

1
2 847 in apical position. Cytoplasm with phagosomes and small vacuoles (Fig. 21E).

3
4 848 Porocytes tubular cylindrical (height 4.6-8.9 μm , width 2.8-2.9 μm), connecting external
5
6 849 milieu with choanocyte tube (Fig. 21F). Nucleus oval to spherical (diameter 2.5 μm), sometime
7
8 850 with nucleolus. Cytoplasm with spherical electron-dense inclusions identical with inclusions of
9
10 851 exopinacocytes, phagosomes and small vacuoles (Fig. 21F).

11
12
13 852 Sclerocytes amoeboid, size 7.6 $\mu\text{m} \times 2.9 \mu\text{m}$ (Fig. 22A). Nucleus usually oval or pear-shaped
14
15 853 (diameter 2.3 μm), sometimes with single nucleolus. Well-developed Golgi apparatus and rough
16
17 854 endoplasmic reticulum. Cytoplasm usually with phagosomes and/or lysosomes (Fig. 22A).

18
19
20 855 Amoebocytes of different shape (from oval to amoeboid) without special inclusions, size 5.7
21
22 856 $\mu\text{m} \times 2.6 \mu\text{m}$ (Fig. 22B). Nucleus spherical (diameter 2.5 μm), sometimes with nucleolus.

23
24 857 Myocytes fusiform cells, size 16.5 $\mu\text{m} \times 3.3 \mu\text{m}$, located in mesohyl. Nucleus oval (2.4 μm
25
26 858 $\times 1.9 \mu\text{m}$), without nucleolus (Fig. 21D). Cytoplasm with mitochondria, ribosomes, small vesicles
27
28 859 and cytoplasmic myofilaments. Myofilaments grouped in bundles (diameter 0.25–0.32 μm) that
29
30 860 are located along the long axis of the cell (Fig. 21D).

31
32
33
34 861 Two morphotypes of bacterial symbionts in mesohyl. Morphotype 1 numerous (Fig. 22C,
35
36 862 D). Bacteria large rod-shaped slightly curved, diameter 0.4-0.5 μm , length 2.7 μm . Cell is double,
37
38 863 smooth and covered with fibers, cytoplasm transparent with vacuolar inclusions, nucleoid region
39
40 864 filamentous (Fig. 22C, D).

41
42
43 865 Morphotype 2 abundant (Fig. 22E, F). Bacteria small rod-shaped bacteria, diameter 0.19 μm ,
44
45 866 length 1.9-2.1 μm . Cell wall smooth, cytoplasm transparent, nucleoid region filamentous (Fig.
46
47 867 22E, F).

48
49
50 868 *Distribution:* Arctic species. In the White Sea quite rare, found in low intertidal and upper subtidal
51
52 869 zones up to 5-10 m depth, on kelps and rocks.

53
54 870 *Reproduction:* In White Sea specimens, collected in mid-June to the end of the August contained
55
56 871 oocytes at different stages (mostly, early) of development (Figs 21B, 22G).

1
2 872 *Remarks: Leucosolenia creepae* sp. nov. well differs from other *Leucosolenia* species in both
3
4 873 external characters and the morphology of spicules. In *L. somesii*, diactines are of two types: (1)
5
6 874 smooth diactines, which are variable in length and (2) short and highly spined ones. In
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8
9 875 *Leucosolenia creepae* sp. nov., we identified only one type of diactines, which have spines on the
10
11 876 outer tip and are variable in length. However, in *Leucosolenia creepae* sp. nov., spines are more
12
13 877 expressed in small and medium-size diactines but become hardly visible in longer diactines of
14
15 878 ~250-300 µm in length. In *L. somesii*, all medium-size diactines have smooth tips (Fig. 24B).
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17
18 879 *Leucosolenia creepae* sp. nov. forms a sparse basal reticulation with few oscular tubes, while the
19
20 880 cormus of *L. somesii* is formed by a dense reticulation of extremely branched winding tubes. From
21
22 881 all other North Atlantic and Arctic *Leucosolenia* species, *Leucosolenia creepae* sp. nov. differs by
23
24 882 the absence of lanceolate diactines. The mesohyl cell composition of *Leucosolenia creepae* sp.
25
26 883 nov. includes only amoebocytes, myocytes and sclerocytes, differing this species from
27
28 884 sympatrically living *L. corallorrhiza* and *L. variabilis* (Table S2).
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34 886 ***Leucosolenia somesii* (Bowerbank, 1874)**

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36 887 (Figs 23-24; Table 9)

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38
39 888 *Type material:* Lectotype BMNH 1925.11.2.24, paralectotype BMNH 1925.11.2.25, slides of the
40
41 889 same: BMNH 1956.4.26.35.

42
43 890 *Type locality:* Brighton Aquarium.

44
45 891 *Material studied:* 1 specimen, ZMA Por. 17572 (external morphology, skeleton organisation,
46
47 892 spicules) (Table S1).

48
49
50 893 *External morphology:* Length up to 12 cm. Cormus formed by dense reticulation of extremely
51
52 894 branched winding tubes (Fig. 23A). Surface hispid. Colouration of living and preserved specimens
53
54 895 grayish white. Examined specimen lacks oscular tubes. According to the original description
55
56 896 (Bowerbank, 1874), sponge have numerous small and large oscular tubes, bearing spicular crown.
57
58 897 Oscular tubes erect and slightly curved, gradually narrowing to oscular rim.
59
60

898 *Spicules*: Diactines (Fig. 24A-C). Two populations of diactines: (1) Curved smooth diactines (Fig.
899 24A, C), mean length 425.5 μm , mean width 9.9 μm (Table 9). Slightly curved, smooth, variable
900 in length, lacking lanceolate tips, with undulated tip. (2) Straight spiny diactines (Fig. 24B, C),
901 mean length 90.0 μm , mean width 3.3 μm (Table 9). Short, straight, lacking lanceolate tips, with
902 numerous spines in distinct rows (Fig. 24B).

903 Triactines (Fig. 24D, E). Sagittal, mostly T-shaped, but V-shaped also occurs (mean angle
904 131.7°) (Table 9), unpaired actine usually shorter than paired actines, but longer unpaired actines
905 rarely occur (mean length: 127.4 μm – unpaired, 155.2 μm – paired) (Table 9). Paired and unpaired
906 actines equal in width (mean width: 8.2 μm – unpaired, 8.0 μm – paired) (Table 9). Abnormal
907 triactines in common, sometimes with undulated rays (Fig. 24E).

908 Tetractines (Fig. 24F). Quite rare. Sagittal, mostly T-shaped, but V-shaped also occurs
909 (mean angle 139.3°) (Table 9), unpaired actine usually shorter than paired actines, but longer
910 unpaired actines rarely occur (mean length: 156.0 μm – unpaired, 178.7 μm – paired, 21.5 μm –
911 apical) (Table 9). Apical actine curved and smooth. All actines more or less equal in width (mean
912 width: 9.1 μm – unpaired, 9.5 μm – paired, 10.0 μm – apical) (Table 9).

913 *Skeleton*: Very dense net predominantly formed by triactines, oriented in different directions,
914 tetractines rare (Fig. 23B). Both trichoxea populations cover tubes surface in large number,
915 orienting in different directions and making it hispid. Skeleton of osculum was not studied.

916 *Cytology*: No material was available for cytological studies.

917 *Distribution*: Boreal species. Described from Brighton Aquarium with confirmed reports from
918 Netherlands (van Soest *et al.*, 2007). Probably it has wider distribution in North-East Atlantic.

919 *Reproduction*: No data on reproduction time are available.

920 *Remarks*: *Leucosolenia somesii* was considered a minor synonym of *L. variabilis* until a recent
921 study by van Soest *et al.* (2007) was published. They showed valuable differences between these
922 two species, based on a large number of specimens, including the type material. Here we provide
923 its first molecular data and an updated morphological description. Our novel data confirms that *L.*

1
2 924 *somesii* represents a distinct species, based on both morphological and molecular analyses. The
3
4 925 reexamination of spicules of specimen ZMA Por. 17572 studied by van Soest *et al.* (2007)
5
6 926 confirms **the** strong correspondence of its specular characteristics to the paralectotype BMNH
7
8 927 1956.4.26.35 (Fig. 23C, D). According to our phylogenetic reconstruction, the most **closely related**
9
10
11 928 species is Arctic *Leucosolenia creepae* sp. nov., with which *Leucosolenia somesii* shares some
12
13 929 specific morphological features: echinate external appearance due to the high number of non-
14
15 930 lanceolate diactines protruding to the external surface, **dimensions of tri- and tetractines**. The
16
17 931 discussion of their differences is given above under *Leucosolenia creepae* sp. nov. description.
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19 932 From all other North Atlantic and Arctic *Leucosolenia* species, *L. somesii* differs **by the** absence
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21 933 of lanceolate diactines.
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934 Discussion

935 *Leucosolenia* taxonomy

936 The calcarean biodiversity in the Arctic region remains poorly studied. In the beginning of
937 the XX century, only three asconoid species were detected in the White and Barents Seas
938 (Breitfuss, 1898a): *Ascandra variabilis*, *A. contorta* and *A. fabricii*. This view was even more
939 simplified in subsequent works, designating that only *Leucosolenia complicata* occurs in these
940 seas (Koltun, 1952). The present study demonstrates that *L. complicata* is restricted to the North-
941 East Atlantic, while in the Arctic, the *Leucosolenia* diversity is represented by at least four species:
942 *Leucosolenia corallorrhiza*, *L. variabilis*, *Leucosolenia creepae* sp. nov. and yet undescribed
943 *Leucosolenia* sp. A. These species represent different clades in the present phylogenetic analysis
944 (Fig. 1): (1) Clade I, which includes the nominative species, *L. corallorrhiza*, *Leucosolenia* sp. A
945 and several phylogenetically distinct lineages formed by Norway and Greenland specimens
946 (*Leucosolenia* sp. B–D), (2) Clade II with the closely related North-Atlantic *L. somesii* and
947 *Leucosolenia creepae* sp. nov. restricted to the White Sea. *Leucosolenia botryooides*, the type
948 species of the genus, shows sister relationships with the latter group, and *L. complicata* is sister to
949 this clade. The phylogenetic signal from different molecular markers gave a similar result (Data
950 S1), supporting the chosen species hypothesis from the concatenated phylogenetic dataset (Figs 1,
951 S1, S2), and the lack of heterozygous sites in the studied markers indicates the absence of
952 hybridization between different species and supports well-established species boundaries.

953 Overall, species within each group show several common characters, which correlate with
954 the level of molecular divergence. Most of the traits traditionally used in calcarean systematics
955 (the general skeleton composition, the spicular set and their fine morphology) supported the
956 species hypothesis obtained from the molecular phylogenetic analysis. Spicular set is the most
957 useful character to delimit species, and general spicular composition and proportions of spicules
958 may also have a certain phylogenetic signal: (i) Lanceolate diactines with or without spines are
959 found within the Clade I (Fig. 1) and in *L. complicata*; (ii) T-shaped tri- and tetractines with shorter

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2 960 unpaired actines are common only in species from the Clade I; (iii) sister species *L. somesii* and
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4 961 *L. creepae* sp. nov. bear generally thinner spicules than other species of the genus; these species
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6 962 also have an echinate appearance due to the high number of long diactines protruding through the
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8
9 963 surface.

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11 964 Here we also show that modern techniques like scanning electron microscopy may give a
12
13 965 new insight into understanding actual biodiversity, as in closely related species, similar spicular
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15 966 types differ by fine features like the presence or absence of spines. This micromorphological
16
17 967 approach has been successfully applied in the taxonomy of calcareous sponges from subclass the
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19 968 *Calcinea* (Azevedo *et al.*, 2009, 2015; Klautau *et al.*, 2016). For example, although both
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21 969 *Leucosolenia creepae* sp. nov. and *L. somesii* have similar specular sets, the latter species has two
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23 970 populations of non-lanceolate diactines (spined and smooth), while only a single population of
24
25 971 spined diactines with a continuous reduction of spines in larger diactines is found in *Leucosolenia*
26
27 972 *creepae* sp. nov. The same was shown for *L. variabilis* and *L. corallorrhiza*: both species have
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29 973 lanceolate and sometimes spined diactines, but in *L. variabilis* we also detected thin long and
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31 974 highly spined trichoxeas. These differences in diactine types are obvious with the help of SEM
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33 975 techniques but may be overlooked during investigation using light microscopy alone.

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39 976 The proportion of spicular types in different parts of cormus and the form of actines in tri-
40
41 977 and tetractines (straight vs. bent) may also be important taxonomical characters. Haeckel (1872)
42
43 978 recognised several species as distinct based on these differences. For example, *Ascandra fabricii*
44
45 979 differs from *L. complicata* by the absence of tetractines, and *Asculmis armata* Haeckel, 1870 – by
46
47 980 a low amount of triactines. Furthermore, Haeckel described two varieties of *L. complicata*: *L.*
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49 981 *complicata* var. *hispida* with straight paired actines and *L. complicata* var. *ameboides* in which
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51 982 paired actines are bent. Also, some varieties – *cervicornis*, *confervicola*, *arachnoides*, *hispidissima*
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53 983 – were distinguished for *L. variabilis* (Haeckel, 1872). Although these names are currently
54
55 984 accepted as valid species within *Leucosolenia* (de Voogd *et al.*, 2023), these forms were for a long
56
57 985 time designated as morphotypes of either *L. complicata* or *L. variabilis* (Minchin, 1904; Burton,

1
2 986 1963) and no integrative study of Norway species **has** been conducted yet to support or reject their
3
4 987 validity. At the same time, our results show that *Leucosolenia* sp. A differs from *L. corallorrhiza*
5
6 988 by the low amount of tetractines and by bent paired actines in triactines. Since we suspect that
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8
9 989 *Leucosolenia* sp. A also represents a distinct species, all **the** above-mentioned characters should
10
11 990 be taken into account for further taxonomical revisions of the North Atlantic and Arctic
12
13 991 *Leucosolenia*.

14
15 992 In addition to the proportion of spicules in different parts of a skeleton, we identified
16
17 993 several differences in the spiculation of **the** oscular crown among different species. The oscular
18
19 994 rim bears numerous diactines protruding **through** the surface and forming a crown. The crown may
20
21 995 be short (max length 50-100 μm , in most species) or extremely long (up to 500 μm , in
22
23 996 *Leucosolenia creepae* sp. nov.). This character was not tracked in previous works on *Leucosolenia*
24
25 997 systematics, as researchers commonly pointed **at the** smooth or echinate surface of the oscular tube
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27 998 **without** considering the spiculation of **the** oscular rim. The only exception is a recent description
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29 999 of *Leucosolenia salpinx* van Soest, 2017 as in this species the extended, long oscular crown is a
30
31 1000 notable diagnostic trait (van Soest, 2017). At the same time, forms with more or less echinate
32
33 1001 surface **may be** found in *L. corallorrhiza* in the White Sea, making its external appearance similar
34
35 1002 to that **of** sympatrically occurring *Leucosolenia creepae* sp. nov. In this case, the oscular rim
36
37 1003 spiculation is more useful **for accurate** identification of these species in the field. However, the
38
39 1004 phylogenetic value of this character is a subject for further studies, as **the current** results lack data
40
41 1005 on *L. somesii*, *L. botryoides* and *Leucosolenia* sp. A.

42
43 1006 Another source of species-specific traits is **the** cytological structure of **the** studied
44
45 1007 *Leucosolenia* species. It is well known that cytological characters, **such as the** cell types with
46
47 1008 inclusions, are very important for Demospongiae and Homoscleromorpha species without skeleton
48
49 1009 identification (*e.g.*, Muricy *et al.*, 1996; Ereskovsky *et al.*, 2011, 2017b; Gazave *et al.*, 2013;
50
51 1010 Willenz *et al.*, 2016). Despite **the fact**, that the set of cell types is **generally** similar among studied
52
53 1011 *Leucosolenia* species, some species have characteristic cytological features. Both *L. corallorrhiza*
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1
2 1012 and *L. variabilis* have unique types of mesohyl cells - cells with inclusions (granular and/or
3
4 1013 spherulous) (Table S2), while *L. complicata* and *Leucosolenia creepae* sp. nov. lack such cells.
5
6 1014 **There are** no cytological data for *L. somesii* and *Leucosolenia* sp. **A.**, but it is reasonable to assume
7
8 1015 that considering the phylogenetic position of these species, *Leucosolenia* sp. **A.** should have cells
9
10 1016 with inclusions and *L. somesii* should not. We have for the first time clearly shown the presence
11
12 1017 of myocytes in all species studied, as well as the presence of endopinacocytes in the oscular ring.
13
14 1018 However, further broad studies of cytology in the genus *Leucosolenia* **are** required to evaluate the
15
16 1019 phylogenetic value of these characters.

17
18 1020 **The** composition of symbiotic bacteria also shows variation among studied species, both
19
20 1021 in **the** number of **bacterial** morphotypes and their morphology (Table S2). The differences in
21
22 1022 composition of symbiotic bacteria are obvious even in the case of closely related sympatric
23
24 1023 species, *e.g.*, *L. corallorrhiza* and *L. variabilis*. Considering the stability of the core microbiome
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26 1024 for a particular sponge species across **various localities** and different environmental conditions
27
28 1025 (Webster & Thomas, 2016), it **could** become a useful character for delimiting calcareous sponge
29
30 1026 species. **Species-specific** traits in microbiome composition **could** already be revealed by
31
32 1027 cytological studies, but **the** metabarcoding approach enables much more detailed analysis and
33
34 1028 precise comparison of microbiome composition (Ribeiro *et al.*, 2023), and should be preferred
35
36 1029 when possible.

1030 ***Integrative taxonomy of Calcaronea***

1031 The systematics of subclass Calcaronea is currently facing many challenges due to **the**
1032 broad implementation of molecular methods in taxonomical studies. Traditional taxonomical
1033 schemes were primary typologic (Borojevic *et al.*, 1990, 2000) and **the** high level of morphological
1034 homoplasy obscured **the** actual evolutionary relationships of high-level taxa within Calcarea
1035 (Manuel *et al.*, 2003). As a result, contemporary studies have **partially** shown the broad
1036 incongruence of morphology-based classification **with** newly reconstructed molecular phylogenies
1037 (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012; Voigt & Wörheide, 2016; Alvizu *et al.*, 2018).

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2 1038 However, these analyses strongly supported the monophyly of both subclasses, Calcinea and
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4 1039 Calcaronea. In the most recent molecular phylogeny of Calcaronea based on broad taxon sampling
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6 1040 and two ribosomal markers, the para- and polyphyly of most families and genera were shown,
7
8 1041 along with the high hidden diversity (Alvizu *et al.*, 2018). The phylogenetic studies within
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10 1042 Calcaronea are also complicated by the unusual architecture of the mitochondrial genome in this
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12 1043 group, which is organised in individual linear chromosomes and has a unique genetic code and
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14 1044 accelerated rates of sequence evolution (Lavrov *et al.*, 2013, 2016b). This hampers using of the
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16 1045 mitochondrial markers, in particular the standard barcode cytochrome c oxidase subunit I (COI).
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18 1046 Accordingly, the phylogenetic and taxonomical studies were primarily based on the nuclear
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20 1047 ribosomal genes and internal transcribed spacers (ITS1, ITS2) (Voigt *et al.*, 2012; Klautau *et al.*,
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22 1048 2013, 2020, 2021; Azevedo *et al.*, 2015, 2017; Sanamyan *et al.*, 2022). Among ribosomal markers,
23
24 1049 the C-region of 28S was suggested as a barcode marker for delimitation of taxa on species- and
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26 1050 generic levels (Voigt & Wörheide, 2016) and was successfully applied in subsequent integrative
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28 1051 studies (Alvizu *et al.*, 2019; Córdor-Luján *et al.*, 2019; Klautau *et al.*, 2020, 2021). In the present
29
30 1052 study, this marker was used for species delimitation as well, but it gave doubtful results at the
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32 1053 lowest taxonomical level. There was an obvious genetic break between some closely related
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34 1054 species, *e.g.*, *L. somesii* and *Leucosolenia creepae* sp. nov., but in the case of *Leucosolenia* species
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36 1055 from Clade I, the interspecific differences may be equal to intraspecific values, like in *L.*
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38 1056 *corallorrhiza*, *L. variabilis* and *Leucosolenia* sp. A (Table 2, Fig. 2). As a result, automatic species
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40 1057 delimitation approaches like ASAP failed to find a barcode gap and delimit species within this
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42 1058 group, suggesting either oversplitting or overlamping scenarios. Therefore, while the C-region
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44 1059 gives a good resolution at the species-level and is useful for detection of the genetic breaks among
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46 1060 species, it cannot be considered equivalent to standard mitochondrial COI. The same challenges
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48 1061 in detecting of a barcode gap in the C-region dataset were previously shown in the larger taxon
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50 1062 sampling of Calcaronea (Alvizu *et al.*, 2018). At the same time, SSU data do not provide sufficient
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52 1063 divergence rates to test the automatic species delimitation methods (Data S1, S2, see also Alvizu
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2 1064 *et al.*, 2018). Another part of **the** nuclear ribosomal operon, ITS1 and ITS2, often contain numerous
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4 1065 allele indels in Calcaronea, which complicate the PCR and sequencing **processes** (Wörheide *et al.*,
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6 1066 2004; Voigt & Wörheide, 2016) and therefore **are** not useful for testing the large taxon sampling.
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8
9 1067 In this study, the first molecular analysis of calcareous sponges using the nuclear histone H3
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11 1068 marker **was performed**. Within *Leucosolenia*, the H3 showed higher substitution rates at both intra-
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13 1069 and interspecific levels than ribosomal markers (Tables 2, 3; Fig. 2), and topologies of **the** H3-
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15 1070 based and ribosomal trees were congruent. High substitution rates enable **the** usage of ASAP, and
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17 1071 its results fully confirmed the phylogenetic species hypothesis even within **Clade I** (Data S2). This
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19 1072 suggests the H3 marker may be a powerful tool for taxonomical studies within calcaronean
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21 1073 sponges in addition to the widely used LSU, and is more useful for delimitation of closely related
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23 1074 species. Also, histone H3 is a protein-coding gene with a conservative protein sequence, which
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25 1075 eases a verification of possible **incorrect** base-calling during sequencing. The congruent topologies
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27 1076 of single-gene trees based on LSU and H3 markers also indicate that the latter may further improve
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29 1077 the resolution and support of multi-locus phylogenetic trees within Calcaronea.
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1078 ***Patterns of biodiversity and biogeography***

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36 1079 **The obtained** results indicate that there is an obvious connectivity between **the** calcaronean
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38 1080 fauna of the White Sea and Greenland, since specimens of *L. corallorrhiza* and *Leucosolenia* sp.
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40 1081 from these localities are conspecific in the molecular phylogenetic analysis. Although both
41
42 1082 localities are parts of the Arctic regions, this is **an** unexpected result in the case of the genus
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44 1083 *Leucosolenia*. Members of this genus have short-living larvae, **which are** not capable **of** dispersal
45
46 1084 to a great distance (Anakina, 1981) so such wide distribution ranges accommodated by long-
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48 1085 distance **deposition of larvae** via existing marine currents **seem very** unlikely. The connectivity of
49
50 1086 adult forms is also prevented by the deep and wide Fram Strait (max depth 2545 m) between
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52 1087 Greenland and Svalbard, hampering the migration of shallow water forms with short-living larvae
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54 1088 or direct development via the North Atlantic route (Meyer-Kaiser *et al.*, 2022). We may suggest
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56 1089 two possible explanations **for** such connectivity. Likely both *Leucosolenia corallorrhiza* and
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2 1090 *Leucosolenia* sp. A have a circumpolar distribution and **may be** found in other regions across the
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4 1091 Arctic. Another explanation is that the connectivity of distant populations is achieved **by**
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6 1092 **transportation** via ballast waters or on ship bottoms as a common part of the Arctic fouling
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8 1093 communities. Unfortunately, the exact mechanism cannot be evaluated based on **the** molecular
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10 1094 data presented in this study, as the population structure is not evident from **the** conservative nuclear
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12 1095 markers used.

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15 1096 For now, possible calcarean faunal connections **between** the Arctic and the **Boreal** North-
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17 1097 East Atlantic waters **cannot be evaluated** due to **the** low number of sequenced specimens from
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19 1098 these regions. *Leucosolenia* diversity in **Norwegian** waters estimates 10 species (de Voogd *et al.*,
20
21 1099 2023), among which at least *Leucosolenia variabilis* and *L. corallorrhiza* **inhabit** the Arctic. At
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23 1100 the same time, neither *L. complicata* nor *L. botryoides* were found in the White Sea, and
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25 1101 *Leucosolenia creepae* sp. nov. and *L. somesii* represent two closely related and morphologically
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27 1102 similar but distinct species, suggesting the temperate Atlantic fauna is distinct. The integrative
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29 1103 studies of the Barents Sea **biodiversity** are therefore **of crucial** importance for **a** precise account of
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31 1104 possible faunal links.

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1129 **Statements and Declarations**

1130 *Competing Interests*

1131 Authors declare no competing interests.

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1133 *Data Availability Statement*

1
2 1134 All new sequences obtained in the study were deposited in **GenBank**, **GenBank accession numbers**
3
4 1135 **are listed in Table S1**. Unedited Maximum **Likelihood** single-gene trees (LSU, H3, **18S and**
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6 1136 **concatenated datasets**) are available in the Supplementary materials. **Generated raw data on the**
7
8 1137 **external morphology, skeleton organisation, spicule types and cytology of studied specimens are**
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10 1138 **available in the Mendeley Data repository ([10.17632/4pkf7x4jb9.1](https://doi.org/10.17632/4pkf7x4jb9.1) and [10.17632/r7f8zmdh2n.1](https://doi.org/10.17632/r7f8zmdh2n.1)).**
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For Review Only

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1380 **Tables**1381 **Table 1.** Amplification and sequencing primers and PCR conditions.

Marker	Primers	PCR conditions	Reference
28S rRNA	28S-C2F	5 min - 94°C, 35x[1 min - 95°C, 45s - 50°C, 1 min - 72°C], 7 min - 72°C	Chombard et al., 1998
	GAA AAG AAC TTT GRA RAG AGA GT		
	28S-D2R	TCC GTG TTT CAA GAC GGG	
18S rRNA	18S-328F	5 min - 94°C, 35x[1 min - 94°C, 1 min - 50°C, 2 min - 72°C], 7 min - 72°C	Alvizu et al., 2018
	CCTGGTGATCCTGCCAG		
	18S-HI + R		
	CAACTAAGAACGGCCATGCAC		
	18S-329R		
	TAA TGA TCC TTC CGC AGG TT		
Histone H3	Por_h3f	5 min - 94°C, 35x[15 s - 95°C, 30s - 50°C, 45 s - 72°C], 7 min - 72°C	This study
	ATG GCC CGT ACC AAG CAG ACT GC		
	Por_h3r		
	ATA TCC TTG GGC ATG ATG GTG AC		

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1383 **Table 2.** Uncorrected intra- and interspecific *p*-distances of 28S marker in the genus *Leucosolenia*.

1384 Intraspecific distances are highlighted in bold.

	<i>L. complicata</i>	<i>L. corallorrhiza</i>	<i>Leucosolenia</i> sp. A	<i>L. variabilis</i>	<i>L. creepae</i>	<i>L. somesii</i>
<i>L. complicata</i>	0-0.4					
<i>L. corallorrhiza</i>	6.3-8	0-1.3				
<i>Leucosolenia</i> sp. A	6.8-7.6	0.4-1.7	0			
<i>L. variabilis</i>	5.1-7.2	0.4-1.3	0.8-1.3	0-0.4		
<i>L. creepae</i>	5.1-5.9	8.9-10.5	8.9-9.3	8.9-9.3	0-0.4	
<i>L. somesii</i>	5.5-5.9	10.5-11.8	10.1	10.1	2.5-3	-

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Table 3. Uncorrected intra- and interspecific *p*-distances of H3 marker in the genus *Leucosolenia*.
Intraspecific distances are highlighted in bold.

	<i>L. complicata</i>	<i>L. corallorrhiza</i>	<i>Leucosolenia</i> sp. A	<i>L. variabilis</i>	<i>L. creepae</i>
<i>L. complicata</i>	0-0.4				
<i>L. corallorrhiza</i>	7.9-8.8	0-0.8			
<i>Leucosolenia</i> sp. A	8.4-9.6	2.5-3.3	0.4-0.8		
<i>L. variabilis</i>	7.9-9.2	3.3-5	2.9-4.2	0-1.3	
<i>L. creepae</i>	3.8-4.6	9.6-10.5	9.6-10.5	9.2-10.9	0-1.7

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Table 4. Spicule dimensions of *Leucosolenia complicata* (Montagu, 1814)

Spicule	Length (µm)					Width (µm)					Angle (°)				
	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n
<i>Curved lanceolate diactines</i>	147.1	263.7	389.3	58.8	46	6.6	9.5	14.4	1.4	47	-	-	-	-	-
<i>Trichoxeas</i>	66.0	127.3	248.7	40.3	18	1.5	2.4	3.5	0.6	17	-	-	-	-	-
<i>Triactines</i>															
<i>Unpaired actine</i>	75.3	113.5	149.4	19.4	40	3.7	6.3	10.5	1.3	41	-	-	-	-	-
<i>Paired actines</i>	62.3	94.9	122.0	13.1	81	4.1	6.8	11.8	1.3	81	120.4	125.7	137.9	4.4	28
<i>Tetractines</i>															
<i>Unpaired actine</i>	30.6	109.3	156.7	24.1	56	3.7	6.7	9.7	1.2	57	-	-	-	-	-
<i>Paired actines</i>	14.2	93.7	170.2	20.4	109	3.5	6.9	10.0	1.2	108	115.7	123.5	134.0	4.4	53
<i>Apical actine</i>	8.9	23.8	49.7	11.1	56	2.0	5.3	10.1	1.4	57	-	-	-	-	-

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Table 5. Spicule dimensions of *Leucosolenia corallorrhiza* (Haeckel, 1872)

Spicule	Length (µm)					Width (µm)					Angle (°)				
	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n
<i>Curved lanceolate diactines</i>	73.5	179.0	455.0	74.2	27	2.8	6.0	11.4	1.7	27	-	-	-	-	-
<i>Triactines</i>															
<i>Unpaired actine</i>	37.1	70.5	100.0	14.9	91	3.5	6.5	10.5	1.4	91	-	-	-	-	-
<i>Paired actines</i>	36.1	82.7	133.3	20.4	165	3.1	6.5	12.5	1.5	164	124.6	142.9	151.2	6.0	28
<i>Tetractines</i>															
<i>Unpaired actine</i>	46.8	68.8	98.8	16.0	14	3.5	5.6	7.9	1.2	15	-	-	-	-	-
<i>Paired actines</i>	37.6	80.7	128.1	22.5	23	2.4	5.8	8.4	1.6	25	144.8	151.4	159.3	4.0	14
<i>Apical actine</i>	11.4	22.9	42.9	10.7	16	3.6	5.5	9.2	1.3	16	-	-	-	-	-

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1395 **Table 6.** Spicule dimensions of *Leucosolenia variabilis* **Haeckel, 1870**

Spicule	Length (µm)					Width (µm)					Angle (°)				
	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n
Curved smooth															
<i>lanceolate</i>	123.7	306.7	482.4	100.1	27	5.7	9.8	13.9	1.8	27	-	-	-	-	-
<i>diactines</i>															
Strait spiny															
<i>lanceolate</i>	111.4	151.4	191.2	28.7	5	2.4	3.1	3.8	0.5	10	-	-	-	-	-
<i>diactines</i>															
<i>Trichoxeas</i>	fragments, up to 362.4					0.6	0.9	1.3	0.2	17	-	-	-	-	-
Triactines															
<i>Unpaired actine</i>	46.5	122.3	208.6	31.7	63	5.0	8.1	11.6	1.5	63	-	-	-	-	-
<i>Paired actines</i>	39.0	127.9	180.2	29.3	130	4.0	8.5	12.7	1.7	128	122.1	138.5	150.6	4.8	42
Tetractines															
<i>Unpaired actine</i>	101.3	147.6	196.2	28.4	12	6.4	8.5	11.1	1.5	12	-	-	-	-	-
<i>Paired actines</i>	92.5	142.0	172.5	22.3	25	6.0	9.1	11.5	1.5	26	134.0	142.2	155.3	4.6	34
<i>Apical actine</i>	16.5	22.8	31.1	5.2	14	4.7	5.9	8.5	1.4	15	-	-	-	-	-

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1397 **Table 7.** Spicule dimensions of *Leucosolenia* sp. **A.**

Spicule	Length (µm)					Width (µm)					Angle (°)				
	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n
Curved spiny															
<i>lanceolate</i>	131.9	189.1	311.0	48.2	15	5.8	7.2	8.8	0.8	15	-	-	-	-	-
<i>diactines</i>															
<i>Curved smooth</i>	-	515.0	-	83.4	2	-	11.6	-	1.7	2					
<i>diactines</i>															
Triactines															
<i>Unpaired actine</i>	81.6	118.5	179.7	22.2	42	6.3	8.9	11.1	1.1	43	-	-	-	-	-
<i>Paired actines</i>	80.5	125.1	174.8	22.4	74	6.6	9.1	11.6	1.0	84	132.9	146.5	160.7	5.6	44
Tetractines															
<i>Unpaired actine</i>	82.4	114.3	157.1	27.2	5	7.9	8.6	9.9	0.7	7	-	-	-	-	-
<i>Paired actines</i>	79.3	113.2	132.0	16.0	14	7.0	8.5	10.2	0.9	14	137.4	140.8	146.2	3.3	5
<i>Apical actine</i>	25.7	30.0	34.2	4.2	3	5.8	7.1	8.5	1.2	5	-	-	-	-	-

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1400 **Table 8.** Spicule dimensions of *Leucosolenia creepae* sp. nov.

Spicule	Length (μm)					Width (μm)					Angle ($^\circ$)				
	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n
<i>Spiny diactines</i>	83.3	194.9	478.8	98.6	81	2.9	5.1	9.6	1.4	81	-	-	-	-	-
Triactines															
<i>Unpaired actine</i>	29.9	80.7	125.8	18.8	87	3.0	5.4	8.8	1.1	89	-	-	-	-	-
<i>Paired actines</i>	37.9	94.2	148.6	20.9	169	2.7	5.9	9.0	1.2	173	118.5	131.1	137.7	4.7	29
Tetractines															
<i>Unpaired actine</i>	31.8	85.1	137.5	25.9	15	4.1	6.2	7.7	1.3	15	-	-	-	-	-
<i>Paired actines</i>	52.9	95.3	136.2	20.6	30	3.7	6.3	8.1	1.2	31	131.0	139.5	146.1	4.5	13
<i>Apical actine</i>	12.7	25.6	43.7	9.8	18	2.9	5.2	8.4	1.3	18	-	-	-	-	-

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1402 **Table 9.** Spicule dimensions of *Leucosolenia somesii* (Bowerbank, 1874)

Spicule	Length (μm)					Width (μm)					Angle ($^\circ$)				
	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n	Min	Mean	Max	SD	n
<i>Curved smooth diactines</i>	148.0	424.5	649.8	116.0	68	6.5	9.9	12.3	1.3	88	-	-	-	-	-
<i>Straight spiny diactines</i>	68.0	90.0	105.8	8.9	58	2.1	3.3	4.1	0.4	39	-	-	-	-	-
Triactines															
<i>Unpaired actine</i>	58.3	127.4	186.1	27.0	60	6.5	8.2	10.3	1.0	64	-	-	-	-	-
<i>Paired actines</i>	86.2	155.2	226.0	28.4	111	5.9	8.0	10.7	1.0	131	117.1	131.7	139.9	4.2	67
Tetractines															
<i>Unpaired actine</i>	103.5	156.0	206.9	32.9	10	8.4	9.1	10.5	0.6	13	-	-	-	-	-
<i>Paired actines</i>	156.0	178.7	209.2	14.5	23	7.8	9.5	11.1	0.7	24	133.5	139.3	149.1	3.9	14
<i>Apical actine</i>	16.6	21.5	29.3	4.2	8	8.1	10.0	11.2	1.0	7	-	-	-	-	-

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2 1406 **Figure captions**
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4 1407 **Figure 1.** The molecular phylogenetic hypothesis of the genus *Leucosolenia* based on the Bayesian
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6 1408 analysis of the concatenated dataset (28S, 18S and H3 markers). Initial species names are used on
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8 1409 the tips, bold font indicates original specimens used in this study. Each putative species-level clade
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10 1410 is coloured according to the revised species hypothesis, the suggested revised species names are
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12 1411 provided on the right. All calcaronean species except representatives of the genus *Leucosolenia*
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14 1412 are collapsed into a single group, 'Calcaronea rest'. Numbers above branches indicate posterior
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16 1413 probabilities from the Bayesian Inference (> 0.9), numbers below branches – bootstrap support
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18 1414 from the Maximum Likelihood (>70).
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23 1415
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25 1416 **Figure 2.** Medium parsimony network analysis (TCS algorithm) of *Leucosolenia* species. The
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27 1417 relative size of circles is proportional to the number of sequences of that same genotype. A – 28S
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29 1418 alignment. Colours of the circles refer to the geographic origin of each genotype. Coloured
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31 1419 backgrounds and species names indicate the revised species hypothesis. B – H3 alignment. Colours
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33 1420 of the circles refer to the revised species hypothesis.
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37 1422 **Figure 3.** *Leucosolenia complicata* (Montagu, 1814). External morphology and skeleton. A –
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39 1423 general morphology (WS11661); B – skeleton of oscular rim (WS11662); C – skeleton of oscular
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41 1424 tube (WS11662); D – skeleton of cormus (WS11662). c – cormus, d – diverticulum, o – osculum,
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43 1425 or – oscular rim, ot – oscular tube.
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50 1427 **Figure 4.** *Leucosolenia complicata* (Montagu, 1814). Spicule types, scanning electron
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52 1428 microscopy. A – curved lanceolate diactines; B, C – trichoxeas; D – triactines; E – tetractine.
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56 1430 **Figure 5.** *Leucosolenia complicata* (Montagu, 1814). Body wall structure and cell types of
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58 1431 bordering tissues. A, B – semi-thin sections of sponge body wall; C – exopinacocyte; D –
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2 1432 endopinacocyte; E – choanocytes; F – porocyte. Scale bars: A – 50 μm , B – 20 μm , C – F – 2 μm .

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4 1433 ch – choanocytes, chd – choanoderm, en – endopinacocyte, ex – exopinacocyte, exp –

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6 1434 exopinacoderm, f – flagellum, m – mesohyl, mv – microvilli, n – nucleus, po – porocyte.

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11 1436 **Figure 6.** *Leucosolenia complicata* (Montagu, 1814). Mesohyl cell types and symbiotic bacteria.

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13 1437 A – sclerocyte; B – amoebocyte; C – symbiotic bacteria, morphotype 1; D – symbiotic bacteria,

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15 1438 morphotype 2. Scale bars: A, B – 2 μm , C, D – 0.5 μm . am – amoebocytes, m – mesohyl, n –

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17 1439 nucleus, sp – spicule.

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22 1441 **Figure 7.** *Leucosolenia corallorrhiza* (Haeckel, 1872). External morphology and skeleton. A –

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24 1442 general morphology (WS11642); B – skeleton of oscular rim (WS11653); C – skeleton of oscular

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26 1443 tube (WS11653); D – skeleton of cormus (WS11653). c – cormus, d – diverticulum, o – osculum,

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28 1444 oc – oscular crown, ot – oscular tube.

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33 1446 **Figure 8.** *Leucosolenia corallorrhiza* (Haeckel, 1872). Spicule types, scanning electron

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35 1447 microscopy. A – curved lanceolate diactines; B – tips of diactines, I and II refer to the zones

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37 1448 marked on A, white arrowheads mark spines; C – tetractine; D – triactines.

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42 1450 **Figure 9.** *Leucosolenia corallorrhiza* (Haeckel, 1872). Body wall structure and cell types of

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44 1451 bordering tissues. A, B – semi-thin sections of body wall of sponge; C – exopinacocyte; D –

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46 1452 endopinacocyte; E – choanocytes, inset – junctions between choanocytes; F – porocyte. Scale bars:

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48 1453 A – 50 μm , B – 20 μm , C - F – 2 μm , inset – 0.2 μm . am – amoebocyte, ch – choanocytes, chd –

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50 1454 choanoderm, en – endopinacocyte, exp – exopinacoderm, f – flagellum, m – mesohyl, mv –

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52 1455 microvilli, n – nucleus, ph – phagosome, po – porocytes, sb – symbiotic bacteria.

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2 1457 **Figure 10.** *Leucosolenia corallorrhiza* (Haeckel, 1872). Mesohyl cell types and symbiotic
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4 1458 bacteria. A – sclerocytes, inset – septate junctions between sclerocytes; B – amoebocytes; C, D –
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6 1459 granular cells in the body wall; E – granular cell; F – degraded granular cell; G – myocytes, inset
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8 1460 – bundles of myofilaments; H, I – symbiotic bacteria, morphotype 1. Scale bars: A, B, E, F – 2
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10 1461 μm , C – 50 μm , C – 20 μm , G – 5 μm , H – 0.5 μm , I – 1 μm . ch – choanocytes, chd – choanoderm,
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12 1462 ex – exopinacocyte, exp – exopinacoderm, f – flagellum, gc – granular cells gr – granule, m –
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14 1463 mesohyl, mf – myofibrils, mv – microvilli, my – myocytes, n – nucleus, po – porocytes, sb –
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16 1464 symbiotic bacteria, sp – spicule.
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22 1466 **Figure 11.** *Leucosolenia variabilis* Haeckel, 1870. External morphology and skeleton. A – general
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24 1467 morphology (WS11731); B – skeleton of oscular rim (WS11731); C – skeleton of oscular tube; D
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26 1468 – skeleton of cormus (WS11643). c – cormus, d – diverticulum, o – osculum, or – oscular rim, ot
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28 1469 – oscular tube.
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33 1471 **Figure 12.** *Leucosolenia variabilis* Haeckel, 1870. Spicule types, scanning electron microscopy.
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35 1472 A – curved smooth lanceolate diactines; B – triactines; C – abnormal triactines; D – tetractine.
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40 1474 **Figure 13.** *Leucosolenia variabilis* Haeckel, 1870. Trichoxeas. A, B – general view of trichoxea
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42 1475 fragments; C – enlarge view of trichoxea, white arrowhead marks spines. tx – trichoxeas.
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47 1477 **Figure 14.** *Leucosolenia variabilis* Haeckel, 1870. Body wall structure and cell types of bordering
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49 1478 tissues. A, B – semi-thin sections of body wall of sponge; C – exopinacocytes; D – choanocytes;
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51 1479 E – porocyte; F – endopinacocyte. Scale bars: A – 50 μm , B – 20 μm , C, D – 5 μm , E, F – 2 μm .
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54 1480 am – amoeboid cell, ch – choanocytes, chd – choanoderm, ex – exopinacocyte, exp –
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56 1481 exopinacoderm, f – flagellum, m – mesohyl, mv – microvilli, n – nucleus, po – porocyte, sb –
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58 1482 symbiotic bacteria, sc – spherulous cells.
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2 1483
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4 1484 **Figure 15.** *Leucosolenia variabilis* Haeckel, 1870. Mesohyl cell types and symbiotic bacteria. A
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6 1485 – amoebocytes; B – large amoeboid cell; C – granular cell; D – myocyte, inset – bundles of
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8 1486 myofilaments; E – spherulous cell; F – spherulous cells in the body wall; G – sclerocyte; H –
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10 1487 symbiotic bacteria, morphotype 1. I – symbiotic bacteria, morphotype 2. J – symbiotic bacteria,
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12 1488 morphotype 3. Scale bars: A – 2 µm, B – 5 µm, C-E – 2 µm, F – 50 µm, G – 2 µm, H-J – 1 µm.
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14 1489 am – amoebocyte, bam – large amoeboid cell, ch – choanocytes, gc – granular cell, gr – granule,
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16 1490 m – mesohyl, mf – myofibrils, n – nucleus, sc – spherulous cells, sp – spicule.
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22 1492 **Figure 16.** *Leucosolenia variabilis* Haeckel, 1870. Spicule types in the White Sea specimen (A)
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24 1493 and in the specimens from the British Natural History Museum collection (B-D). A – spicules from
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26 1494 WS11731; B – spicules from BMNH 1906.12.1.40; C – spicules from BMNH 1906.12.1.50; D –
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28 1495 spicules from the syntype BMNH 1910.1.1.421.a. d – diactines, tx – trichoxeas.
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34 1497 **Figure 17.** *Leucosolenia* sp. A. External morphology and skeleton. A – general morphology
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36 1498 (WS11752); B – skeleton of oscular rim (WS11770); C – skeleton of oscular tube (WS11770); D
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38 1499 – skeleton of cormus (WS11752). c – cormus, d – diverticulum, o – osculum, or – oscular rim, ot
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40 1500 – oscular tube.
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45 1502 **Figure 18.** *Leucosolenia* sp. A. Spicule types, scanning electron microscopy. A – curved smooth
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47 1503 diactines; B – curved spiny lanceolate diactines triactines; C – tips of diactines, I and II refer to
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49 1504 the zones marked on A and B, white arrowheads mark spines; D – triactines; E – abnormal
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51 1505 triactines; F – tetractine.
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56 1507 **Figure 19.** *Leucosolenia creepae* sp. nov. External morphology and skeleton. A – general
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58 1508 morphology (WS11702, holotype); B – skeleton of oscular rim (WS11762); C – skeleton of
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2 1509 oscular tube (WS11728); D – skeleton of cormus (WS11655). c – cormus, d – diverticulum, o –
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4 1510 osculum, oc – oscular crown, ot – oscular tube.
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9 1512 **Figure 20.** *Leucosolenia creepae* sp. nov. Spicule types, scanning electron microscopy. A – spiny
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11 1513 diactines; B – tips of diactines, I, II, III and IV refer zones marked on A, white arrowheads mark
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13 1514 spines; C – triactines; D – abnormal triactines; E – tetractines.
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18 1516 **Figure 21.** *Leucosolenia creepae* sp. nov. Body wall structure and cell types of bordering tissues.
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20 1517 A, B – semi-thin sections of body wall of sponge; C – exopinacocyte; D – endopinacocyte and
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22 1518 myocyte, inset - bundles of myofilaments in the myocyte; E – choanocytes. F – porocyte. Scale
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24 1519 bars: A – 50 μm , B – 20 μm , C – 2 μm , D – 5 μm , E, F – 2 μm . am – amoeboid cell, ch –
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26 1520 choanocytes, chd – choanoderm, en – endopinacocyte, ex – exopinacocyte, exp – exopinacoderm,
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28 1521 f – flagellum, m – mesohyl, mf – myofibrils, mv – microvilli, my – myocytes, n – nucleus, oo –
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30 1522 oocyte, ph – phagosome, po – porocyte, sb – symbiotic bacteria.
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36 1524 **Figure 22.** *Leucosolenia creepae* sp. nov. Mesohyl cell types and symbiotic bacteria. A –
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38 1525 sclerocyte; B – amoebocyte; C, D – symbiotic bacteria, morphotype 1; E, F – symbiotic bacteria,
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40 1526 morphotype 2; G – young oocyte. Scale bars: A, B – 2 μm , C - F – 0.5 μm , G – 5 μm . am -
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42 1527 amoeboid cell, ch – choanocytes, m – mesohyl, n – nucleus, oo – oocyte, sb – symbiotic bacteria,
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44 1528 sp – spicule.
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50 1530 **Figure 23.** *Leucosolenia somesii* (Bowerbank, 1874). External morphology and skeleton. A –
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52 1531 general morphology (ZMA Por. 17572); B – skeleton of cormus (ZMA Por. 17572); C, D –
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54 1532 spicules from BMNH 1956.4.26.35. c – cormus, d – diverticulum.
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2 1534 **Figure 24.** *Leucosolenia somesii* (Bowerbank, 1874). **ZMA Por. 17572.** Spicule types, scanning
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4 1535 electron microscopy. A – curved smooth diactines; B – straight spiny diactines; C – tips of
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6 1536 diactines, I and II refer to the zones marked on A and B, white arrowheads mark spines; D –
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8 1537 triactines; E – abnormal triactines; F – tetractines.
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For Review Only

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2 1538 **SUPPLEMENTARY MATERIAL**
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4 1539 **Data S1.** Unedited Maximum Likelihood trees (LSU, H3, 18S and concatenated datasets).
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7 1540 **Data S2.** Results of ASAP analysis based on LSU and H3 datasets.
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9 1541 **Table S1.** List of *Leucosolenia* specimens used in the study. Voucher numbers, collection dates
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11 1542 and localities, and GenBank accession numbers are given.
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14 1543 **Table S2.** The cell types and symbiotic bacteria of the studied *Leucosolenia* species.
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16 1544 **Figure S1.** The molecular phylogenetic hypothesis of the genus *Leucosolenia* based on the
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18 1545 Bayesian analysis of the concatenated dataset (28S, 18S and H3 markers). Numbers above
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20 1546 branches indicate posterior probabilities from the Bayesian Inference (> 0.9), numbers below
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22 1547 branches – bootstrap support from the Maximum Likelihood (>70).
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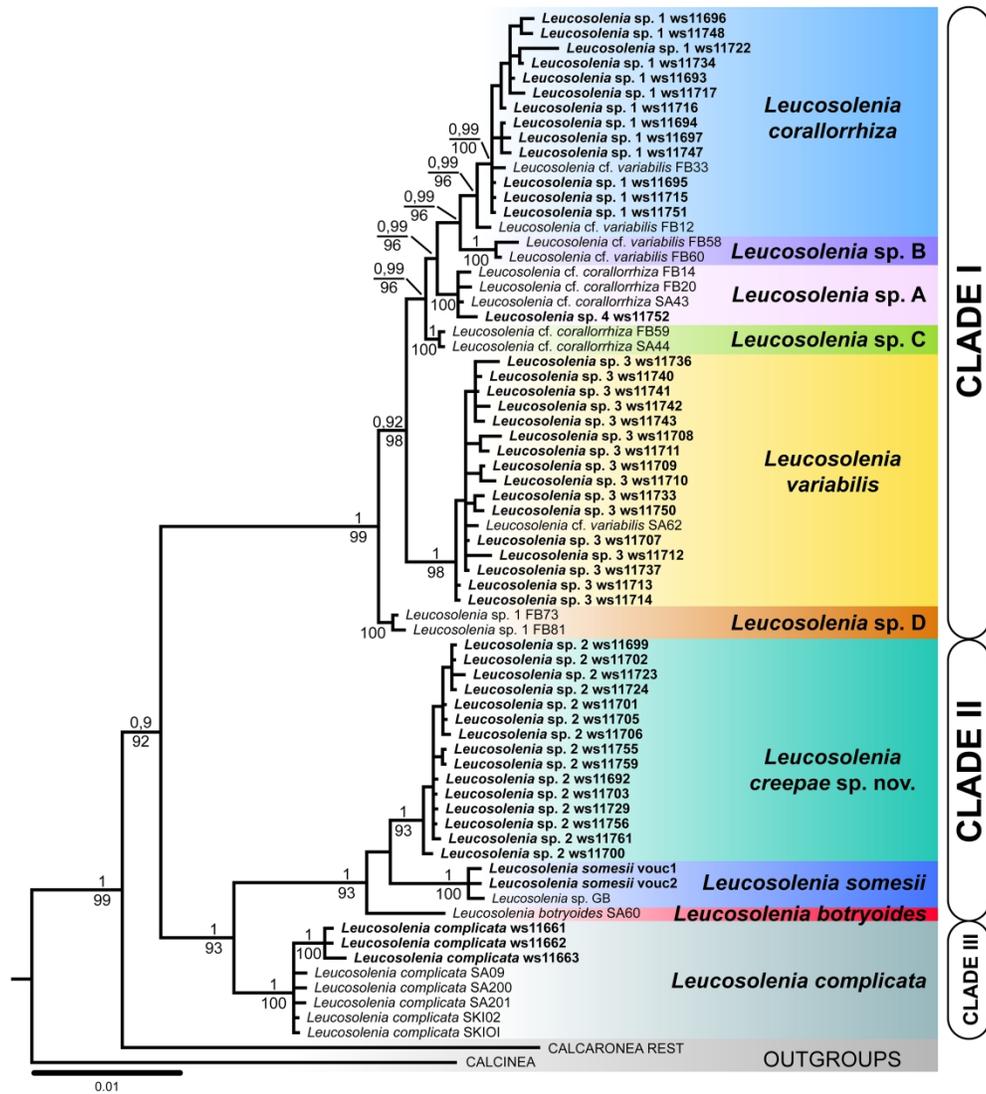
25 1548 **Figure S2.** The molecular phylogenetic hypothesis of the genus *Leucosolenia* based on the
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27 1549 Bayesian analysis of the concatenated dataset (28S and 18S markers). Numbers above branches
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29 1550 indicate posterior probabilities from the Bayesian Inference (> 0.9), numbers below branches –
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31 1551 bootstrap support from the Maximum Likelihood (>70).
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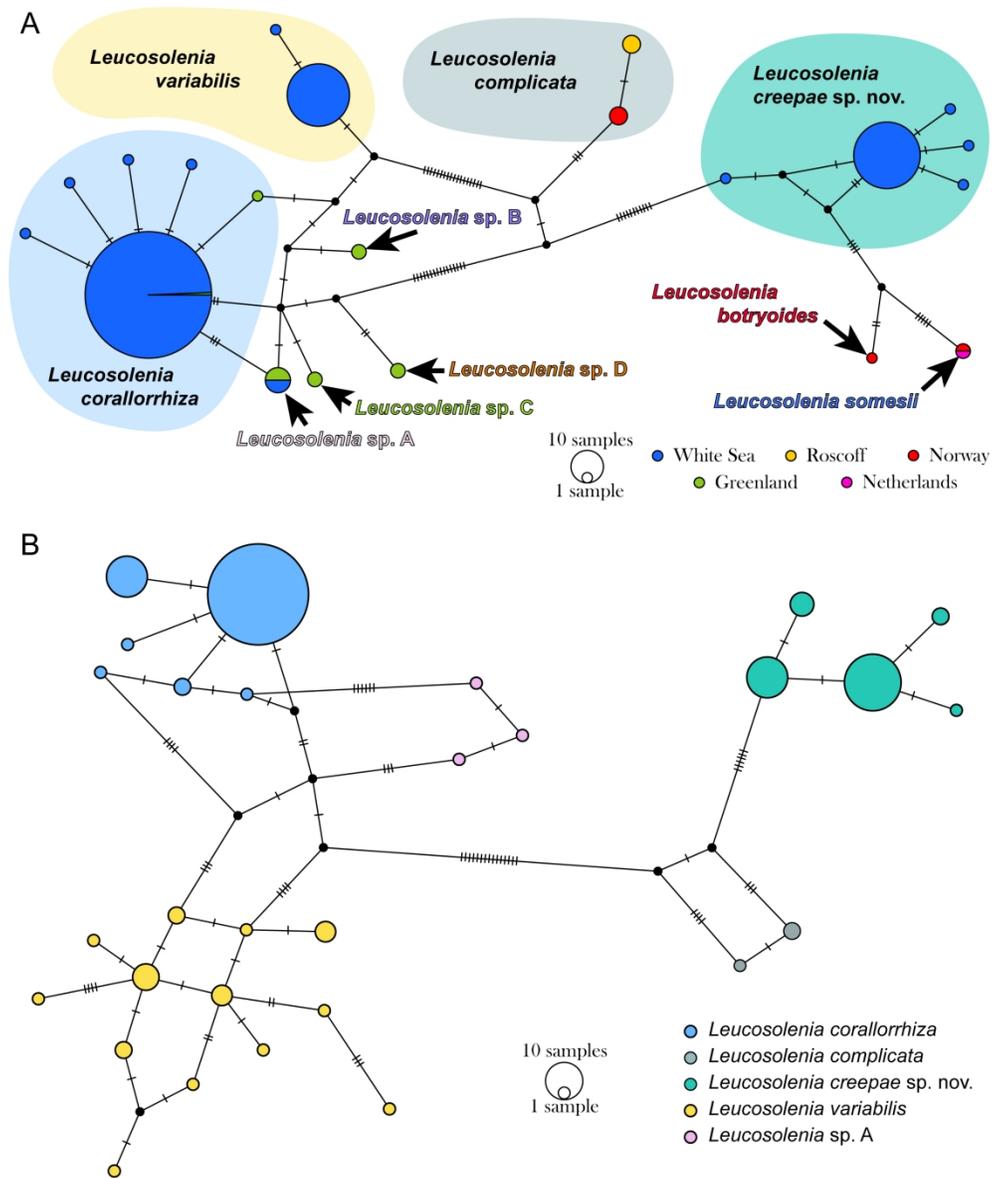


Figure 2. Medium parsimony network analysis (TCS algorithm) of *Leucosolenia* species. The relative size of circles is proportional to the number of sequences of that same genotype. A – 28S alignment. Colours of the circles refer to the geographic origin of each genotype. Coloured backgrounds and species names indicate the revised species hypothesis. B – H3 alignment. Colours of the circles refer to the revised species hypothesis.

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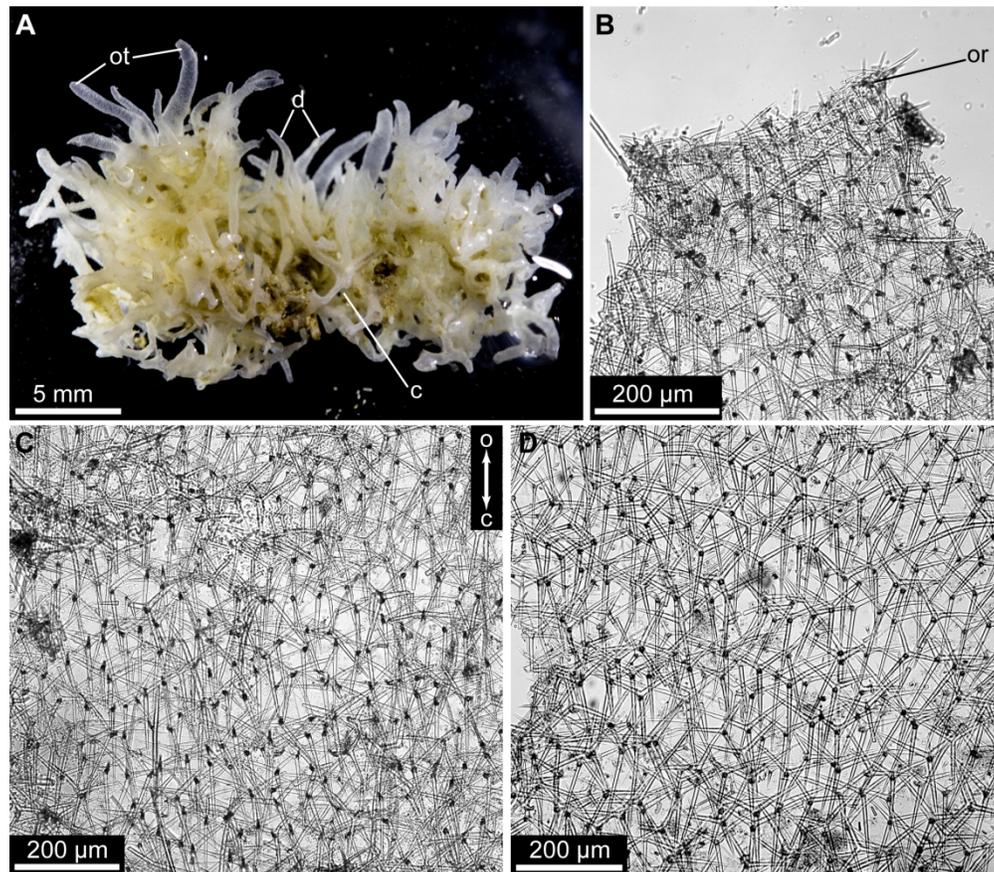


Figure 3. *Leucosolenia complicata* (Montagu, 1814). External morphology and skeleton. A – general morphology (WS11661); B – skeleton of oscular rim (WS11662); C – skeleton of oscular tube (WS11662); D – skeleton of cormus (WS11662). c – cormus, d – diverticulum, o – osculum, or – oscular rim, ot – oscular tube.

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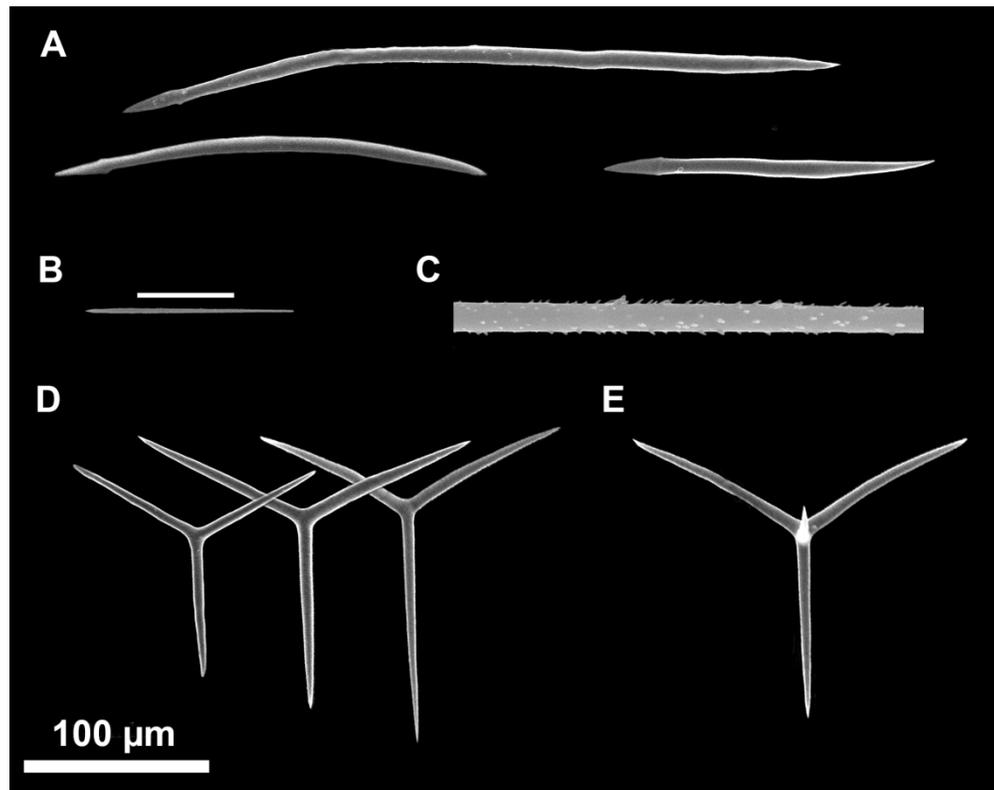


Figure 4. *Leucosolenia complicata* (Montagu, 1814). Spicule types, scanning electron microscopy. A – curved lanceolate diactines; B, C – trichoxeas; D – triactines; E – tetractine.

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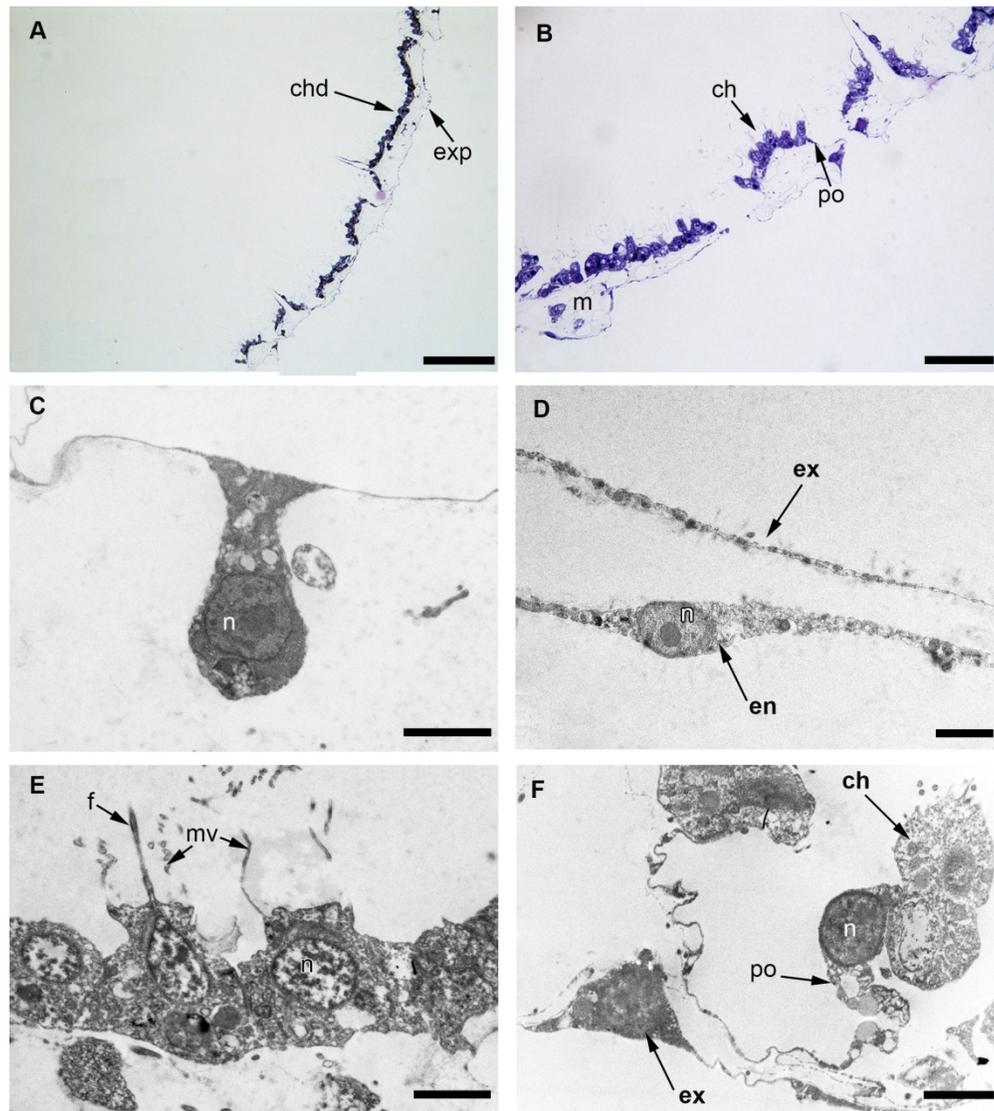


Figure 5. *Leucosolenia complicata* (Montagu, 1814). Body wall structure and cell types of bordering tissues. A, B – semi-thin sections of sponge body wall; C – exopinacocyte; D – endopinacocyte; E – choanocytes; F – porocyte. Scale bars: A – 50 μm , B – 20 μm , C – F – 2 μm . ch – choanocytes, chd – choanoderm, en – endopinacocyte, ex – exopinacocyte, exp – exopinacoderm, f – flagellum, m – mesohyl, mv – microvilli, n – nucleus, po – porocyte.

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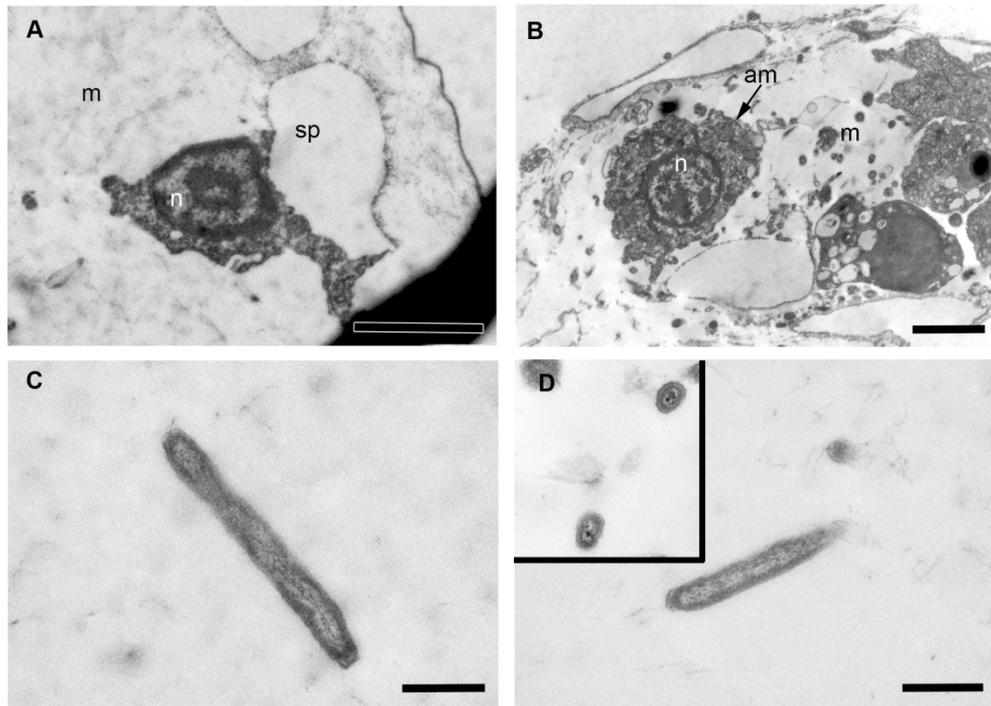


Figure 6. *Leucosolenia complicata* (Montagu, 1814). Mesohyl cell types and symbiotic bacteria. A – sclerocyte; B – amoebocyte; C – symbiotic bacteria, morphotype 1; D – symbiotic bacteria, morphotype 2. Scale bars: A, B – 2 μm , C, D – 0.5 μm . am – amoebocytes, m – mesohyl, n – nucleus, sp – spicule.

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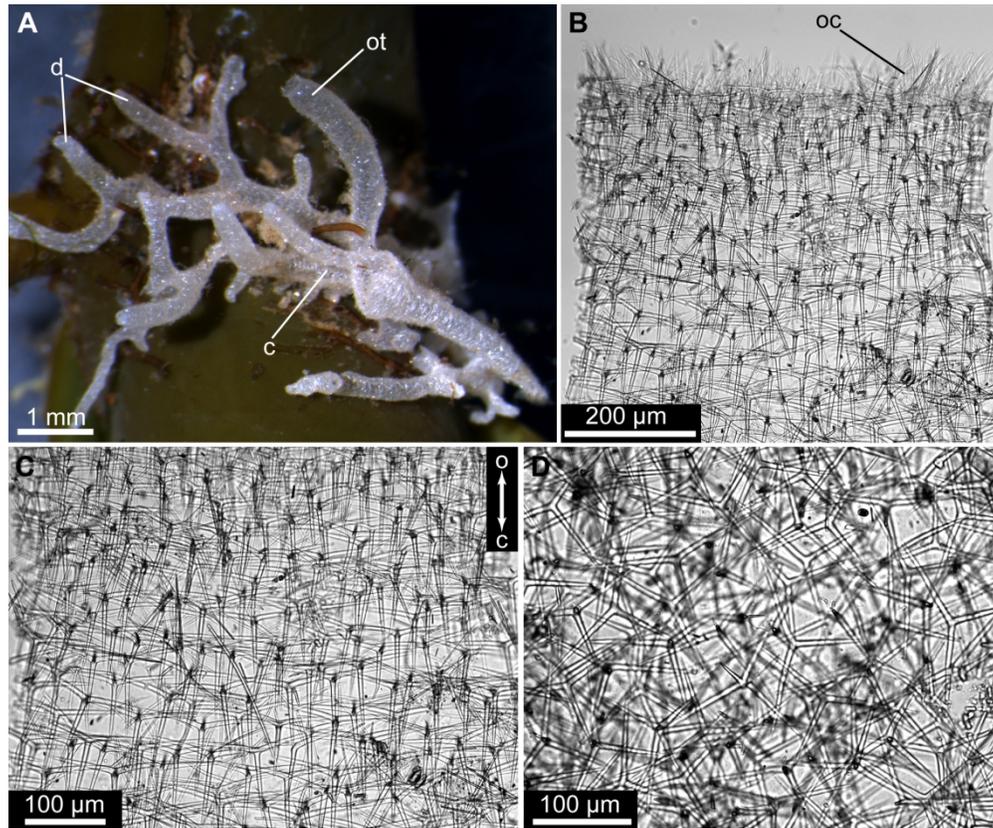


Figure 7. *Leucosolenia corallorrhiza* (Haeckel, 1872). External morphology and skeleton. A – general morphology (WS11642); B – skeleton of oscular rim (WS11653); C – skeleton of oscular tube (WS11653); D – skeleton of cormus (WS11653). c – cormus, d – diverticulum, o – osculum, oc – oscular crown, ot – oscular tube.

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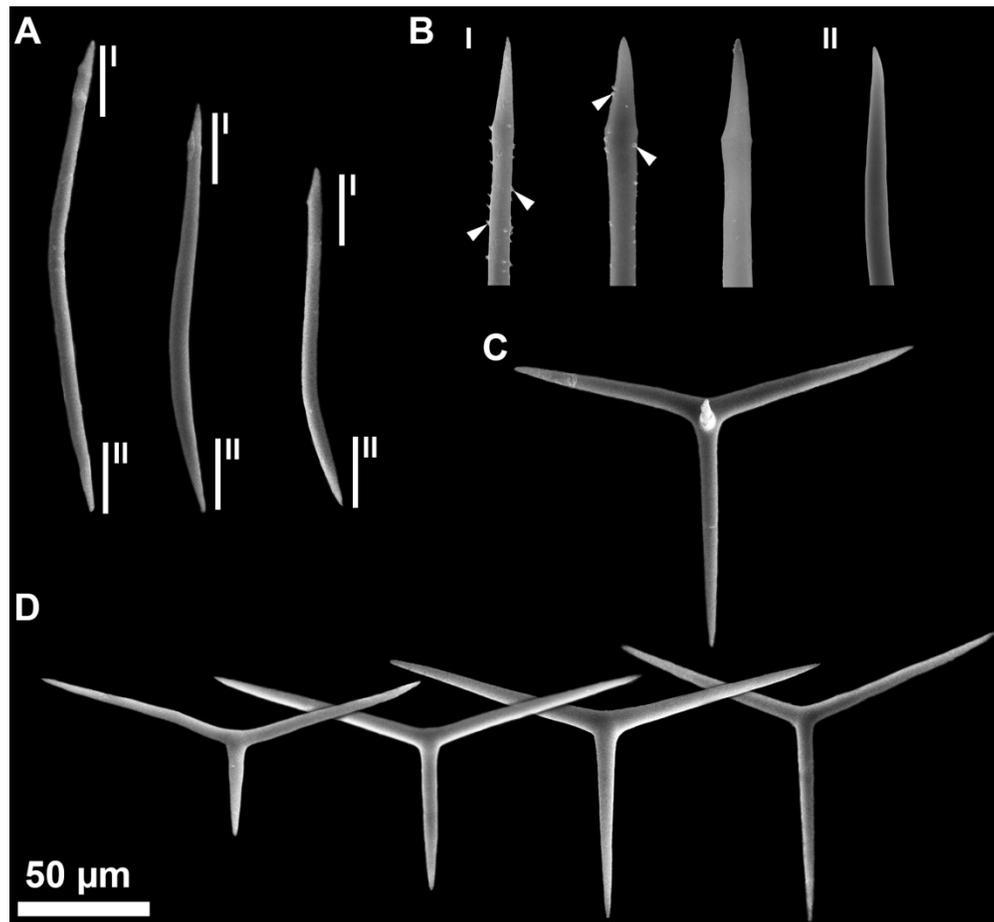


Figure 8. *Leucosolenia corallorrhiza* (Haeckel, 1872). Spicule types, scanning electron microscopy. A – curved lanceolate diactines; B – tips of diactines, I and II refer to the zones marked on A, white arrowheads mark spines; C – tetractine; D – triactines.

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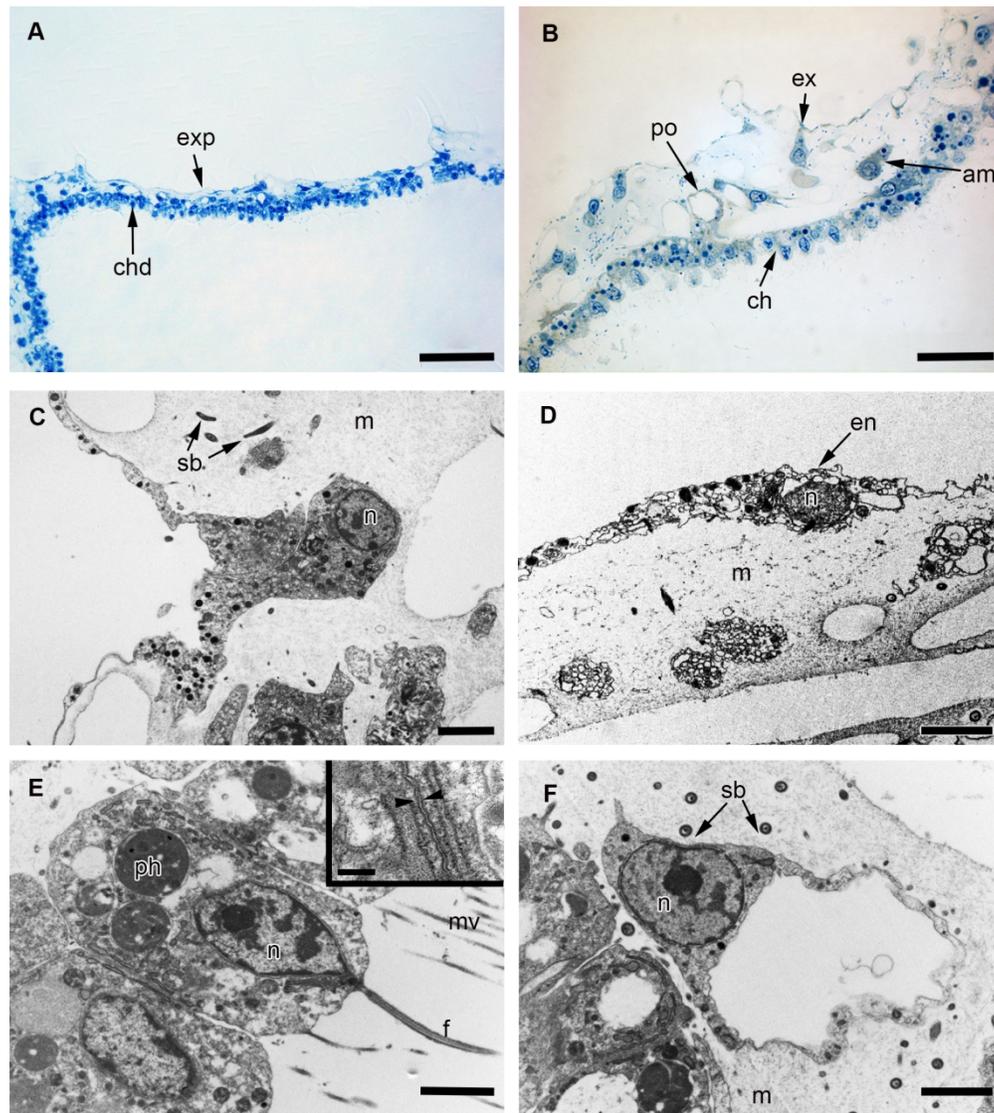


Figure 9. *Leucosolenia corallorrhiza* (Haeckel, 1872). Body wall structure and cell types of bordering tissues. A, B – semi-thin sections of body wall of sponge; C – exopinacocyte; D – endopinacocyte; E – choanocytes, inset – junctions between choanocytes; F – porocyte. Scale bars: A – 50 μm , B – 20 μm , C – F – 2 μm , inset – 0.2 μm . am – amoebocyte, ch – choanocytes, chd – choanoderm, en – endopinacocyte, exp – exopinacoderm, f – flagellum, m – mesohyl, mv – microvilli, n – nucleus, ph – phagosome, po – porocytes, sb – symbiotic bacteria.

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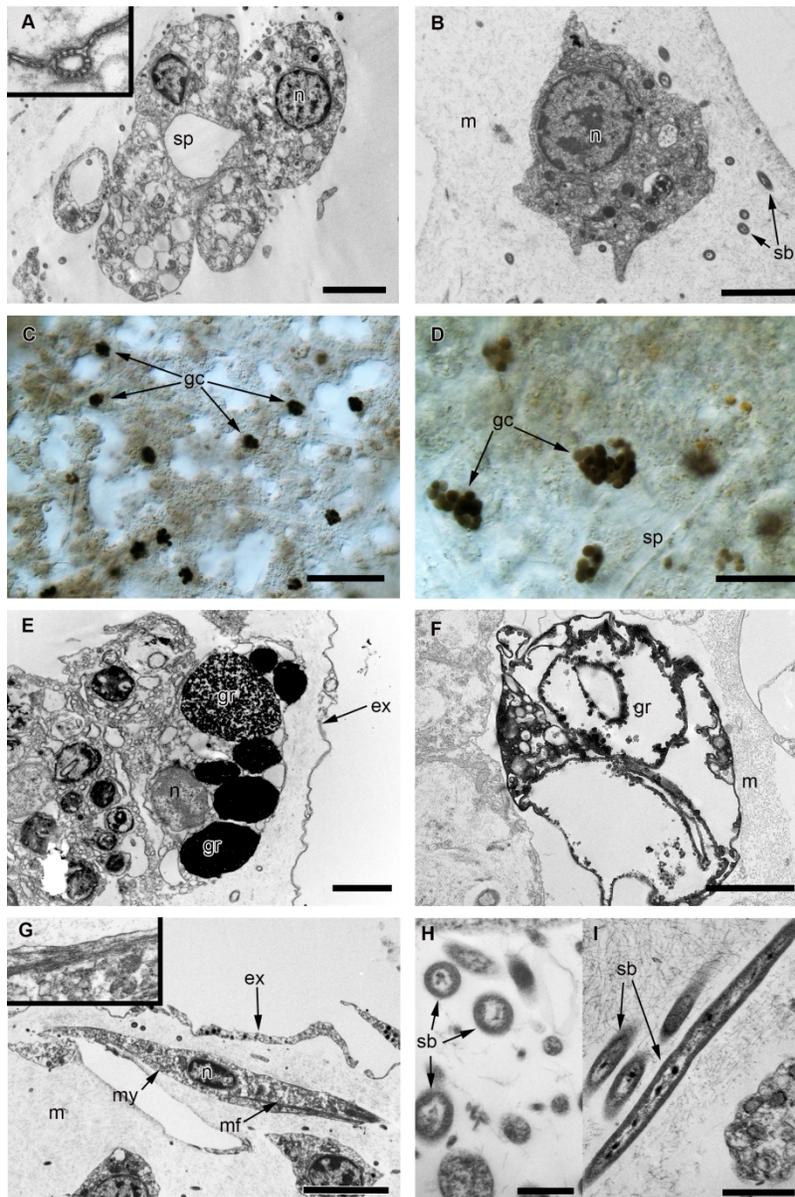


Figure 10. *Leucosolenia corallorrhiza* (Haeckel, 1872). Mesohyl cell types and symbiotic bacteria. A – sclerocytes, inset – septate junctions between sclerocytes; B – amoebocytes; C, D – granular cells in the body wall; E – granular cell; F – degraded granular cell; G – myocytes, inset – bundles of myofilaments; H, I – symbiotic bacteria, morphotype 1. Scale bars: A, B, E, F – 2 μ m, C – 50 μ m, C – 20 μ m, G – 5 μ m, H – 0.5 μ m, I – 1 μ m. ch – choanocytes, chd – choanoderm, ex – exopinacocyte, exp – exopinacoderm, f – flagellum, gc – granular cells gr – granule, m – mesohyl, mf – myofibrils, mv – microvilli, my – myocytes, n – nucleus, po – porocytes, sb – symbiotic bacteria, sp – spicule.

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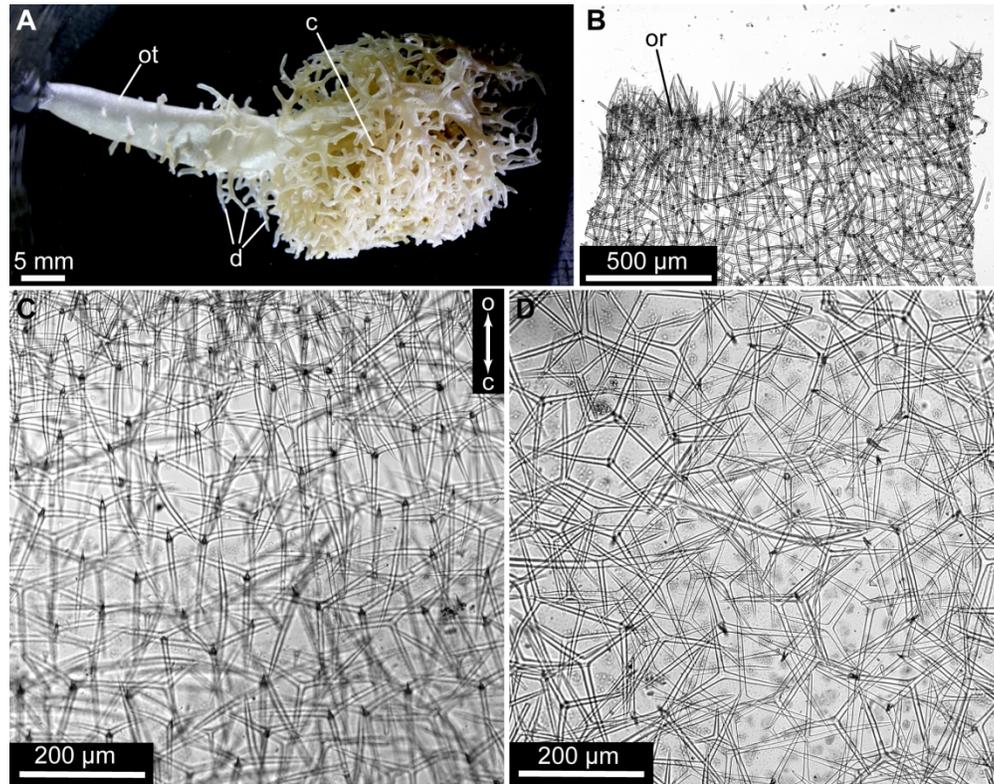


Figure 11. *Leucosolenia variabilis* Haeckel, 1870. External morphology and skeleton. A – general morphology (WS11731); B – skeleton of oscular rim (WS11731); C – skeleton of oscular tube; D – skeleton of cormus (WS11643). c – cormus, d – diverticulum, o – osculum, or – oscular rim, ot – oscular tube.

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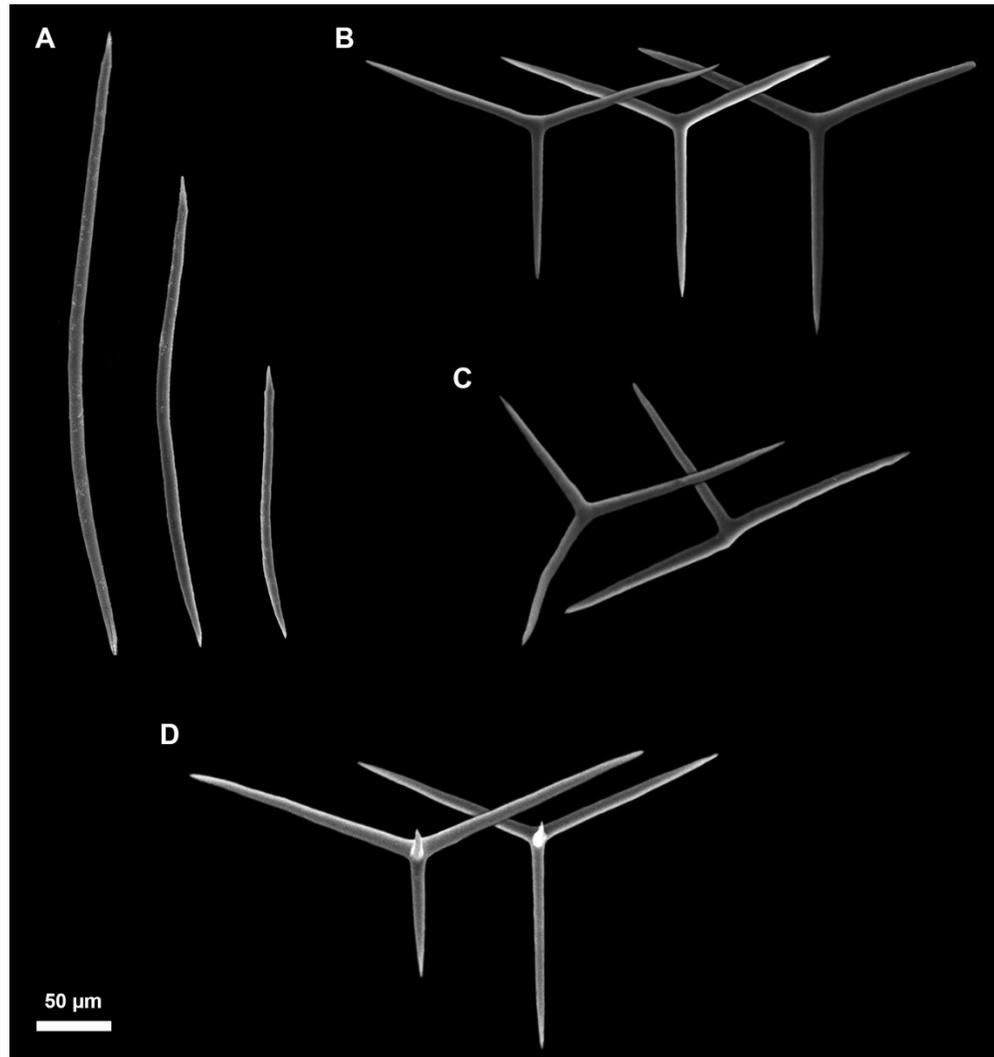


Figure 12. *Leucosolenia variabilis* Haeckel, 1870. Spicule types, scanning electron microscopy. A – curved smooth lanceolate diactines; B – triactines; C – abnormal triactines; D – tetractine.

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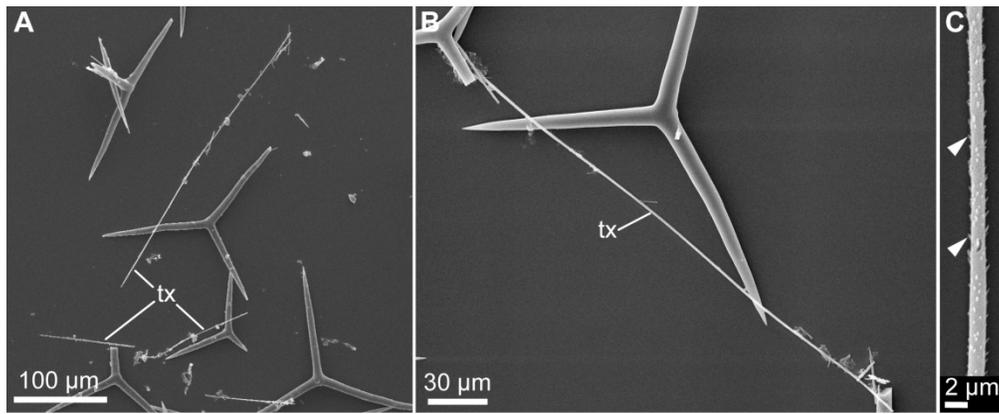


Figure 13. *Leucosolenia variabilis* Haeckel, 1870. Trichoxeas. A, B – general view of trichoxea fragments; C – enlarge view of trichoxea, white arrowhead marks spines. tx – trichoxeas.

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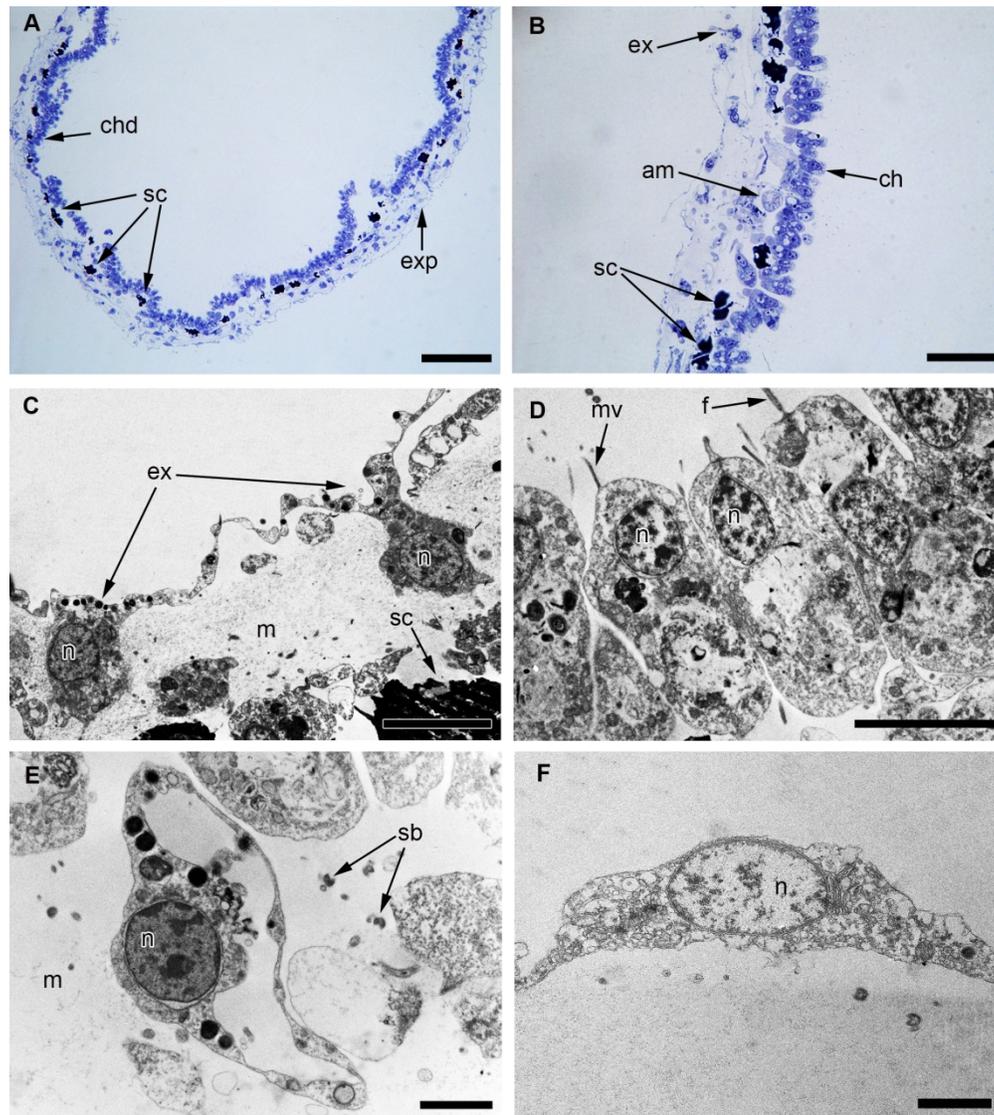


Figure 14. *Leucosolenia variabilis* Haeckel, 1870. Body wall structure and cell types of bordering tissues. A, B – semi-thin sections of body wall of sponge; C – exopinacocytes; D – choanocytes; E – porocyte; F – endopinacocyte. Scale bars: A - 50 μ m, B - 20 μ m, C, D - 5 μ m, E, F - 2 μ m. am - amoeboid cell, ch - choanocytes, chd - choanoderm, ex - exopinacocyte, exp - exopinacoderm, f - flagellum, m - mesohyl, mv - microvilli, n - nucleus, po - porocyte, sb - symbiotic bacteria, sc - spherulous cells.

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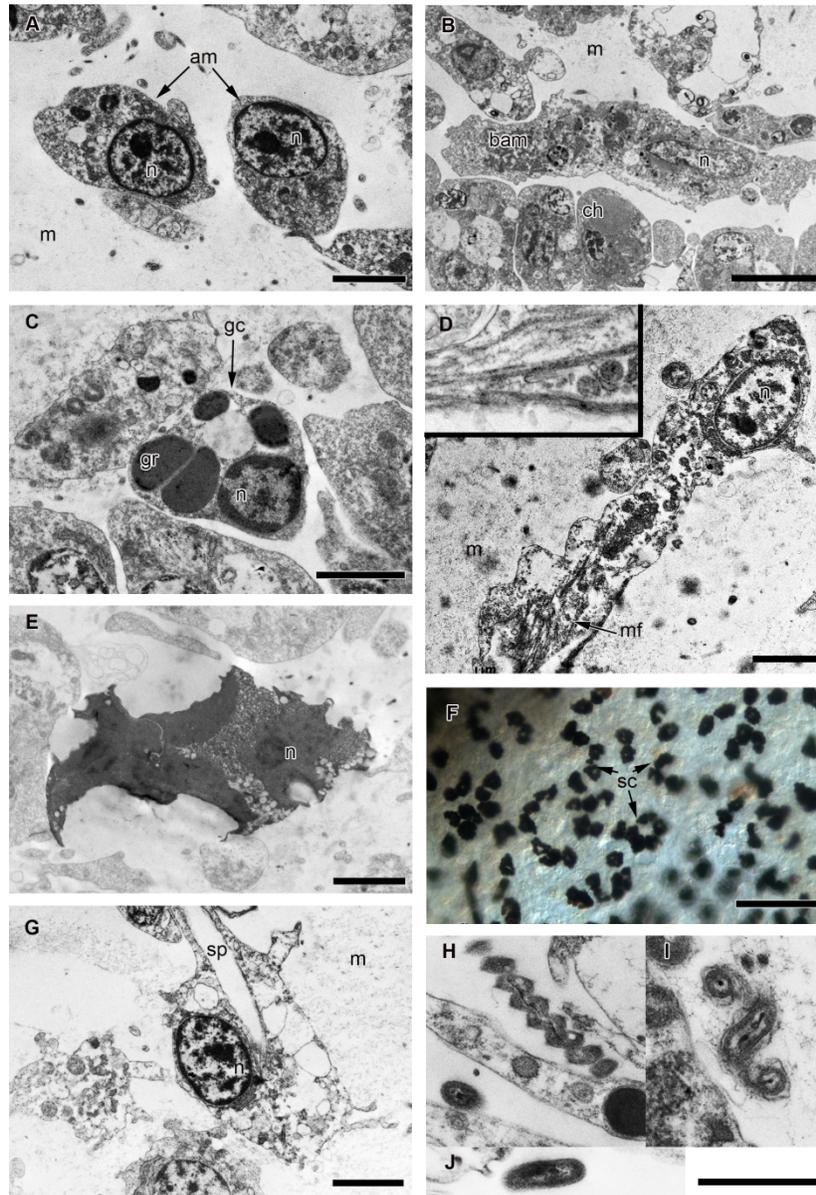


Figure 15. *Leucosolenia variabilis* Haeckel, 1870. Mesohyl cell types and symbiotic bacteria. A – amoebocytes; B – large amoeboid cell; C – granular cell; D – myocyte, inset - bundles of myofibrils; E – spherulous cell; F – spherulous cells in the body wall; G – sclerocyte; H – symbiotic bacteria, morphotype 1. I – symbiotic bacteria, morphotype 2. J – symbiotic bacteria, morphotype 3. Scale bars: A - 2 μm , B - 5 μm , C-E - 2 μm , F - 50 μm , G - 2 μm , H-J - 1 μm . am - amoebocyte, bam - large amoeboid cell, ch - choanocytes, gc - granular cell, gr - granule, m - mesohyl, mf - myofibrils, n - nucleus, sc - spherulous cells, sp - spicule.

173x253mm (300 x 300 DPI)

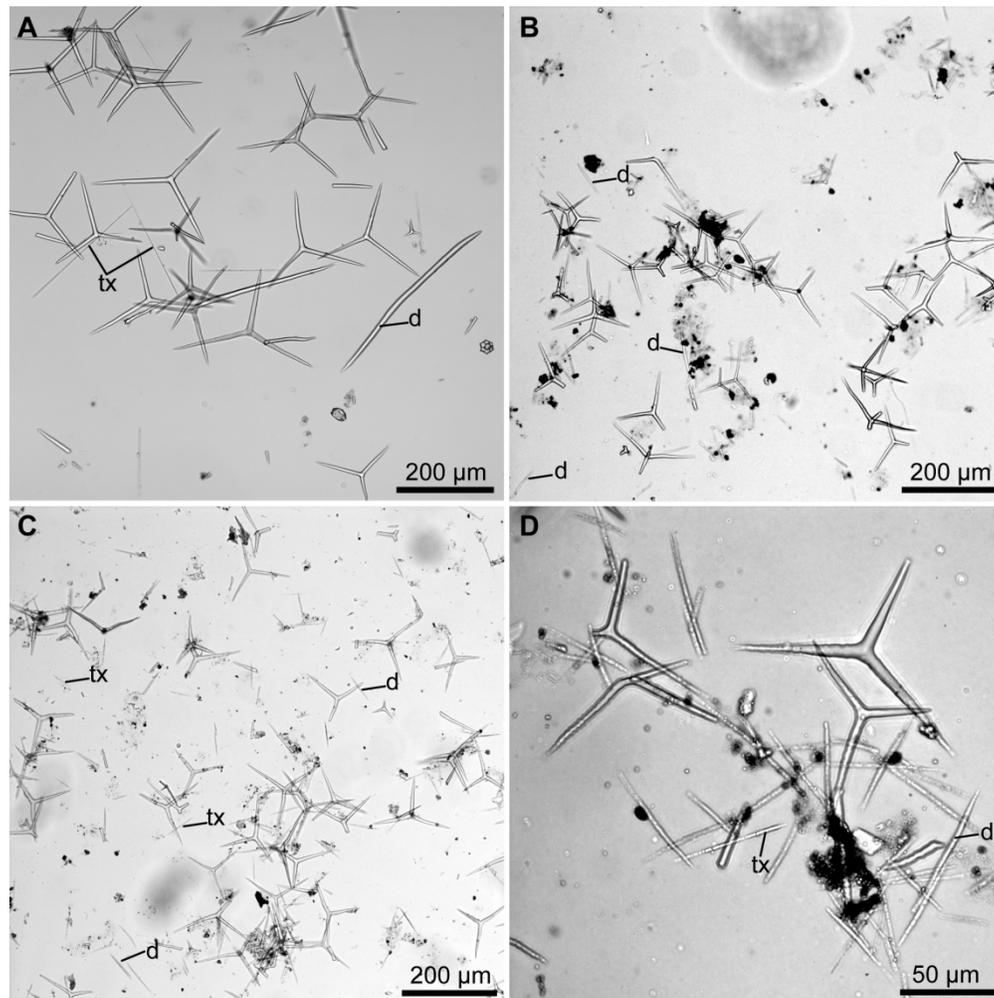


Figure 16. *Leucosolenia variabilis* Haeckel, 1870. Spicule types in the White Sea specimen (A) and in the specimens from the British Natural History Museum collection (B-D). A – spicules from WS11731; B – spicules from BMNH 1906.12.1.40; C – spicules from BMNH 1906.12.1.50; D – spicules from the syntype BMNH 1910.1.1.421.a. d – diactines, tx – trichoxeas.

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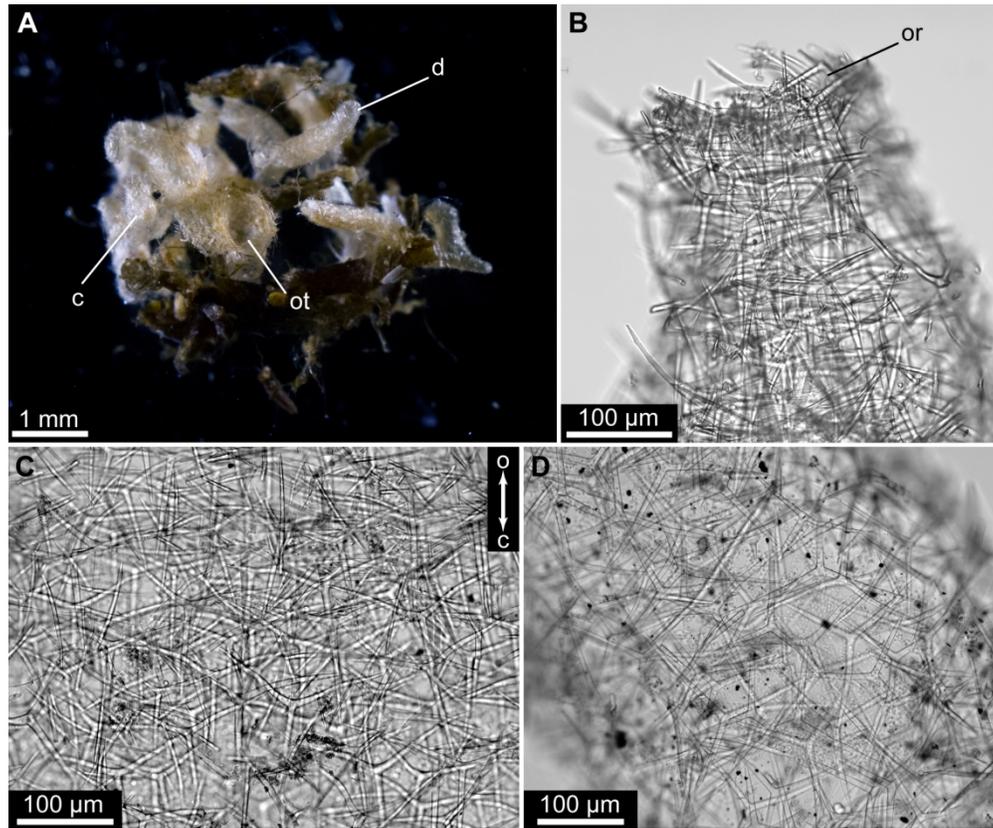


Figure 17. *Leucosolenia* sp. A. External morphology and skeleton. A – general morphology (WS11752); B – skeleton of oscular rim (WS11770); C – skeleton of oscular tube (WS11770); D – skeleton of cormus (WS11752). c – cormus, d – diverticulum, o – osculum, or – oscular rim, ot – oscular tube.

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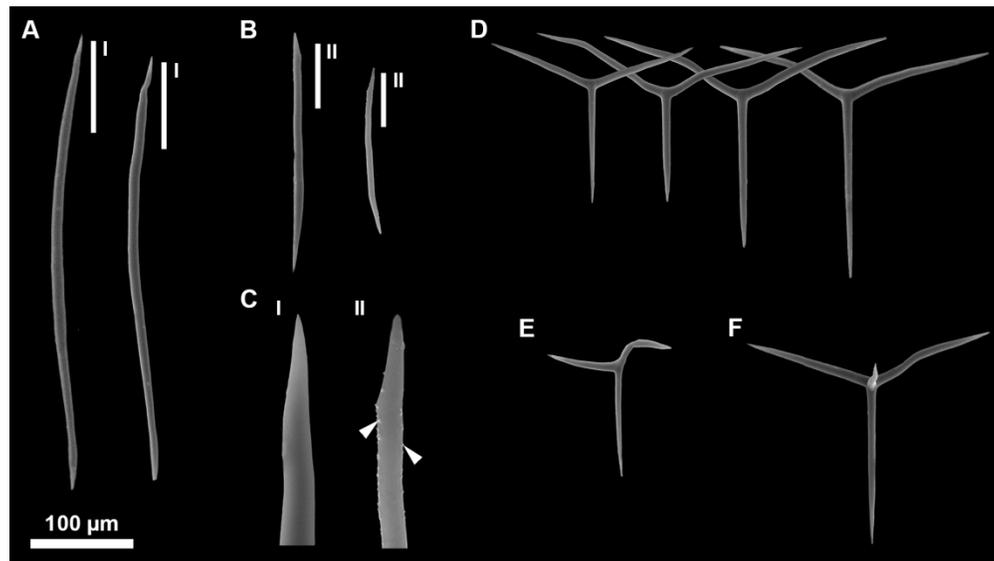


Figure 18. *Leucosolenia* sp. A. Spicule types, scanning electron microscopy. A – curved smooth diactines; B – curved spiny lanceolate diactines triactines; C – tips of diactines, I and II refer to the zones marked on A and B, white arrowheads mark spines; D – triactines; E – abnormal triactines; F – tetractine.

173x97mm (300 x 300 DPI)

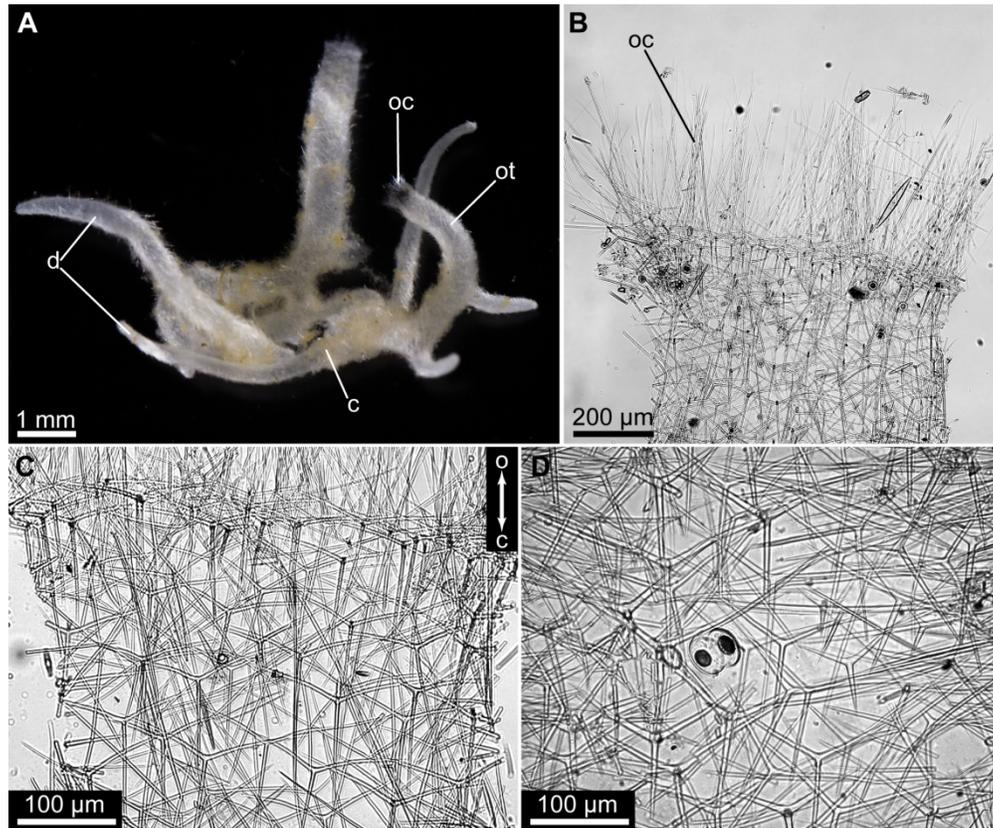


Figure 19. *Leucosolenia creepae* sp. nov. External morphology and skeleton. A – general morphology (WS11702, holotype); B – skeleton of oscular rim (WS11762); C – skeleton of oscular tube (WS11728); D – skeleton of cormus (WS11655). c – cormus, d – diverticulum, o – osculum, oc – oscular crown, ot – oscular tube.

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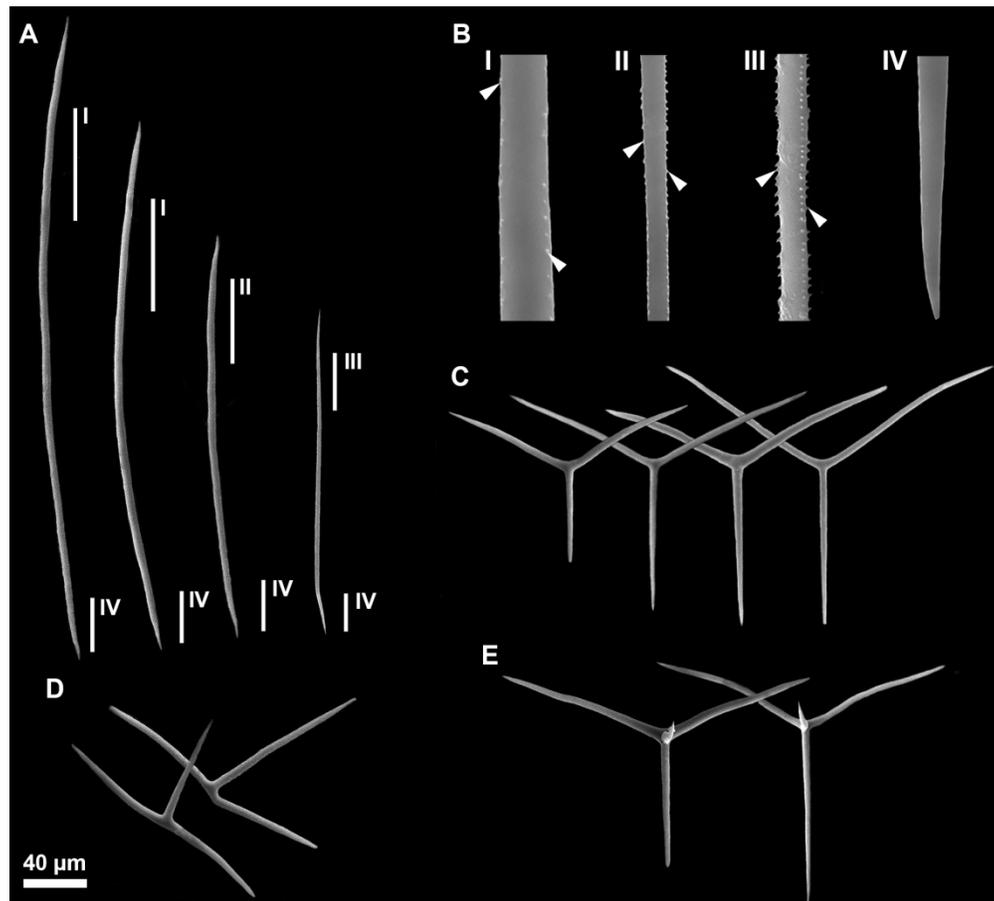


Figure 20. *Leucosolenia creepae* sp. nov. Spicule types, scanning electron microscopy. A – spiny diactines; B – tips of diactines, I, II, III and IV refer zones marked on A, white arrowheads mark spines; C – triactines; D – abnormal triactines; E – tetractines.

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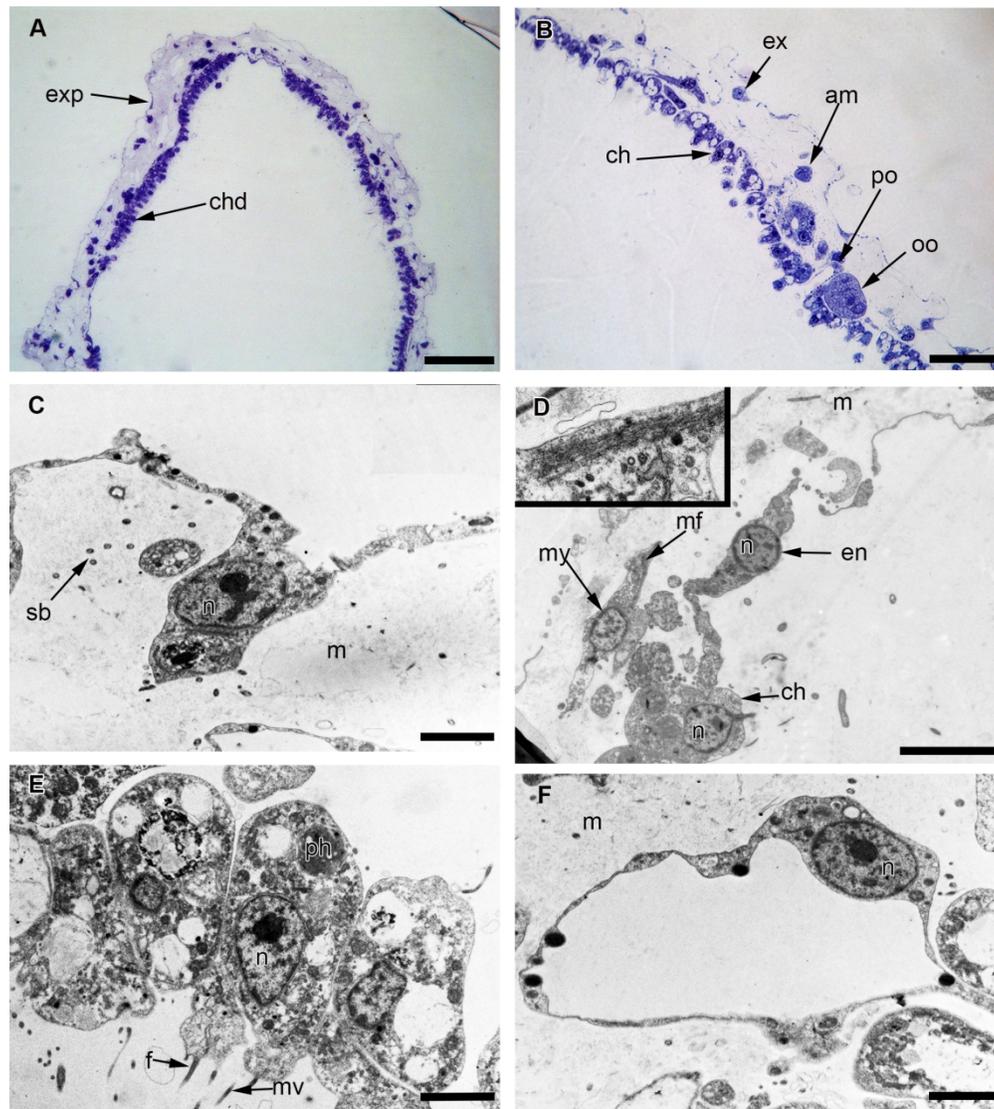


Figure 21. *Leucosolenia creepae* sp. nov. Body wall structure and cell types of bordering tissues. A, B – semi-thin sections of body wall of sponge; C – exopinacocyte; D – endopinacocyte and myocyte, inset – bundles of myofibrils in the myocyte; E – choanocytes. F – porocyte. Scale bars: A – 50 μ m, B – 20 μ m, C – 2 μ m, D – 5 μ m, E, F – 2 μ m. am – amoeboid cell, ch – choanocytes, chd – choanoderm, en – endopinacocyte, ex – exopinacocyte, exp – exopinacoderm, f – flagellum, m – mesohyl, mf – myofibrils, mv – microvilli, my – myocytes, n – nucleus, oo – oocyte, ph – phagosome, po – porocyte, sb – symbiotic bacteria.

173x193mm (300 x 300 DPI)

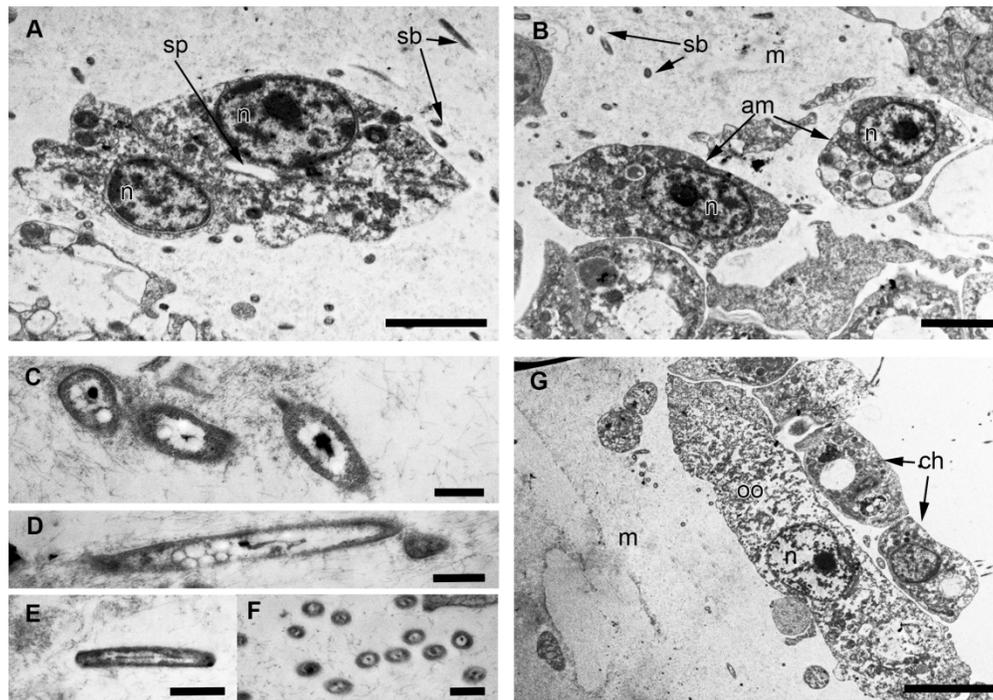


Figure 22. *Leucosolenia creepae* sp. nov. Mesohyl cell types and symbiotic bacteria. A – sclerocyte; B – amoebocyte; C, D – symbiotic bacteria, morphotype 1; E, F – symbiotic bacteria, morphotype 2; G – young oocyte. Scale bars: A, B – 2 μm , C – F – 0.5 μm , G – 5 μm . am – amoeboid cell, ch – choanocytes, m – mesohyl, n – nucleus, oo – oocyte, sb – symbiotic bacteria, sp – spicule.

173x121mm (300 x 300 DPI)

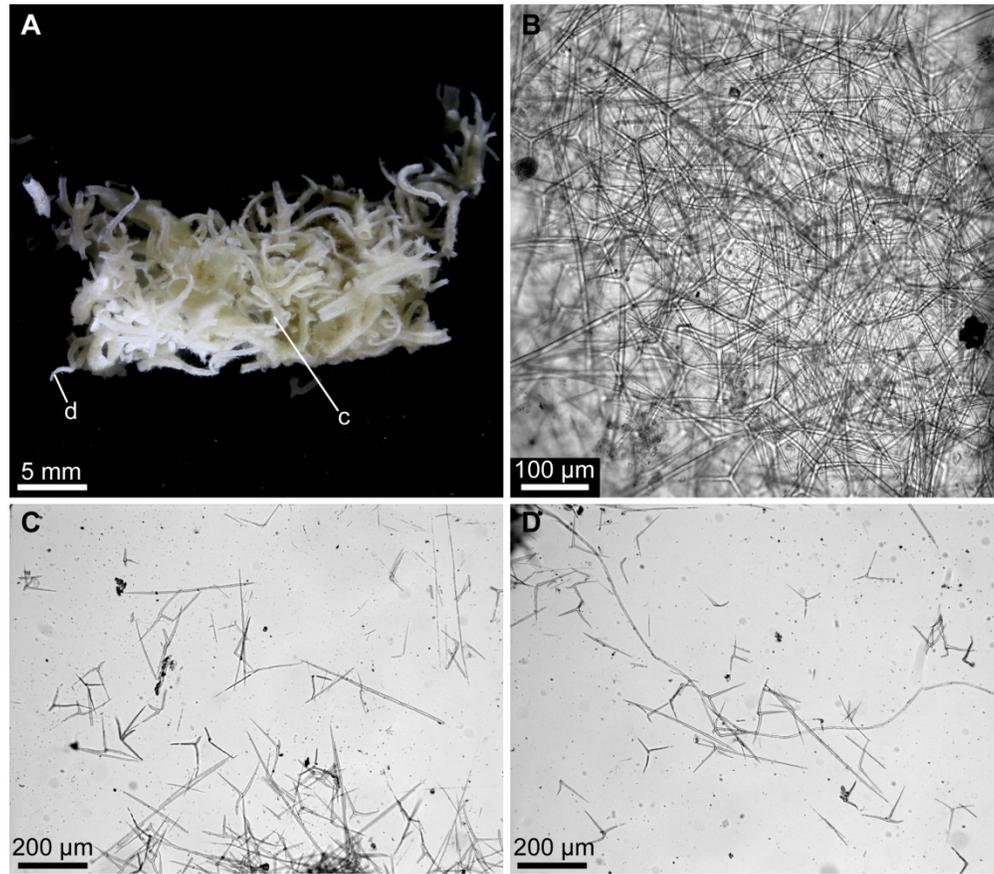


Figure 23. *Leucosolenia somesii* (Bowerbank, 1874). External morphology and skeleton. A – general morphology (ZMA Por. 17572); B – skeleton of cormus (ZMA Por. 17572); C, D – spicules from BMNH 1956.4.26.35. c – cormus, d – diverticulum.

174x152mm (300 x 300 DPI)

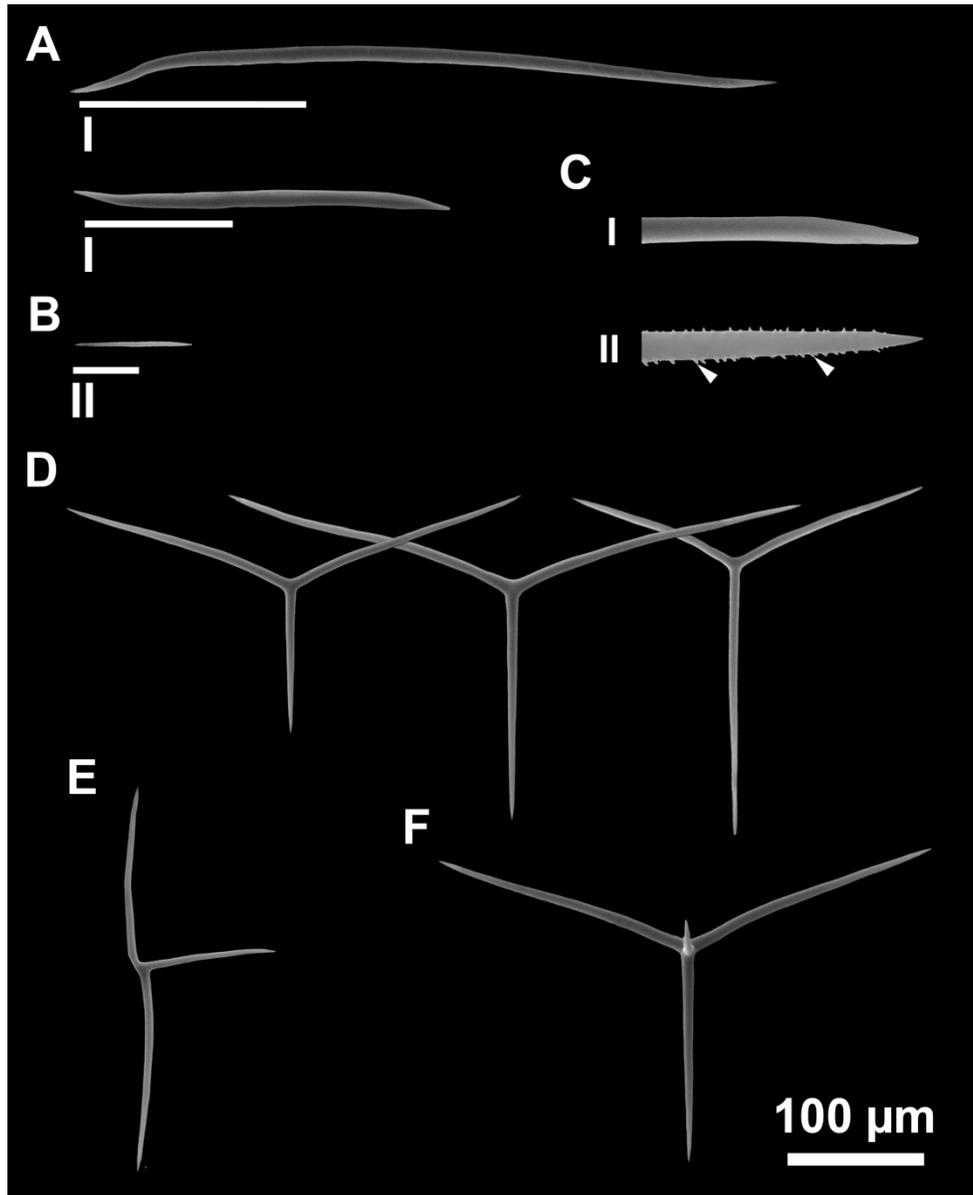


Figure 24. *Leucosolenia somesii* (Bowerbank, 1874). ZMA Por. 17572. Spicule types, scanning electron microscopy. A – curved smooth diactines; B – straight spiny diactines; C – tips of diactines, I and II refer to the zones marked on A and B, white arrowheads mark spines; D – triactines; E – abnormal triactines; F – tetractines.

173x213mm (300 x 300 DPI)