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Two new species of calcareous sponges (Porifera: Calcarea) from the deep Antarctic Eckström Shelf and a revised list of species found in Antarctic waters

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Abstract

The paper reports on two new species of calcareous sponges (Porifera, Calcarea) from the Antarctic Weddell Sea, Clathrina brandtae sp. nov. and Leucetta delicata sp. nov., collected at 600 m depth during the ANT XXIV/2-SYSTCO expedition in January 2008. The new species are described based on a combination of morphological and molecular data. With these new additions the number of species of calcareous sponges reported from south of 50°S (~south of the Polar Front) reaches 50 species. We report an exceptionally high degree of endemism within the group, and as many as 44 out of the 50 species of calcareous sponges are solely confined to Antarctic waters. An updated list of species of calcareous sponges from the area is provided.

Key words: Clathrina brandtae sp. nov., Leucetta delicata sp. nov., SYSTCO, endemic species, Weddell Sea, deep-water sponges

Introduction

The calcareous sponges, or Calcarea Bowerbank, 1864, comprises the two sub-classes Calcinea Bidder, 1898 and Calcaronea Bidder, 1898. They all have a mineral skeleton composed entirely of calcium carbonate, consisting of free or rarely linked and cemented diactine, triactine, tetractine or polyactine spicules, sometimes in combination with a solid basal calcitic skeleton. More than almost 700 species have been described world-wide (van Soest et al. 2012), but recent investigations in poorly studied areas have indicated that the species diversity may be much higher (Wörheide & Hooper 1999; Rapp 2006; Azevedo et al. 2009; Rapp et al. 2011). The calcareous sponges are small sponges (millimeter to centimeter scale) and have traditionally been regarded as shallow-water organisms (van Soest et al. 2012). However, calcareous sponges have also been reported from bathyal and abyssal depths in the North Atlantic, the Southern Ocean and the North Pacific (Hansen 1885; Borojevic & Graat-Kleeton 1965; Koltun 1970; Barthel & Tendal 1993; Janussen et al. 2003; Rapp et al. 2011). Even though the calcareous sponges appear to be present in deep waters in all major oceans it is only in the Norwegian- and Greenland Seas that they represent a highly diverse and abundant part of the abyssal fauna (Rapp & Tendal, unpublished data).

The monophyletic origin of the calcareous sponges has been supported by a range of molecular phylogenies (Adams et al. 1999; Manuel et al. 2003; Dohrmann et al. 2006; Voigt et al. 2012). However, several of the traditionally accepted groups at order- family- and genus level have recently been shown to be non-monophyletic (Dohrmann et al. 2006; Rossi et al. 2011; Voigt et al. 2012).

Calcarea from the deep Antarctic shelf have been reported only on a few occasions, mostly from single specimens. For example, Koltun (1976) identified two species from a depth of 603 m and 640 m off Enderby Land and Adelie Land respectively, both in the Indian Ocean sector of Antarctica. Tanita (1959) sampled one calcareous
sponge from a depth of 570 m off Queen Mauds Land (Atlantic sector). Tendal described eight species from 11 stations dredged from depths of 400 to 890 m off Coats Land/Cape Norvegia in the eastern Weddell Sea (Tendal unpublished; partly mentioned without depth indications in Barthel et al. (1997)). The ANDEEP I-III expeditions (2002 and 2005) and SYSTCO I campaign provided new insight into the bathyal and abyssal benthic zone of the Southern Ocean, including numerous new records of the Porifera (e.g. Janussen et al. 2004; Brandt et al. 2007; Janussen & Tendal 2007; Plotkin & Janussen 2008; Janussen & Reiswig 2009; Göcke & Janussen 2011). Of particular interest was the discovery of five isolated specimens of calcareous sponges, three new to science, collected from five different bathyal and abyssal stations in the Weddell Sea (Rapp et al. 2011).

**TABLE 1.** List of species of Calcarea recorded in Antarctic waters south of 50°S (in alphabetical order). The list is based on original literature, World Porifera Database (van Soest et al. 2012), examination of original material and collections held by the authors and a re-evaluation of the records used in a recent publication on the distribution of sponges in the Southern Oceans (Downey et al. 2012). All together 50 species of calcareous sponges are considered to be part of the Antarctic sponge fauna (here defined as below 50 °S). As many as 44 species are considered endemic to Antarctic waters (in bold) while only six out of these 50 species have also been found elsewhere.

| Species                   | Year and Authors |
|---------------------------|------------------|
| Achramorpha glacialis     | Jenkin, 1908     |
| Achramorpha grandinis     | Jenkin, 1908     |
| Achramorpha nivalis       | Jenkin, 1908     |
| Achramorpha truncata      | Topsent, 1908    |
| Ascalitis abyssus         | Rapp et al., 2010|
| Ascalitis procumbens      | Lendenfeld, 1885 |
| Breitfussia chartacea     | Jenkin, 1908     |
| Breitfussia vitiosa       | Brøndsted, 1931  |
| Clathrina brandae sp. nov.|                  |
| Clathrina broendstedi     | Rapp et al., 2010|
| Dermatreton hodgsoni      | Jenkin, 1908     |
| Dermatreton scotti        | Jenkin, 1908     |
| Granitia hirsuta          | Topsent, 1907    |
| Granitia transgrediens    | Brøndsted, 1931  |
| Jenkina articulata       | Brøndsted, 1931  |
| Jenkina glabra           | Brøndsted, 1931  |
| Jenkina hiberna           | Jenkin, 1908     |
| Leucandra comata          | Brøndsted, 1931  |
| Leucandra conica          | Lendenfeld, 1885 |
| Leucandra frigida         | Jenkin, 1908     |
| Leucandra gausapata       | Brøndsted, 1931  |
| Leucandra levis           | Poléjaeff, 1883  |
| Leucandra mawsoni         | Dendy, 1918      |
| Leucascus leptoraphis     | Jenkin, 1908     |
| Leucusetta antarctica     | Dendy, 1918      |

The Antarctic sponge fauna has been characterized by a high degree of endemism (Janussen & Tendal 2007; Downey et al. 2012), many eurybathic species (Janussen & Tendal 2007) and a circumpolar distribution pattern (Janussen & Tendal 2007; Janussen & Rapp 2011; Downey et al. 2012). The Weddell Sea area is of special interest from a zoogeographical point of view as it seems to be a strong link between the sponge faunas of the Antarctic and South America (Downey et al. 2012). During the recent ANT XXIV/2 (SYSTCO I) expedition (2007/08), a very rich association of invertebrates (Mollusca, Polychaeta and Porifera) was collected from a station (#71/48-1) on the deep Eckström Shelf (eastern Weddell Sea) at 600 m depth. Porifera had the highest biomass and was among the
more abundant groups, primarily due to the presence of large Demospongiae and Hexactinellida (some more than 10 kg in wet weight), especially of the genus *Rossella* (Göcke & Janussen 2011; Göcke & Janussen, this volume). Apart from these, an unusual “mass occurrence” of the calcareous sponges *Jenkina articulata* was also recorded (Janussen & Rapp 2011). We here report on two more species of Calcarea from this locality and describe the new species *Clathrina brandtae* sp. nov., formerly reported as *Clathrina "primordialis"* at several localities in the Antarctic (Jenkin 1908; Brøndsted 1931; Koltun 1976; Barthel et al. 1997) and *Leucetta delicata* sp. nov. Based on published records and examination of published and unpublished collections we present a list of species of calcareous sponges found south of the Antarctic polar front (~ 50°S) (Table 1).

![FIGURE 1. Square: Sampling location in the Weddell Sea (SYSTCO 2008) and type locality of *Clathrina brandtae* sp. nov. and *Leucetta delicata* sp. nov. Asterisks: additional known records of *Clathrina brandtae* sp. nov.](image)

**Material and methods**

The material was collected on January the 12th 2008 with an Agassiz trawl (1.5 m width, 10 mm mesh size) during the ANT-XXIV/2, SYSTCO I Expedition on the Eckström Shelf (start trawling 70° 23.94' S, 8° 19.14' W, 602.1 m, end trawling 70° 23.89' S, 8° 18.67' W, 595.2 m), Eastern Weddell Sea, Antarctica (Figure 1). For more details on this station (# 71/48-1) and on other Agassiz trawl catches, see Janussen (2010) and Janussen et al. (2010). Spicule preparations and spicule measurements were made following standard procedures for calcareous sponges (e.g. Rapp 2006), and thick sections were produced from epoxy embeddings, following the standard procedures by Boury-Esnault & Bézac (2007) and Plotkin et al. (2012). Specimens were stained with Toluidine blue after
sectioning. The studied material is deposited at the Forschungsinstitut und Naturmuseum Senckenberg (SMF-No. 11866-11868). For barcoding purposes the internal transcribed spacer (ITS) was sequenced. DNA was extracted from ethanol-preserved specimens using the Qiagen Kit following the manufacturer’s instructions. The entire region comprising the two spacers (ITS1 and ITS2) and the 5.8S ribosomal DNA was amplified by PCR with primers anchored on 18S (5’ TCA TTT AGA GGA AGT AAA AGT CG 3’) and 28S (5’ GTT AGT TTC TTT TCC GCC GCT T 3’) (Lôbo-Hajdu et al. 2004) following the procedures described in detail in Rossi et al. (2011), only with minor changes in the PCR steps. Our PCR steps included 5 min at 95 °C, 35 cycles of 30 sec at 94 °C, 30 sec at 50-55 °C, and 1 min at 72 °C. The obtained sequences were edited using the Lazergene program package (v.6; DNASTAR, Inc., Madison, WI, USA). The Poriferan origin of the sequences was checked by BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

Systematics

Class Calcarea Bowerbank, 1864

Subclass Calcinea Bidder, 1898

Order Clathrinida Hartman, 1958 emend.

Family Clathrinidae Minchin, 1900

Genus Clathrina Gray, 1867

Type species: Clathrina clathrus (Schmidt, 1864).

Genus diagnosis. Clathrinidae in which the choanoderm is flat or rarely raised into conuli by the apical actines of the tetractines, but never forms true folds, at least when the sponge is in the extended state. The full-grown cormus comprises anastomosed tubes. The skeleton is composed of regular, equiangular and equiradiate triactines and/or tetractines, to which diactines and/or tripods may be added (from Borojevic et al. 2002).

Clathrina brandtae sp. nov.

(Figure 2, Table 2)

Synonyms and citations: Clathrina primordialis (Jenkin 1908 pars: 6; Brøndsted 1931 pars: 4-9; Koltun 1976: 163; Barthel et al. 1997: 46–47); Clathrina sp. nov. 14 (Klautau et al. 2013).

Type locality: Eckström Shelf, Eastern Weddell Sea, Antarctica. SYSTCO station 71/48-1 at 70° 23.94’ S, 8° 19.14’ W, 602.1 m depth to 70° 23.89’ S, 8° 18.67’ W, 595.2 m depth.

Type material: Holotype SMF 11867, paratype SMF 11866 (fragmented specimen), both from the type locality.

Additional material examined: EASIZ Expedition. Weddell Sea (1 incomplete specimen), st ps 39/24, 21.02.1996, 71°S8.15’W, 118–123 m depth (Barthel et al. 1997).

Etymology: Named after Professor Angelika Brandt for her great efforts organizing the ANDEEP and SYSTCO expeditions.

Description: Clathrinidae composed of a clathroid body of very irregularly and loosely anastomosing tubes. The cormus is narrower at the base than in the oscular region. The holotype is 1.5 cm high and 1 cm wide. There is one apical osculum. The paratype and additional material examined are fragmentary and the true shape of the cormus is not clear. Colour in life is yellowish beige and almost white in alcohol. No granular cells could be observed. Consistency is soft.

The skeleton is composed of regular triactines and regular to subregular tetractines. Tetractines is the most
abundant spicule type (Figure 2B). Spicules are intermingled without any organization (Figure 2C). However, the spicules of the single tubes are overlapping and therefore form a thicker layer than is found in the most simply built species of *Clathrina*. The apical actines of the tetractines are pointing into the interior of the asconoid tubes. Triactines are most common in the outermost layer of the tube wall. Otherwise there is no special orientation of the spicules.

**Spicules:** Triactines: They are regular with slender and sharply pointed and conical actines (102-153-200 μm x 7 μm) (Table 2, Figure 2).

| Spicule                | Length (μm) | Width (μm) |
|------------------------|-------------|------------|
|                        | min | max | mean | ± | mean | s | n |
| **Holotype, SMF 11867**|     |     |      |   |       |   |   |
| Triactines             | 102 | 200 | 153  | ±25.8 | 7.7 | ±0.5 | 30 |
| Tetractines            | 100 | 235 | 153  | ±23.9 | 6.5 | ±1.0 | 30 |
| Apical actine          | 50  | 138 | 82   | ±21.5 | 5.0 | ±0.7 | 30 |
| **Paratype, SMF 11866**|     |     |      |   |       |   |   |
| Triactines             | 123 | 200 | 159  | ±21.6 | 8.3 | ±1.7 | 30 |
| Tetractines            | 135 | 225 | 164  | ±20.2 | 7.3 | ±0.7 | 30 |
| Apical actine          | 50  | 113 | 87   | ±5.1  | 5.1 | ±0.9 | 30 |

**TABLE 2.** Spicule measurements of *Clathrina brandtae* sp. nov. Measurements are given as minimum, mean and maximum values. σ = standard deviation. n = number of spicules measured.

**FIGURE 2.** *Clathrina brandtae* sp. nov. A. Preserved Holotype from the Weddell Sea (SMF 11866) (os = osculum). B. Spicules of *C. brandtae* sp. nov. From left: ste = subregular tetractine, rte = regular tetractine, rtri = regular triactine and aa = apical actine of a tetractine. C. Wall of asconoid tube seen from the interior.

Tetractines: The tetractines are similar to triactines. Some are slightly subregular. Apical actine is long, smooth, slightly curved and sharp (100-153-235 μm x 6.5 μm, apical actine 50-87-113 μm x 5.1 μm) (Table 2, Figure 2).
Molecular identification: ITS1-5.8S-ITS2 sequences of the paratype has been deposited in GenBank under the accession number KC874655.

BLAST search confirmed the poriferan origin of the sequence, with *Clathrina contorta* (Minchin, 1905) as the closest match.

**Distribution:** Antarctic. Weddell Sea (this article and Barthel *et al.* 1997), Kemp Land (Koltun 1976), Kaiser Wilhelm II-Land (Brøndsted 1931) and the Winter Quarters (Jenkin 1908).

**Remarks:** The name *Clathrina primordialis* (Haeckel, 1872) has erroneously been used for different species of *Clathrina* from most oceans, representing the majority of *Clathrina* species with a skeleton consisting of spicules with sharply pointed actines (e.g. Arnesen 1900; Brøndsted 1931; Burton 1963). However, it is now well established that *C. primordialis* is a Mediterranean species with a skeleton solely composed of triactines (Klautau & Valentine 2003), and recent examination of material from a wide geographical range, previously identified as *C. primordialis*, have resulted in description of a number of new species of *Clathrina* (e.g. Klautau & Borojevic 2001; Rapp *et al.* 2001; Rapp 2006).

So far only one additional species of *Clathrina* is known from the Antarctic, namely *Clathrina broendstedi* Rapp *et al.*, 2011 (Table 1). *C. brandtiae* sp. nov. and *C. broendstedi* are very different as tetractines is the dominant spicule type in the former and the latter has only triactines. The loose and irregular anastomosis of tubes, large size of spicules, in combination with tetractines being the most abundant spicule type is rare within the genus. Based on organization of the cormus, organization of spicules as well as spicule size, *C. biscayae* Borojevic & Boury-Esnault, 1987 appears to be the closest. However, in *C. biscayae* the spicules are mainly parasagittal while in *C. brandtiae* sp. nov. they are regular. Recently it has been shown that the genus *Clathrina* is not monophyletic (Rossi *et al.* 2011; Voigt *et al.* 2012). Based on molecular and morphological evidence Rossi *et al.* (2011) suggested that the genus should comprise only species with a skeleton composed of triactines, and species with additional di- and tetractines should be transferred to other genera and/or new genera should be erected. However, as none of the new clades were defined and given names, we choose to place *C. brandtiae* sp. nov. provisionally in the genus *Clathrina*, awaiting formal description of the different groups within the old “*Clathrina*”. In a molecular phylogeny based on the D2 region of 28S as well as the entire region comprising the two spacers (ITS1 and ITS2) and the 5.8S ribosomal DNA, *C. brandtiae* clusters within a well-defined group also containing *C. biscayae* and with *Clathrina contorta* as the most closely related species (Klautau *et al.* 2013).

**Family Leucettidae de Laubenfels, 1936**

**Genus Leucetta** Haeckel, 1872

Type species: *Leucetta primigenia* Haeckel, 1872 (by original designation).

**Genus diagnosis:** Leucettidae with a homogeneous organization of the wall and a typical leuconoid aquiferous system. There is neither a clear distinction between the cortex and the choanoskeleton, nor is a distinct layer of subcortical inhalant cavities present. The atrium may be reduced to a system of exhalant canals that open directly into the osculum or may be a large cavity (modified from Borojevic *et al.* 2002).

*Leucetta delicata* sp. nov. (Figure 3, Table 3)

**Type locality:** Eckström Shelf, Eastern Weddell Sea, Antarctica. SYSTCO station 71/48-1 at 70° 23.94' S, 8° 19.14' W, 602.1 m depth to 70° 23.89' S, 8° 18.67' W, 595.2 m depth.

**Type material:** Holotype SMF 11868, from the type locality.

**Etymology:** Named from the easily compressible and delicate structure of this species of *Leucetta*.

**Additional material examined:** BMNH25.11.1.35a. *Pericharax carteri* var. *homorrhaphis* (Polejaeff, 1883). Challenger collection. Tristan da Cunha. Antarctic. Discovery Collection. BMNH 10.2.7.1a. *Leucandra microrhaphis* Haeckel, 1872. Antarctic Belgica Expedition. BMNH07.8.6.65a. *Leucandra primigenia* var. *leptoraphis* (Jenkin, 1908) (now defined as *Leucascus leptoraphis* (Cavalcanti *et al.* 2013)). SMF 10359. *Leucetta*
**Description:** The holotype is an ovoid and massive tube reaching 2.2 cm in height and 1.2 cm in width. Colour in life and in alcohol is beige (Figure 3A). Surface is smooth. Consistence is soft and compressible. The single apical osculum is naked. Below the osculum there is a small atrial cavity, smooth to slightly echinated because of single actines of choanosomal triactines piercing the atrial wall (may be an artefact due to collection or fixation). Numerous exhalant canals are dispersed in the atrium. The aquiferous system is leuconoid and the skeleton is disorganized, as typical in the genus. The cortex and the atrial walls are thin (Figure 3D–E), while the choanosome is thick. Triactines are the dominating spicules while very rare tetractines are found sparsely scattered in the choanosome. Sub-cortical spaces are present but poorly developed.

**FIGURE 3.** *Leucetta delicata* sp. nov. A. Preserved Holotype (SMF 11868). os = osculum. B. Spicules from *L. delicata* sp. nov. Bottom left: rte = regular tetractine with very short apical actine. Remaining spicules: rtri = regular triactines of variable size. C. Cross section of the body wall. cx = cortex. ca = canal. atr = atrium. D. Cross section of the cortical region. cx = cortex. cch and arrow = choanocyte chamber (scale bar as in E). E. Cross section of the atrial region. atr = atrium.
Spicules: Triactines: They are regular, equiradiate and equiangular. Actines are conical, with gradually tapering and sharply pointed tips. The size is variable, but the majority of the bigger spicules are tangentially arranged in the cortex. Some larger triactines can also be found in the choanosome (299-392-505 μm x 23.4 μm) (Table 3, Figure 3).

Tetractines: The basal system of these spicules is similar to those of the triactines. Apical actines are very short and irregular (134-213-309 μm x 13.1 μm, apical actine up to 15 μm long) (Table 3, Figure 3).

**TABLE 3.** Spicule measurements of holotype of *Leucetta delicata* sp. nov. Measurements are given as minimum, mean and maximum values. σ = standard deviation. n = number of spicules measured.

| Spicule          | Length (μm) | Width (μm) |
|------------------|-------------|------------|
|                  | min | max | mean | s  | mean | s  | n  |
| Triactines       | 299 | 505 | 392  | ±42.6 | 23.4  | ±3.9 | 50 |
| Tetractines      | 134 | 309 | 213  | ±62.5 | 13.1  | ±1.7  | 9  |
| Apical actine    | -   | 15  | -    | -    | -     | -    | 2  |

Molecular identification: A ITS1-5.8S-ITS2 sequence of the holotype has been deposited in GenBank under the accession number KC874654. BLAST search confirmed the poriferan origin of the sequence, with *Leucetta chagosensis* Dendy, 1913 as the closest match.

**Distribution:** Weddell Sea, known only from the type locality.

**Remarks:** Five species of *Leucetta* are at present known from Antarctic waters, namely *L. antarctica* Dendy, 1918; *L. apicalis* Brøndsted, 1931; *L. gelatinosa* (Jenkin, 1908); *L. pyriformis* (Burton, 1932) and *L. weddelliana* Rapp et al., 2011. Recent work on *Leucetta*, including the status of *L. microrhaphis* and *L. primigenia* has resulted in more limited distribution of these species and they are no longer considered part of the Antarctic fauna (Valderrama et al. 2009). Material representing species previously published as *Leucetta* spp or species potentially representing *Leucetta* from adjacent waters were studied to avoid confusion with *L. delicata* sp. nov. (listed under “additional material examined”). Our *L. delicata* sp. nov. bears the strongest similarities to *L. antarctica* and *L. weddelliana*. *L. weddelliana* and *L. delicata* sp. nov. both have triactines of very variable size, and the spicules cannot be divided into specific size classes. However, the triactines in *L. delicata* sp. nov are almost two times bigger than in *L. weddelliana*. In addition *L. delicata* sp. nov. presents rare tetractines dispersed in its choanoskeleton. *Leucetta antarctica* and *L. delicata* sp. nov. have triactines that are similar. However, the maximum size of triactines in *L. antarctica* is around 360 x 35 μm (Dendy 1918), while the average size is around 390 x 23 μm in *L. delicata* sp. nov. No tetractines have been found in *L. antarctica*, and while the tips of the actines are sharply pointed in *L. delicata* sp. nov they are slightly blunt in *L. antarctica*. In ongoing molecular phylogenetic analyses based on the D2 region of 28S as well as the entire region comprising the two spacers (ITS1 and ITS2) and the 5.8S ribosomal DNA, comprising a high number of clathrinid taxa, *Leucetta delicata* sp. nov. clusters within a well-defined group also containing *L. chagosensis* and with an unidentified Antarctic species of *Leucetta* as the most closely related species (Klautau et al. in preparation).

**Concluding remarks**

Our results support the general trend that Calcarea appears as a highly diverse but not very abundant group of sponges in Antarctic waters (Barthel et al. 1997; Rapp et al. 2011; Downey et al. 2012). Even in the Weddell Sea where the sponge fauna is fairly well-known, a large proportion of the calcareous sponges in new collections represent new taxa. Our review of published information about calcareans in the area, supported by the work of Downey et al. (2012) and World Porifera Database (van Soest et al. 2012), resulted in a list of 50 species considered valid for Antarctic waters below 50°S, which except from the Australian side represents the average position of the Antarctic Polar Front (Table 1). As many as 44 of these species are confined to Antarctic waters and only six species have been reported from elsewhere. Even though many species are represented by single records only, our results clearly indicate that there is an exceptionally high degree of endemism among Antarctic calcareous sponges. The work also revealed a great need of revision of most groups of calcareans in Antarctic waters.
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