Recycling resources: silica of diatom frustules as a source for spicule building in Antarctic siliceous demosponges

ANA RIESGO FLS[1*], SERGI TABOADA[1,2,3], NATHAN J. KENNY[1,4,●],
NADIA SANTODOMINGO FLS[1], JUAN MOLES[5,●], CARLOS LEIVA[1,6], EILEEN COX[1],
CONXITA AVILA[7], LUIS CARDONA[7] and MANUEL MALDONADO[8]

[1]*Department of Life Sciences, The Natural History Museum of London, Cromwell Road, London, SW7 5BD, UK*
[2]*Department of Biology, Faculty of Science, Universidad Autónoma de Madrid, C/ Darwin Street 2, 28049, Madrid, Spain*
[3]*Departament of Life Sciences, Apdo. 20, Universidad de Alcalá, 28805 Alcalá de Henares, Spain*
[4]*Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK*
[5]*Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, 02138 Cambridge, MA, USA*
[6]*Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Avinguda Diagonal 643, 08028, Barcelona, Spain*
[7]*Department Evolutionary Biology, Ecology, and Environmental Sciences, Faculty of Biology, University of Barcelona, Avinguda Diagonal 643, 08028, Barcelona, Spain*
[8]*Department of Marine Ecology. Center of Advanced Studies of Blanes, C/ Acess Cala St. Francesc 14, 17300, Blanes, Spain*

Dissolved silicon (DSi) is biologically processed to produce siliceous skeletons by a variety of organisms including radiolarians, silicoflagellates, choanoflagellates, plants, diatoms and some animals. In the photic ocean, diatoms are dominant consumers over competing other silicifiers. In Antarctica, where DSi is not particularly limiting, diatoms and sponges coexist in high abundances. Interestingly, diatom ingestion by sponges is a regular feeding strategy there. Although it was known that the diatom organic nutrients are readily metabolized by the sponges, what happened to the inorganic diatom silica skeleton remained unexplored. Here, we have conducted a multi-analytical approach to investigate the processing of diatom silica and whether it is reconverted into sponge silica. We have documented widespread diatom consumption by several demosponges, identifying storage vesicles for the diatom-derived silica by electron microscopy and microanalysis. Diatom-consuming sponges showed upregulation of silicatein and silicase genes, which in addition to the $\delta^{30}Si$ values of their silica, supports that the sponges are converting the ingested diatom silica into sponge silica without much further Si fractionation. Our multidisciplinary approach suggests that the reutilization of diatom silica by sponges is a common feature among Antarctic sponges, which should be further investigated in other latitudes and in other silicifiers.

ADDITIONAL KEYWORDS: Antarctica—biogenic silica—biosilicification—Porifera—siliceous skeletons—Southern Ocean.

INTRODUCTION

The biological use of minerals (biomineralization) dates back to Precambrian times *c.* 550 Mya and most likely evolved to provide protection against predation thus boosting the Cambrian explosion of diverse lineages (Cui *et al.*, 2016). Many living organisms use calcium phosphates and carbonates to build their defensive and/or structural skeletons, while comparatively few use silica. Biosilicification occurs in a variety of eukaryotes, from radiolarians and diatoms to plants and sponges (Volcani, 1983; Marron *et al.*, 2013).

*Corresponding author. E-mail: A.Riesgo@nhm.ac.uk

The biosilicification ability, which appears to have emerged independently in most eukaryotic lineages, is relatively well known in diatoms (Hildebrand *et al.*, 2018), but still poorly understood in most other groups. The consumption of dissolved silicon (DSi) in the form of silicic acid by living organisms to produce biological silicon dioxide (biogenic silica, SiO_2) is recognized to play a fundamental role in the biogeochemical cycle of silicon (Si) in both the modern ocean (Treguer *et al.*, 1995; Ragueneau *et al.*, 2000; Maldonado *et al.*, 2005; Struyf *et al.*, 2009; Maldonado *et al.*, 2010a; Maldonado *et al.*, 2012) and through geological time (Maldonado *et al.*, 1999; De La Rocha, 2003; Conley *et al.*, 2017). It is known that a natural or experimental DSi scarcity has detrimental consequences for diatom growth (Yool & Tyrrell, 2003) and sponge spicule formation (Maldonado *et al.*, 1999). Several studies show that the biosilicifying systems of sponges work optimally at DSi concentrations one to two orders of magnitude higher than those of diatoms (Frøhlich & Barthel, 1997; Maldonado *et al.*, 2011; López-Acosta *et al.*, 2016, 2018a) and that rarely occurs in the modern ocean. Consequently, it can be summarized that, unlike for planktonic diatoms, all species of siliceous sponges examined to date are limited by DSi availability in their shallow-water habitats (Frøhlich & Barthel, 1997; Maldonado *et al.*, 2011; López-Acosta *et al.*, 2016, 2018a), given that the modern ocean is depleted in DSi compared to previous conditions through geological time (Maliva & Knoll, 1989; Siever, 1991; Fontorbe *et al.*, 2016). In the Southern Ocean, where DSi is in principle not limiting (Cardinal *et al.*, 2005), diatoms are a major component of the phytoplankton both in diversity and abundance. Likewise, sponges are also major components of the benthos, structuring many Antarctic communities (Dayton *et al.*, 1974; McClintock *et al.*, 2005; Gutt *et al.*, 2013). The Antarctic sponge fauna consists of species that, on average, are more skeletonized (in terms of skeletal silica weight vs. organic weight) than sponges from other marine regions (Barthel, 1995). Unsurprisingly, it is in this particular environment where peculiar associations between diatoms and sponges have been reported (Gaino *et al.*, 1994; Bavestrello *et al.*, 2000; Cattaneo-Vietti *et al.*, 2000; Cerrano *et al.*, 2000, 2004a, b; Regoli *et al.*, 2004; Totti *et al.*, 2005; Núñez-Pons *et al.*, 2012), although diatom-sponge associations are not exclusive to Antarctica [e.g. also found off the north-east coast of Australia (Cox & Larkum, 1983)].

Until relatively recently, Si cycling in the ocean was thought to be largely under the biological control of diatoms (Ragueneau *et al.*, 2000), which use DSi to grow their frustules in a process regulated by sodium ions in marine species (Bhattacharyya & Volcani, 1980). However, over the last decades, the importance of sponges in the Si cycle has been increasingly recognized (Maldonado *et al.*, 2005, 2010a, 2011, 2019; Chu *et al.*, 2011; Tréguer & De La Rocha, 2013; López-Acosta *et al.*, 2018b). Three out of the four major sponge lineages (i.e. classes Demospongiae, Hexactinellida, Homoscleromorpha) need DSi to construct their silica skeletons (i.e. isolated spicules or frameworks). The available knowledge indicates that the DSi is polycondensated enzymatically around a proteinaceous, stick-shaped matrix (traditionally known as axial filament) (Shimizu *et al.*, 1998; Cha *et al.*, 1999; Krasko *et al.*, 2000; Müller *et al.*, 2003; Shimizu *et al.*, 2015). Although the silica forming the diatom frustules dissolves relatively quickly when directly exposed to seawater after diatom death, the silica of sponge spicules exposed to seawater is highly resistant to dissolution in both seawater and alkalis (Lewin, 1961; Maldonado *et al.*, 2005; Chu *et al.*, 2011), and it represents an important silicon sink at the ocean level (Maldonado *et al.*, 2019).

Most sponges are suspension feeders, known to feed on a variety of sources, including dissolved organic carbon (DOC), dissolved organic matter (DOM) and pico- and nanoplanktonic organisms (Reiswig, 1971; Ribes *et al.*, 1999; Pile *et al.*, 2003; de Goeij *et al.*, 2008). Sponges most commonly feed on nanoplankton (smaller than 2 µm), mostly bacteria (Ribes *et al.*, 1999), given the features of their choanocyte chambers and canal systems; however, several studies have already reported grazing on bigger organisms including diatoms, although to a much lesser extent than the prokaryotic fraction (Ribes *et al.*, 1999). Although choanocytes, epithelial flagellated cells lining the canals of most sponges, usually control particle uptake, sometimes the pinacocytes can pick up larger particles (Turon *et al.*, 1997; Maldonado *et al.*, 2010b). Gaino *et al.* (1994) were the first to observe active uptake of diatoms larger than 80 µm by the exopinacocytes of two Antarctic demosponges, *Phorbas glaberrimus* (Topsent, 1917) and *Tedania* (*Tedaniopsis*) *charcoti* Topsent, 1907. In 2004, Cerrano *et al.* (2004a) established that the main relationship between sponges and diatoms in the Southern Ocean was trophic, involving a seasonal use of diatoms as food source during the summer. The ability of sponges to digest diatoms was also observed in freshwater sponges (Frost, 1981; Gaino & Rebora, 2003). In both cases, the authors ambiguously suggested that the frustules might be somehow "incorporated into the sponge structures". Indeed, biosilicification in sponges is detrimentally affected by starvation, indicating that it is an energy consuming process. Therefore, reutilization by the sponge of the silica skeletons of the ingested diatoms may have energetic benefits.

To test such a hypothesis, we investigated the digestion of diatoms by six species of Antarctic demosponges using electron microscopy, analysed the elemental contents of their digestive vesicles compared to the spicules, examined the $\delta^{30}Si$ values of sponge and diatom silica and evaluated the levels of expression for the genes silicatein (directing sponge silica production) and silicase (directing sponge silica digestion for putative re-utilization). Our results will help to better understand the biological intricacies of silicon utilization, opening the possibility of an active biological recycling of silica among silicifying organisms.

MATERIAL AND METHODS

SAMPLE COLLECTION AND PRESERVATION

Samples of the sponge species *Haliclona penicillata* (Topsent, 1908), *Hemigellius pilosus* (Kirkpatrick, 1907), *Isodictya kerguelenensis* (Ridley & Dendy, 1886), *Kirkpatrickia variolosa* (Kirkpatrick, 1907), *Mycale* (*Oxymycale*) *tridens* Hentschel, 1914 and *Phorbas areolatus* (Thiele, 1905) were collected in February 2013 during the ACTIQUIM-4 campaign and in February 2016 during the DISTANTCOM-1 campaign in the south Shetland Islands. All sponges were collected by SCUBA diving on rocky outcrops at 15 m depth at Deception Island (62°59'31.2" S, 60°33'5.07" W).

For comparative genomic analyses, two individuals of *Phorbas tenacior* (Topsent, 1925) were collected in August 2016 in Santa Anna (41°40'22" N, 2°48'11" E, Blanes, Spain), and one individual of *Mycale laevis* (Carter, 1882) was collected by SCUBA diving on coral rock at 5–10 m depth at Bastimentos Island (9°20'44.93" N, 82°12'51.82" W, Bocas del Toro, Panama). Permits to collect samples in Spain were issued by the Spanish Ministry of Economy and Competitivity (CPE-EIA-2011–7 and CPE-EIA-2015–7). Permits for marine invertebrate specimen collection in Panama were issued by the Aquatic Resources Authorities of Panama (PO #212995).

SEM, TEM AND MICROANALYSIS

For transmission and scanning electron microscopy (TEM and SEM), we preserved several tissue replicates of three-four different individuals of all Antarctic species and *P. tenacior* in 2.5% glutaraldehyde in 0.4 mol/L phosphate-buffer saline (PBS) (with NaCl). Samples preserved for SEM and TEM were further rinsed using a solution of 0.6 mol/L NaCl and PBS and fixed for 1 h at 4 ºC in 1% osmium tetroxide-potassium ferrocyanide. No desilicification step was performed on any sample. For SEM, the samples were rinsed in PBS and distilled water several times, dehydrated through an ethanol series, critically point-dried, carbon-coated (20 nm) and imaged with a Zeiss Ultra Plus Field Emission Electron Microscope at the Natural History Museum, London (NHM). Samples preserved for TEM were dehydrated through a series of ethanol and propylene oxide and embedded in Spurr's resin for at least 3 days. Sections were performed using a ULTRACUT ultramicrotome, stained with lead citrate and uranyl acetate and observed with a JEOL 1010 electron microscope with a Gatan module for image digitalization at the Microscopy Unit at the Scientific and Technological Centre, University of Barcelona (CCiT-UB). Observations on the tissues of samples of *P. tenacior* showed that they were devoid of diatoms.

For elemental analysis, unstained thin sections of *M. tridens* and *P. areolatus* were coated with carbon (3–4 nm) to stabilize them at 200 kV for the analysis. Thin sections were then analysed using a JEOL JEM-2100 LaB6 transmission electron microscope with energy dispersed analysis of X-rays (EDX), operating at 200 kV in TEM mode with a spot size of ~15 nm at CCiT-UB. The spectrometer was an Oxford Instruments INCA X-Sight, with Si (Li) detector. Spectra acquisition was accomplished using the INCA Microanalysis Suite v.4.09 software. Images were taken with a Gatan CCD Camera Orius SC1000 and the Digital Micrograph v.1.71.38 software was used.

ISOTOPIC ANALYSIS OF SI IN SPONGES, DIATOMS AND SEAWATER

For isotopic analysis of silicon (Si) in sponge spicules, two replicated samples of *He. pilosus* and *P. areolatus* and one of *Ha. penicillata* were preserved in 70% ethanol and later dried completely. Organic matter was first removed in a 50% HNO_3/10% HCl solution at room temperature overnight, then rinsed three times in distilled water and any lithogenic particles removed by hand. About 10 mg of clean spicule material was re-cleaned for each sample using the same procedure. For isotopic analysis of Si in diatoms from the seawater off Deception Island, three replicates were collected by means of a 53 µm-plankton net of 25 cm diameter on an inflatable boat travelling at reduced speed (<1 knots) for 10 min (*c.* 15 m³ of water per replicate). Diatoms were later preserved frozen at -20 ºC. Finally, for isotopic analysis of the seawater surrounding Antarctic sponges of Deception Island, we collected 1 L samples of seawater that were stored at -20 ºC before analysis. Solid samples (sponge spicules and diatoms) and liquid samples (seawater) were sent to the NERC Isotope Geosciences Laboratory (NIGL) and the Centre for Environmental Geochemistry (CEG) of the British Geological Survey for analysis.

The dissolved $Si(OH)_4$ concentration in ~2 mL of seawater collected around the sponges was determined through inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Perkin Elmer model Optima 8300 at the CCiT-UB. After quantification, seawater samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS) using a silicon tetrafluoride tank gas to check for silica sand-optical (NBS-28) normalization. Determination of biogenic silica from sponge spicules and diatoms (~5 mg for each sample) was carried out using classical fluorination for the liberation of silicon gas at NIGL (Leng & Barker, 2006; Leng & Sloane, 2008). The SiF_4 produced was then cryogenically cleaned using dry ice/acetone slush and collected for analysis by isotope ratio mass spectrometry (IRMS) using a Finnegan MAT253 mass spectrometer for both $\delta^{29}Si$ and $\delta^{30}Si$. Data were reported relative to NBS-28.

The Si from the sample of seawater was extracted using the modified MAGIC method (Hendry *et al.*, 2010) starting with the addition of 2% (v/v) 1 M sodium hydroxide solution, and Si isotope analysis was undertaken using a Thermo Fisher Neptune Plus MC-ICP-MS at NIGL. The primary reference material used was NBS-28, with Diatomite serving as a secondary reference.

TRANSCRIPTOMIC ANALYSIS—RNA EXTRACTION AND TRANSCRIPTOME ASSEMBLY

For comparative genomic analysis between warm- and cold-water sponges, we used the congeneric species *P. areolatus* (Antarctic) and *P. tenacior* (Mediterranean), and *M. tridens* (Antarctic) and *M. laevis* (Caribbean). Only one individual of *M. tridens* and three of *P. areolatus* were preserved in RNAlater (Ambion) at 4 ºC during 1 day and later stored at -80 ºC. Two individuals of *P. tenacior* and one of *M. laevis* were also collected in RNAlater (Ambion), stored at 4 ºC for at least 1 day, and then further transferred to a -80 ºC until processed.

Total RNA of all samples was extracted using a standard protocol with TRIzol (ThermoFisher Scientific, UK) and mRNA later purified with the Dynabeads mRNA Direct Purification Kit (Thermofisher Scientific, UK) according to the manufacturer's protocols. This was used to produce an RNA library for next-generation sequencing using the ScriptSeq RNA-Sea Library Preparation Kit (Illumina, USA). Sequencing was performed on a single run of the Illumina NextSeq 500 Platform by the NHM Sequencing Unit at 2 × 150 bp read length in parallel with several other samples. The raw reads were subject to removal of adaptor sequences and initial quality screening. After analysing these reads with FastQC (Babraham Bioinformatics), we performed additional trimming with Trimmomatic (Bolger *et al.*, 2014) to remove areas of sequence with low Phred scores and any residual sequence (settings: ILLUMINACLIP:Adaptors.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:30, where Adaptors.fa contained the sequences of the adaptors used in sequencing). The remaining paired reads were then used to construct a transcriptome using Trinity v.2.4.0 (Grabherr *et al.*, 2011) using all default options. Raw reads can be accessioned at the Short Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under Bioproject numbers PRJNA572573, PRJNA572575, PRJNA612015 and PRJNA612016.

TRANSCRIPTOMIC ANALYSIS – IDENTIFICATION OF SILICON-RELATED GENES

In order to assess the expression levels of silicatein genes (involved in the production of silica spicules in sponges) and silicase genes (involved in the digestion of biogenic silica, putatively for its reutilization), we performed a comparative transcriptomic study using congeneric species from the genus *Phorbas* because we could collect replicates for both species. The assemblies used in our final analysis are the separate assemblies for *P. areolatus* (Antarctic), comprised of three replicates, and *P. tenacior* (Mediterranean), with two replicate samples (Table 1). Overall annotation for each assembly was performed with tBLASTx (Altschul *et al.*, 1990) and then Gene Ontology terms obtained with Blast2GOPRO (Conesa *et al.*, 2005). Also, gene sequences of silicatein and silicase were manually identified using tBLASTn searches (Altschul *et al.*, 1990) against gene sequences of known homology from sponges and other organisms downloaded from the NCBI *nr* database. Silicatein is a sponge-specific modified cathepsin L protein, and therefore cathepsin L sequences for other organisms were used here as outgroups (see Supporting Information, File S2). Similarly, silicases are known carbonic anhydrases (CA) (Schröer *et al.*, 2003), so several CAs were selected across a wide range of other organisms to serve as outgroups for sponge silicases (see Supporting Information, File S1). These were used as queries against databases created on a local server using BLAST v.2.7.1+ using the makeblastdb command. Genes putatively identified using this method and those obtained from the overall annotation were reciprocally BLASTed against the online NCBI *nr* database using BLASTx for initial confirmation of identity, followed by phylogenetic analysis as described below.

Table 1. Reference transcriptome statistics for the species used later in gene expression analyses: *P. areolatus* and *P. Tenacior*

	Phorbas areolatus	*Phorbas tenacior*
Min contig length	201	201
Max contig length	49 741	7210
Mean contig length	604.79	369.89
Median contig length	328	285
N50 contig length	911	364
Number of contigs	199 374	162 825
Number of contigs > = 1kb	25 943	5065
Number of contigs in N50	28 545	47 727
Number of bases in all contigs	120 579 096	60 227 729
Number of bases in contigs > = 1kb	57 806 865	7 517 921
GC content (%) of contigs	46.74	45.29

Table 2. Number of transcriptome reads used to determine differential expression

	Read count (after cleaning)	Sample accession
Phorbas areolatus		
Replicate 1	28 234 585	PRJNA572573
Replicate 2	18 630 435	
Replicate 3	37 291 892	
Phorbas tenacior		
Replicate 1	27 645 171	PRJNA572575
Replicate 2	11 605 700	

RESULTS

ELECTRON MICROSCOPY IMAGING AND ELEMENTAL ANALYSIS OF DIATOM-SPONGE CELL INTERACTIONS

No diatoms were observed in the tissues of the temperate sponge *P. tenacior*. We identified several species of diatoms living in the surface layers of the Deception Island waters, including species of the genera *Chaetoceros*, *Fragilariopsis*, *Navicula* and *Porosira* (Fig. 1). No signs of silica degradation or frustule dissolution were detected in any of the free-living diatoms observed by SEM. Many morphologically diverse, although not fully identified, diatoms were observed both in the extracellular medium of the mesohyl and within mesohyl cells of the investigated sponges, namely *Ha. penicillata*, *He. pilosus*, *I. kerguelenensis*, *K. variolosa*, *M. tridens* and *P. areolatus* (Figs 2, 3). When the diatoms occurred extracellularly in the mesohyl, sponge cells were often close to them, although not engulfing them (Fig. 2). In all species, we also detected diatoms being digested within sponge cells, although most prevalently in *K. variolosa* and *P. areolatus* (Fig 4). Depending on the size of the diatom, either one (Figs 4D–F, 5A, D) or several (Fig. 4A–C) sponge cells phagocytosed the diatom cells to digest them, forming a digestive vesicle for such purpose. Frequently, silica-like granules appear close to the diatom case fragments (Figs 4F, 5A, D), seemingly derived from a process of silica digestion performed by the sponge (see further sections of *Results* for detection of silicase enzymes). We also observed sponge cells containing electron-dense, silica-like granular accumulations in vesicles of the cytoplasm in *H. pilosus*, *M. tridens* and *P. areolatus* (Figs 5, 6), that later accumulated in the cytoplasm as non-membrane-bound aggregations (Figs 5B, D, 6A). The content of those vesicles (Fig. 6A, B) was consistently identified by elemental microanalysis in *M. tridens* and *P. areolatus* as being silica (Fig. 6E, F).
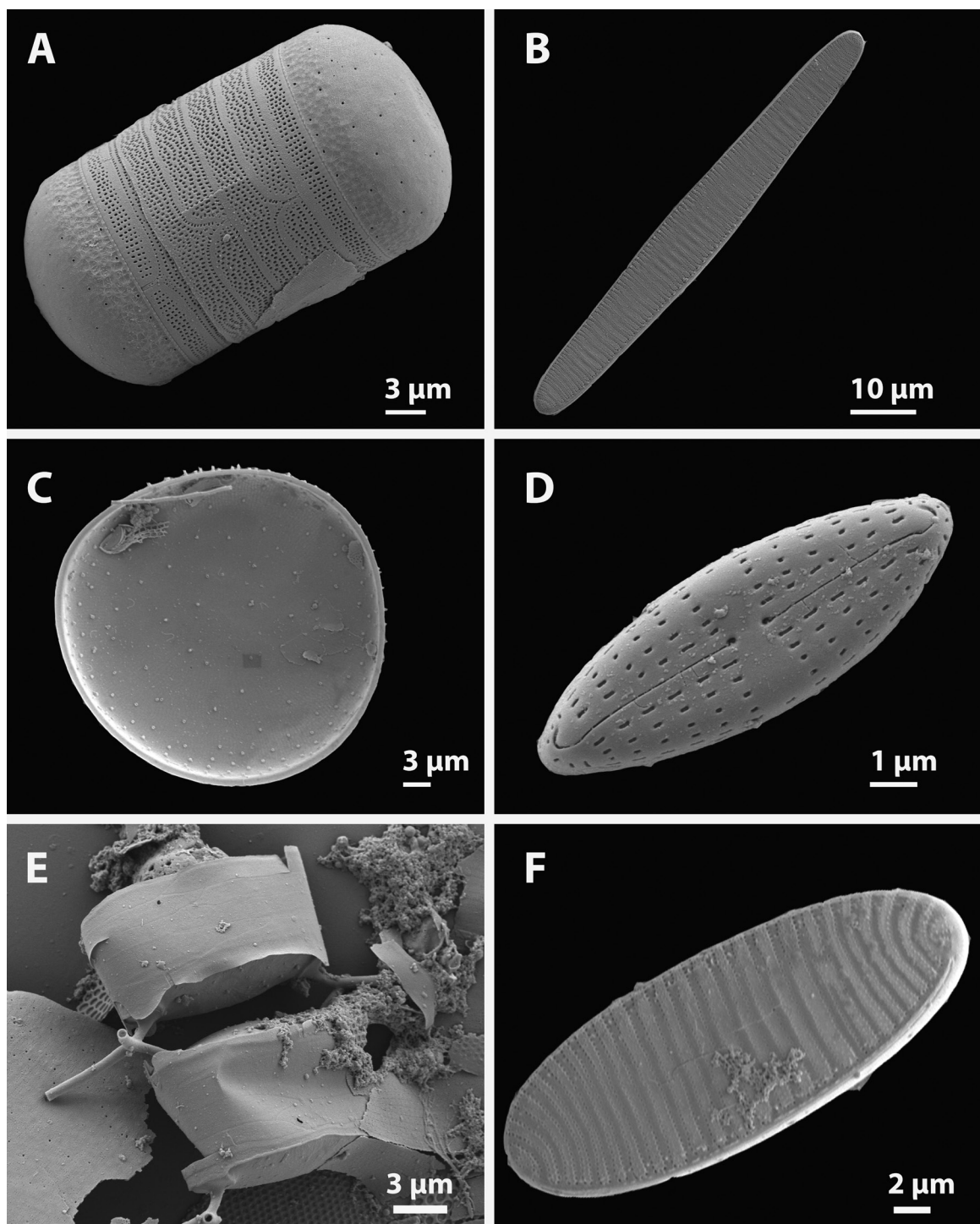
TRANSCRIPTOMIC ANALYSIS—COMPARATIVE EXPRESSION

Comparative expression of the two target genes was assayed by mapping reads (Table 2) from individual replicates to the combined assemblies using bowtie2 (Langmead & Salzberg, 2012) and RSEM (Li & Dewey, 2011) as packaged in the Trinity module (Grabherr *et al.*, 2011) to compare expression in each replicate sample. We then ran cross-sample normalization: Trimmed Mean of M-valies (TMM) and the TMM-normalized TPM (transcripts per million) values for expression are reported here. Significant differences in the expression levels of the genes between species were assayed using a *t*-test in R (R Core Team, 2013).

PHYLOGENETIC ANALYSIS

MAFFT v.7 (Katoh & Standley, 2013) was used to align amino acid sequences of silicatein and silicase, separately, using the L-INS-i strategy (available in Supporting Information, File S3). Sequences were curated using Gblocks (Castresana, 2000) with all lenient settings, followed by removal of sites with gaps. The Bayesian phylogenetic analysis was performed with MrBayes v.3.2.2 x64 software (Ronquist *et al.*, 2012) using mixed models for initial identification of the fittest model for the data. A Markov chain Monte Carlo search was run over at least 1 000 000 generations. Trees were sampled every 1000 generations and the first 25% of trees gathered were discarded as 'burn-in'.

Figure 1. Free-living diatoms of Deception Island. A, external frustule of *Porosira* sp. B, external valve view of *Fragilariopsis* sp. C, internal view of *Porosira* sp. valve. D, external valve of *Navicula* sp. E, two frustules of *Chaetoceros* sp. Note the fused setae linking the cells. F, external view of *Fragilariopsis* sp.

However, when the analysed vesicles were identified as silica deposition vesicles of sclerocytes making spicules (Fig. 6B) rather than mere digestive vesicles (Fig. 6A), the silica was found out to incorporate lead, iron, polonium and aluminium (Fig. 6F). In contrast, relatively pure silica consisting of only silicon and
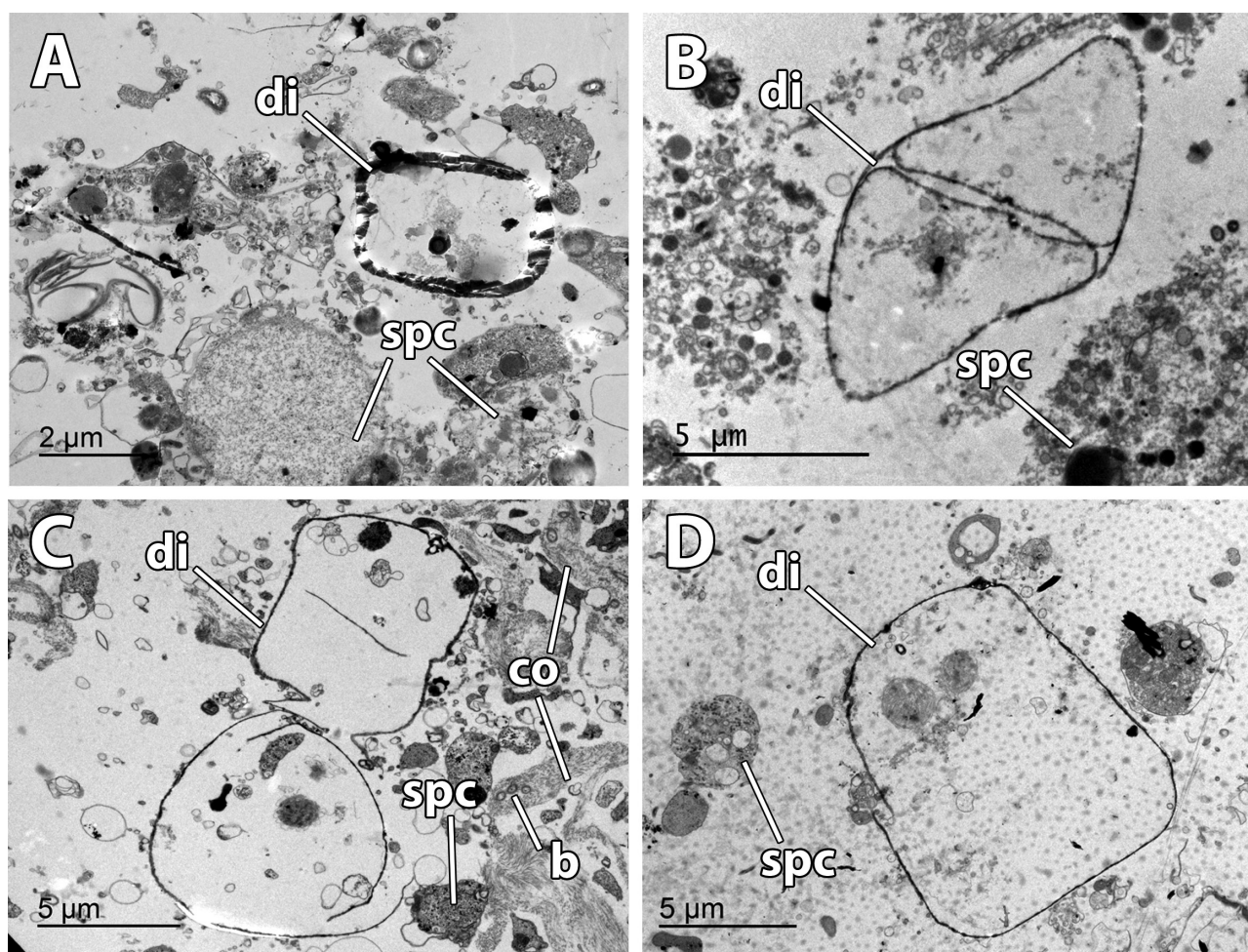
Figure 2. Diatoms within sponge tissues. A, diatom in the mesohyl of *P. areolatus*. B, diatom in the mesohyl of *I. kerguelenensis*. C, diatom in the mesohyl of *Ha. penicillata*. D, diatom in the mesohyl of *K. variolosa*. Abbreviations: b, bacteria; co, collagen; di, diatom; spc, sponge cell.

oxygen (Fig. 6E) was detected in the vesicles identified as digestive organelles. Interestingly, microanalysis of diatom frustules within the sponge tissues detected only silicon and oxygen (Fig. 6C, G), whereas the sponge spicules contained lead, magnesium and iron in addition to silicon and oxygen (Fig. 6D, H) in detectable amounts.

ISOTOPIC ANALYSIS

The DSi concentration of the local water is 5.01 ppm (83.39 µmol/L). Figure 7 and Table 3 show the profiles of measured δ^{30}Si and δ^{29}Si in the sponge spicules, as well as those for diatom frustules collected from the upper layer of the seawater column in Deception Island and the seawater itself. As sponges typically fractionate Si isotopes more intensely than diatoms,

the silica of the sponge spicules (i.e. *Ha. penicillata*, *He. pilosus* and *P. areolatus*) was depleted in ^{30}Si compared to the silica of the frustules of the local diatom species, which in turn were depleted in ^{30}Si compared to the silicic acid dissolved in the local sea water (Table 3). On average, the five δ^{30}Si values obtained for the three diatom-feeding sponges was -1.91 ± 0.29, while a collection of 20 other sponges from the same Antarctic area (Hendry *et al.*, 2010; Wille *et al.*, 2010) reported an average of -2.63 ± 0.89. The fact that the diatom-feeding sponges show, on average, a less intense fractionation than other sponges from adjacent sites supports the view that the former ones are directly re-using Si atoms that have been carried into their silicifying cells through phagocytosis in the form of poorly fractioned diatom silica rather than to standard transport vies for soluble DSi.
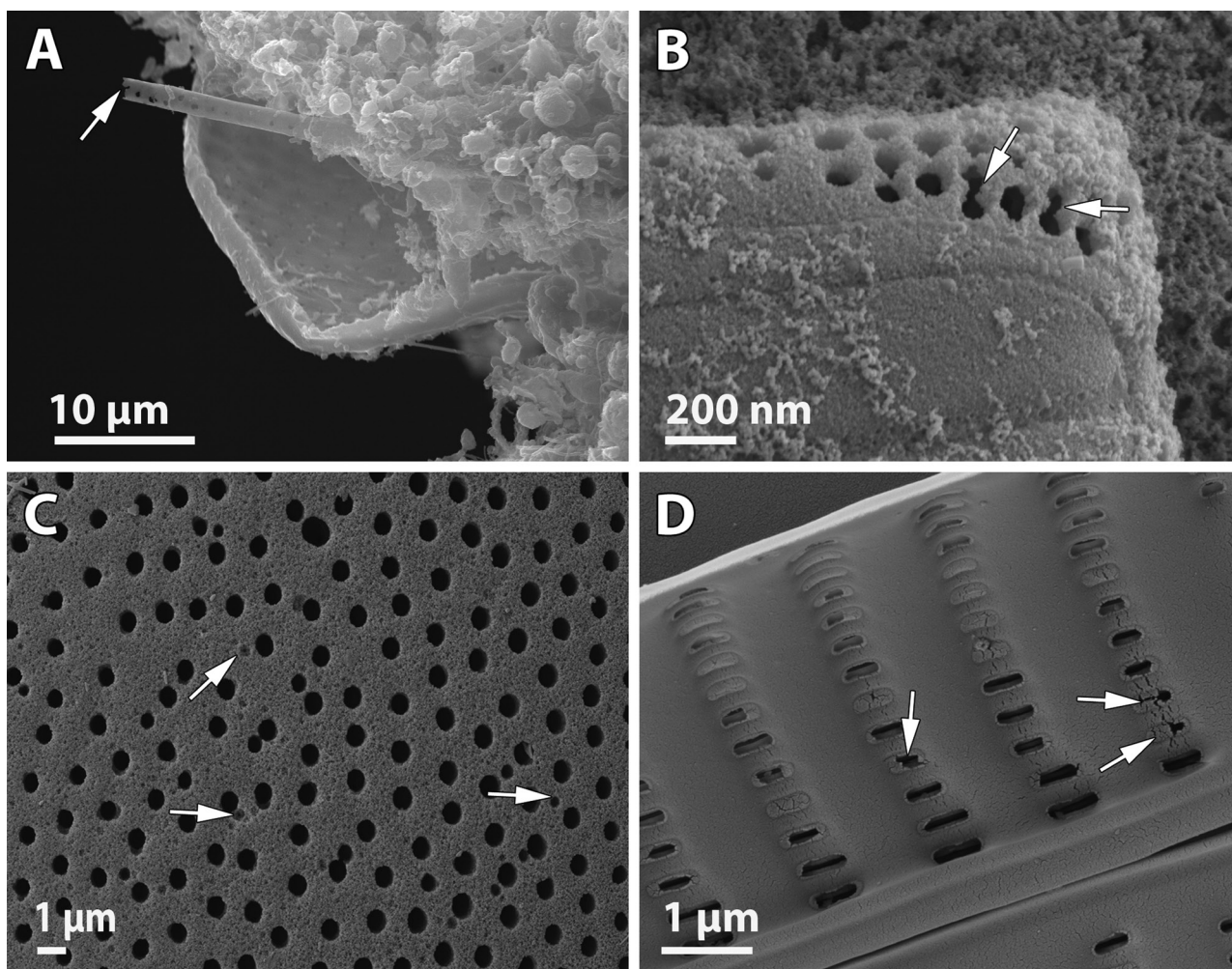
Figure 3. Signs of silica dissolution in diatom frustules and valves. A–B, diatoms in the tissue of *M. tridens*. C, *Podosira* sp. valve in *P. areolatus*. D, *Navicula* sp. valve within *He. pilosus*. Arrows: evidence of silica dissolution or degradation in diatoms.

TRANSCRIPTOMIC AND PHYLOGENETIC ANALYSES

The expression levels of both silicatein and silicase genes were higher in the Antarctic *P. areolatus* than in its temperate congener *P. tenacior* (Table 4). Interestingly, the expression levels of the genes for silicatein ($P = 0.02$) and silicase ($P = 0.01$) were between 10 and 20 times more in one individual of *P. areolatus* than in *P. tenacior* (Table 4).

Silicase is a carbonic anhydrase or CA (Schröer *et al.*, 2003), which is a huge family composed of α-, β-, γ-, δ-, ζ- and η-CA proteins, mostly known from bacteria involved in pH regulation, HCO_3^- reabsorption and CO_2 expiration (Hewett-Emmett, 2000; Sun & Alkon, 2002). We performed a phylogenetic analysis of the translated protein sequence for silicase*s* to understand their evolutionary relationship within the group. Our analysis showed that they formed a well-supported and distinctive monophyletic clade among the carbonic anhydrase protein family (Supporting Information, File S1). Silicases in warm-water and cold-water *Mycale* spp. and *Phorbas* spp. clustered exclusively with silicases from other poecilosclerid sponges (Supporting Information, File S1). Interestingly, only in *P. areolatus* was there an additional, highly-expressed paralogue of silicase that was not present in *P. tenacior* (Table 4; Supporting Information, File S1). Although most of the silicatein sequences of *Mycale* and *Phorbas* seemed to cluster in the same clades, at least two clades contained exclusively *Mycale* sequences for silicatein and no paralogues from any *Phorbas* spp. (Supporting Information, File S2). Our results indicate duplication before species divergence and later loss in *Phorbas* or species-specific expansions in the genus *Mycale*. Interestingly, silicatein genes have expanded in the Antarctic species *P. areolatus*, but not in the Antarctic *M. tridens*, with the silicatein complement of the
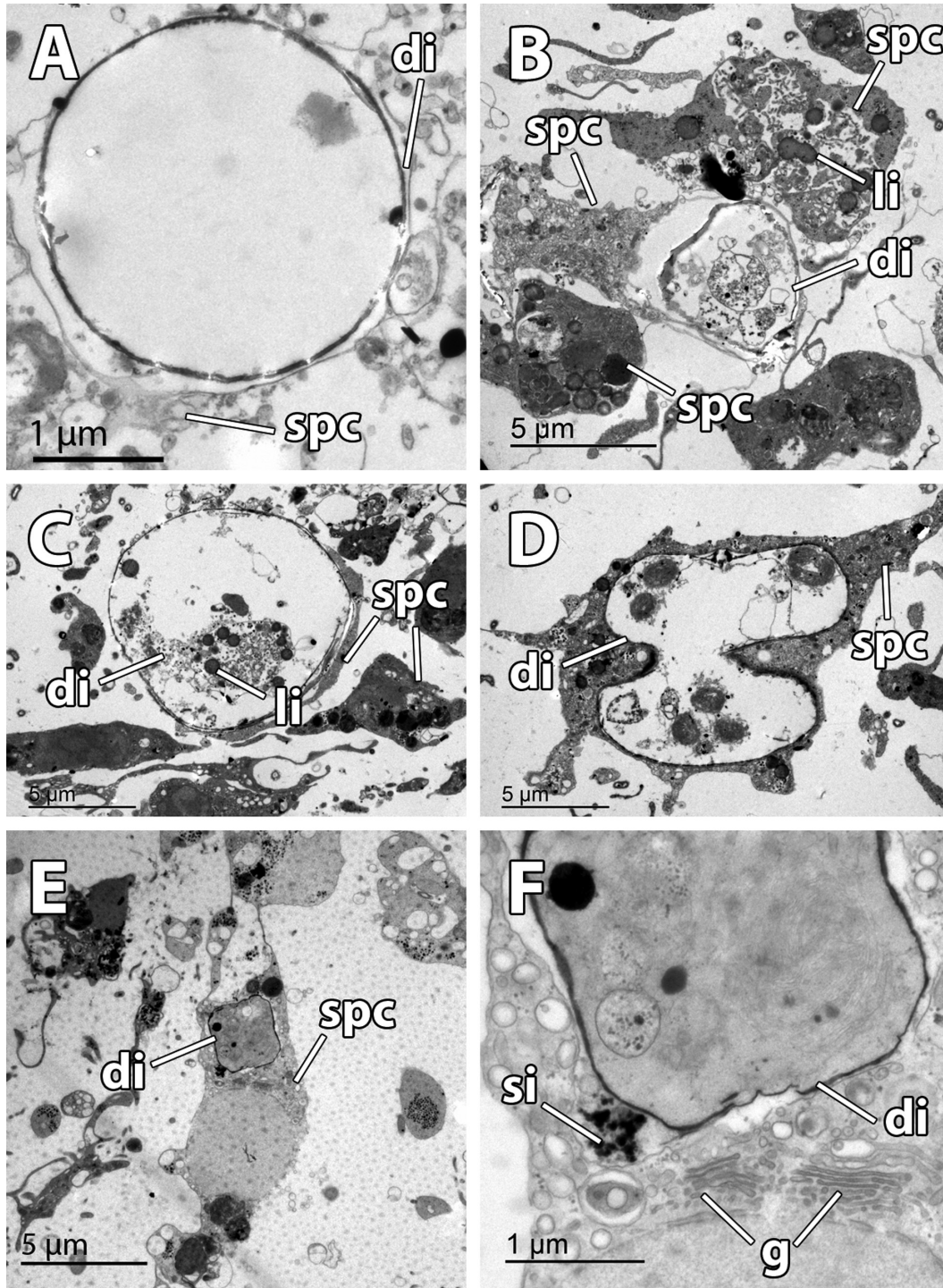
Figure 4. Diatoms (di) ingested by amoeboid sponge cells (spc). A–D, sponge cells (spc) digesting diatoms (di) in *P. areolatus*. Note the large lipid (li) droplets present within diatoms (di) and later accumulated in the cytoplasm of sponge cells (spc). E–F, sponge cells (spc) digesting diatoms (di) in *K. variolosa*. Note the silica-like (si) granules being dissolved from the diatom frustule and the well-developed Golgi apparatus (g).
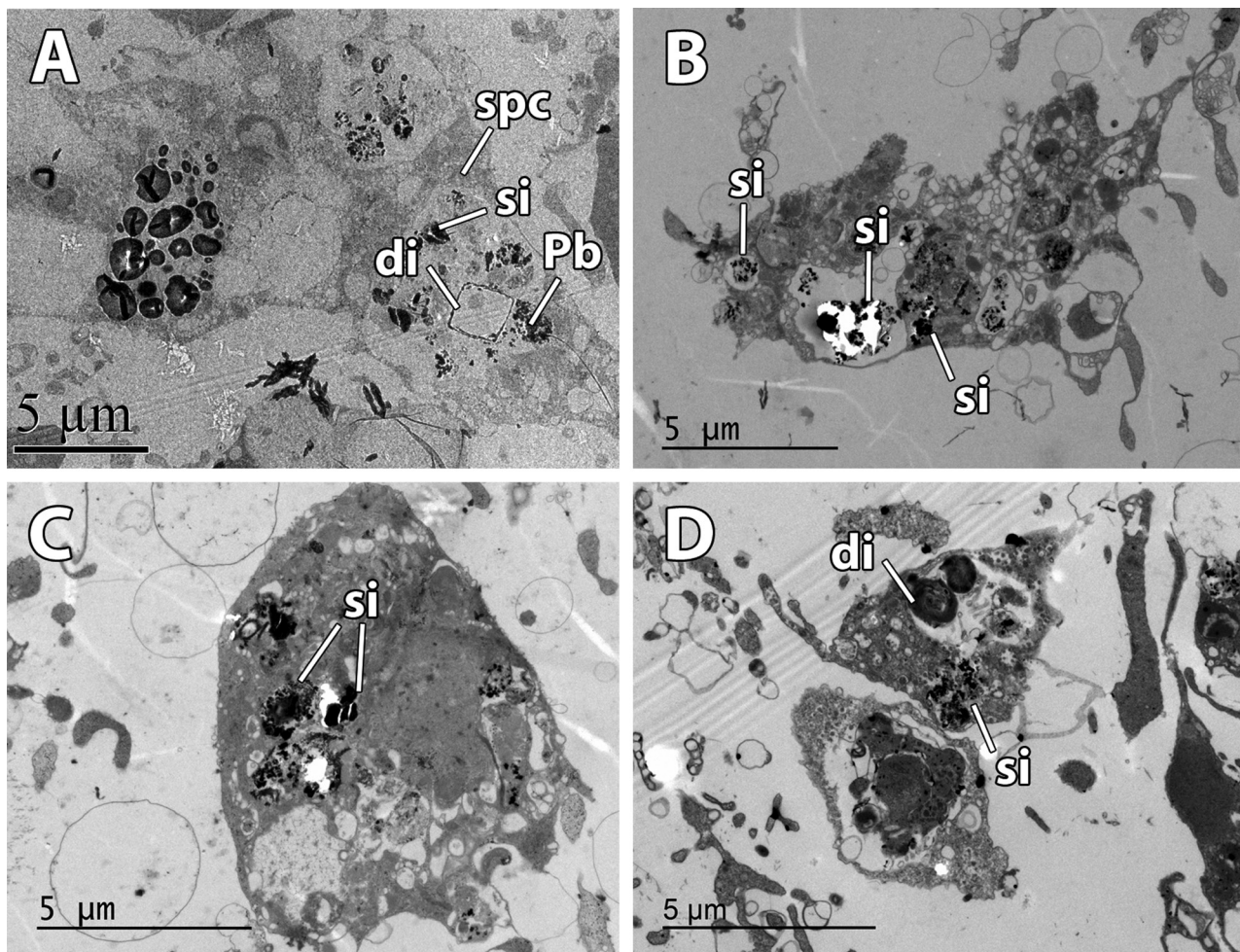
Figure 5. Vesicles with silica-like granules within sponge cells. A, an amoeboid sponge cell (spc) in *P. areolatus* showing a cytoplasm packed with vesicles and an ingested diatom (di) within a large digestive vesicle. Note that three different granule types were identified by microanalysis in the section of this species: silica granules (si), and lead granules (Pb). B–C, highly vesiculated, amoeboid sponge cell in *He. pilosus*. Note the silica-like granules (si) first present within vesicles and later incorporated within the cytoplasm. D, amoeboid sponge cell in *P. areolatus* showing an ingested diatom (di) and silica-like granules (si).

tropical *M. laevis* being more abundant (Supporting Information, File S2).

The simultaneous overexpression of the silicifying enzyme silicatein and its antagonistic silica-digesting enzyme is in full agreement with the hypothesis that the sponges are disassembling the diatom's silica to reuse it in the elaboration of their own siliceous spicules.

DISCUSSION

Our study presents evidence of diatom consumption by at least some Antarctic demosponges, which are able to incorporate and digest large diatom cells, with fragments of their silica frustules ending stored within vesicles of sponge amoeboid cells. The ingested diatoms were not selected by the sponge, but reflected the taxonomic composition to the planktonic diatoms in the area Cerrano *et al.*, 2004a, b; Totti *et al.*, 2005). Diatom consumption has been long reported in sponges. In 1971, Reiswig found that the sponges he studied showed significantly less uptake of armoured cells (mostly diatoms) over 5 μm (and up to 100 μm) than unarmoured cells or bacteria, although some species showed 100% uptake of large diatoms (Reiswig, 1971). In 1981, Frost studied the diatom consumption of a freshwater sponge, finding a preference for particular diatom species, documenting large numbers of empty frustules within the sponge body, and suggesting that the frustules may be "incorporated into the sponge structures" (Frost, 1981), a hypothesis
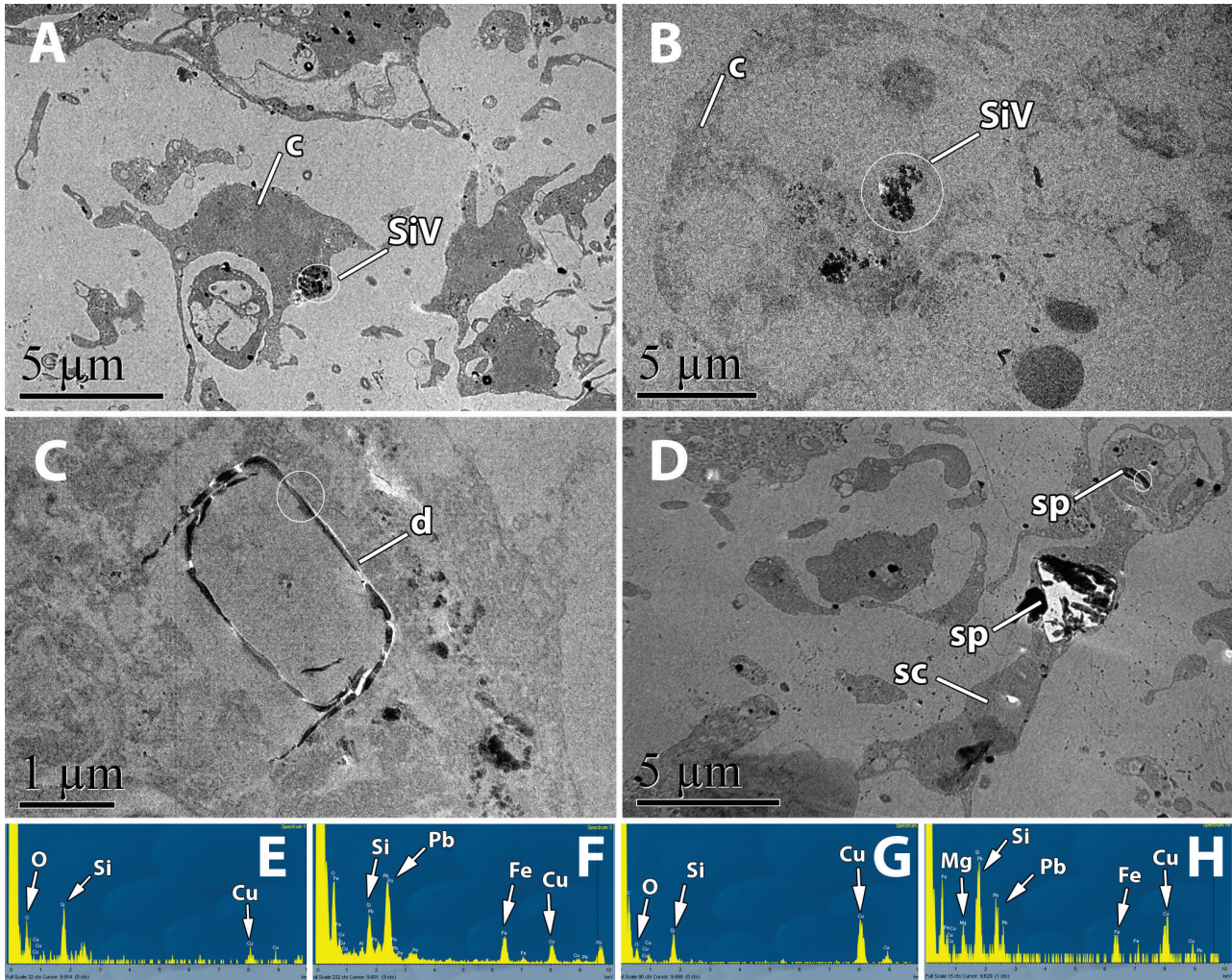
Figure 6. Microanalysis of the content of silica-like vesicles, sponge spicules and diatoms within the sponge tissues. A, sponge cell, probably an amoebocyte (c), of *P. areolatus* showing accumulation of silica-like granules in the cytoplasm (SiV). B, sclerocyte-like cell (c) of *M. tridens* showing accumulation of silica-granules in vesicles (SiV). C, diatom (di) engulfed by a sponge cell in *M. tridens*. D, sclerocyte (sc) of *P. areolatus* making spicules (sp). E, elemental profile of Figure 6A. F, elemental profile of Figure 6B. G, elemental profile of Figure 6C. H, elemental profile of Figure 6D. Note that the EDX probe measurements were taken on the white circles marked in the images.

that was later confirmed (Gaino & Rebora, 2003). In the Mediterranean, diatom grazing by sponges was found to be higher during the winter than during the summer, although still only contributing up to 26% of their diet during the maximum consumption episodes (Ribes *et al.*, 1999). In contrast, diatoms were avidly consumed by several species of Antarctic demosponges, and their frustules were exclusively found in the sponge tissues during the Antarctic summer (Cerrano *et al.*, 2004a, b; Totti *et al.*, 2005; Núñez-Pons *et al.*, 2012). The occasional or frequent consumption of diatoms by at least some demosponges suggests that a mechanism for dealing with the mineral frustule should be in place, either egestion along with other refractory food components or disassembling and reutilization of its silica.

Diatoms are usually large armoured cells (see Fig. 1) and the ability to capture them by filter feeding is related to the size of the inhalant orifices and internal aquiferous canals; also the size of the cells that have to digest them, whether they are choanocytes, amoebocytes or pinacocytes. In general, the filter-feeding of sponges is designed to deal with smaller food particles. Wolfrath and Barthel (1989) found that *Halichondria panicea* (Pallas, 1766) did not ingest anything larger than 4.5 μm and the deep-sea sponge *Thenea abyssorum* Koltun, 1964 could not uptake anything larger than 6 μm. The diatoms occurring in
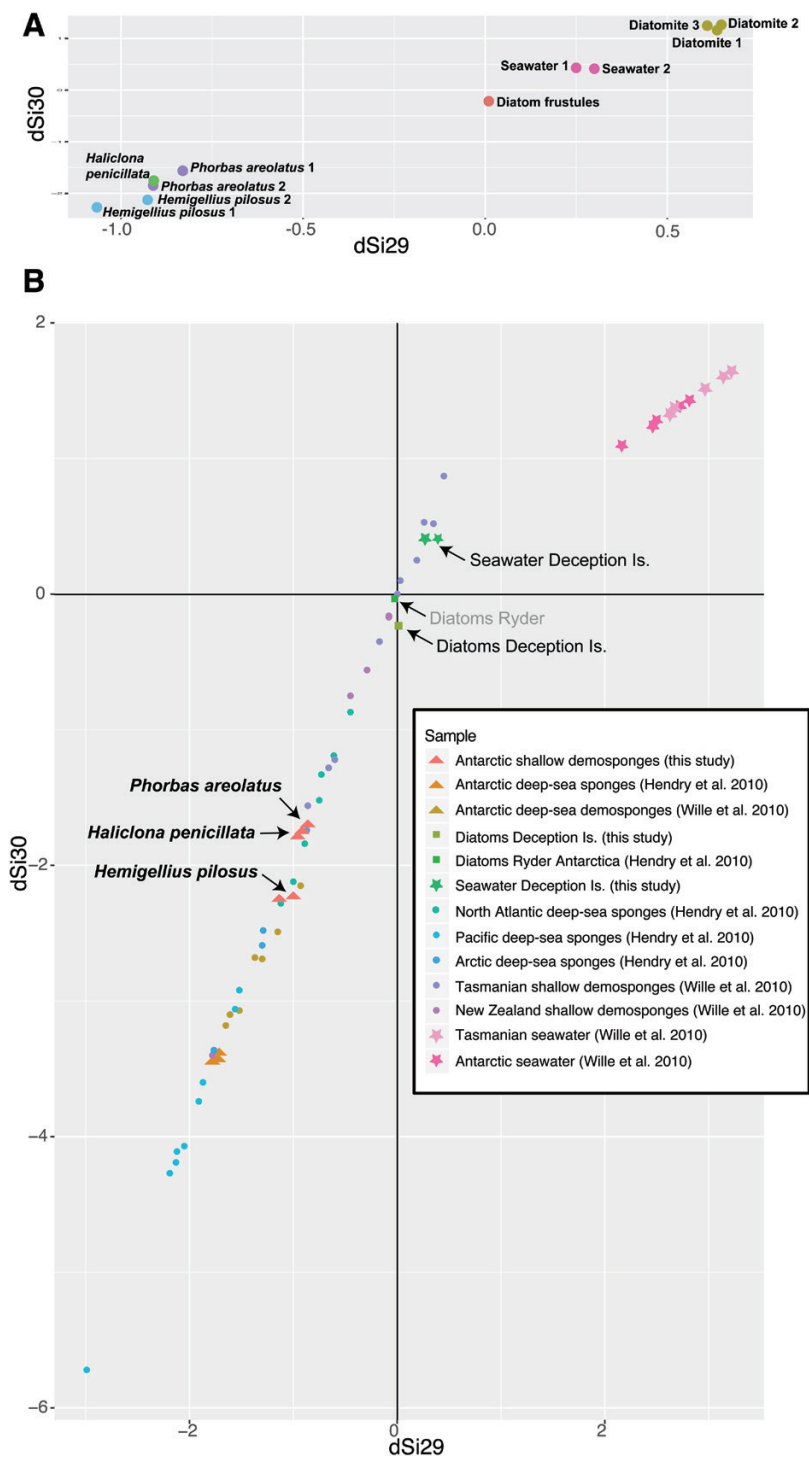
Figure 7. Silicon isotope data of sponge spicules, free-swimming diatoms and seawater. A. Silicon isotope (δ^{30}Si) composition for sponges, diatoms and seawater of Deception Island. B. Comparison of our data for δ^{30}Si/ δ^{29}Si in sponges, diatoms and seawater from Deception Island, with values reported in previous datasets of sponges, diatoms and seawater (Hendry *et al.*, 2010; Wille *et al.*, 2010).

Table 3. Silica fractionation values

Sample identity	dSi29	dSi30	Si29/Si30
Hemigellius pilosus	-1.06	-2.30	1.9
Hemigellius pilosus	-0.93	-2.11	2.0
Phorbas areolatus	-0.83	-1.57	1.6
Phorbas areolatus	-0.91	-1.83	1.7
Haliclona penicillata	-0.91	-1.74	1.6
Diatom frustules	0.01	-0.229	-15.9
Seawater_1	0.25	0.43	0.58
Seawater_2	0.30	0.40	0.75
Diatomite_1	0.64	1.22	0.52
Diatomite_2	0.65	1.26	0.52
Diatomite_3	0.61	1.25	0.49

the mesohyl of the Antarctic species we analysed here could have been taken up by pinacocytes in the canal system, captured by choanocytes, or slipped through slits in choanocyte chambers; however, unfortunately we could not observe or trace the capture mechanism *in vivo*. It was not evident to us that that the Antarctic demosponges had special adaptations in their morphological features aimed to capture diatoms, but of course this should be studied in greater detail.

The elemental composition of the electron-dense particles resulting from the digestion of diatoms was consistent with silica, that is, silicon and oxygen. In contrast, the siliceous spicules of the sponges incorporated additional detectable elements, such as lead, magnesium and iron. Analyses of spicules of other sponges available in the literature testify for both spicules containing exclusively silicon and oxygen (Uriz *et al.*, 2000) and spicules containing detectable and traces of aluminium, potassium, calcium and/ or sodium (Sandford, 2003; Uriz, 2006). Most trace elements present in the herein investigated spicules are also available at high concentrations in the coastal waters of Deception Island (Elderfield, 1972; Deheyn *et al.*, 2005) because of the local geothermal activity (an active volcano). Iron and lead were also detected at high concentrations in benthic organisms such as sea urchins from Deception Island (Deheyn *et al.*, 2005). Traces of elements other than silica have been found in the frustules of the Antarctic diatom *Porannulus contentus* Hamilton, Poulin, Yang & Kloser 1997, in particular iron, copper and aluminium (Hamilton *et al.*, 1997), although such diatoms are usually found on the outside of sponges [*Microxina phakellioides* (Kirpatrick, 1907), *Mycale (Oxymycale) acerata* Kirkpatrick, 1907, *Polymastia isidis* Thiele, 1905 and *Tedania (Tedaniopsis) charcoti*], being only rarely incorporated into their choanosome (inside) (Cerrano *et al.*, 2004a,b; Totti *et al.*, 2005). A possible

explanation for the absence of other trace elements in the frustules of the digested diatoms could be that most of the ingested diatom species grow their frustules out in open-water blooms, rather than in the bottom-water layer around Deception Island. In any case, the results from the different approaches are consistent in pointing towards the digestion of diatoms and accumulation of their silica leftovers in intracellular vesicles, which appear to be later reused for new spicule elaboration.

The δ^{30}Si values of the diatom-feeding sponges, being slightly more negative than those of the known average for Antarctic sponges, also support the view that the silica of the diatom-feedings sponges may well have derived from the direct reutilization of diatom silica, which is typically less fractionated than the sponge silica. Indeed, the DSi concentration around Deception Island is larger than 50 μmol/L and hence, if the investigated sponges were not reusing diatom silica, a stronger fractionation of their silica should be expected (Wille *et al.*, 2010). Recycling of diatom silica, which is enriched in ^{30}Si, could explain the relatively high δ^{30}Si values observed in sponges from Deception Island, particularly in *Ha. penicillata* and *P. areolatus*. These findings support the hypothesis that the diatom silica is not completely redissolved by the sponges to soluble DSi, but disassembled down to the level of silica nanospheres and then reutilized.

The molecular pathways involved in siliceous spicule formation of demosponges are only partially understood (Ehrlich, 2011); however, the simultaneous overexpression of silicatein and silicase detected in the diatom-feeding sponges is also consistent with the digestion of diatom's skeletons and the reutilization of the resulting nanospheres to build new spicules. Because DSi concentrations in the Southern Ocean are relatively high during most of the year (except after major diatom spring blooms), DSi is not limiting to the sponges. Therefore, at first sight there would be no particular reason for the sponges to select for the solid ingested silica of diatoms versus the externally available DSi. However, a potential reason could be that the use of silicase to partially digest the diatom silica to the nanosphere stage and then using the nanospheres to produce new spicules through the action of silicatein could be energetically less costly than any *de novo* spicule production starting from DSi. Indeed, previous studies on sponge silicification have shown that silica nanospheres (75 nm) are commonly produced during the early steps of the spicule formation process (Weaver *et al.*, 2003). In Figure 8, we provide a tentative explanation about where these several steps could happen within the sponges. The traditional route for silica uptake and use in sponges involves a step of silica influx into the cell via co-transporters (Fig. 8). However, in

Table 4. Expression levels (TMM normalized TPM values) for biosilicification-related genes for each sample

| Transcript name | *Phorbas areolatus* | | | | Transcript name | *Phorbas tenacior* | | |
	Individual 1	Individual 2	Individual 3	Average		Individual 1	Individual 2	Average
Silicase								
TRINITY_ DN35977_c0_g1	46.13	5.48	3.80	18.47	TRINITY_ DN28741_c1_g1	0.85	0.55	0.70
TRINITY_ DN6156_c0_g1	17.98	0.78	0.97	6.58	No clear paralogue			
TRINITY_ DN8651_c0_g1	52.72	6.15	9.31	22.73	No clear paralogue			
No clear paralogue					TRINITY_ DN31186_c0_g1	0.89	0.72	0.80
Silicatein								
TRINITY_ DN3898_c0_g2	6.36	4.13	6.03	5.51	TRINITY_ DN6084_c0_g1	0.94	1.21	1.07
TRINITY_ DN34142_c0_g1	6.19	8.31	12.32	8.94	No clear paralogue			
TRINITY_ DN35339_c0_g1	0.80	3.60	3.13	2.51	No clear paralogue			
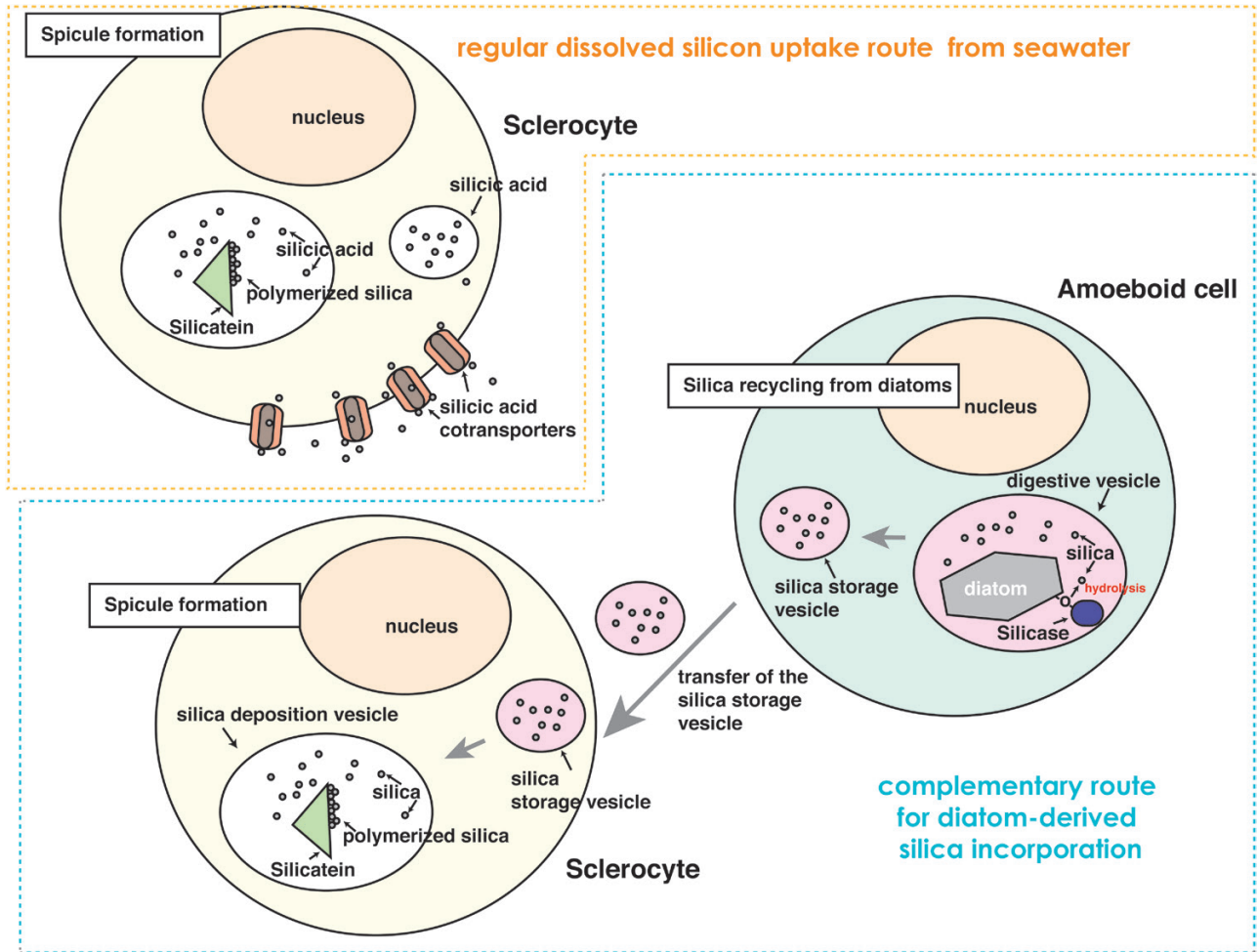
Figure 8. Schematic summary of our hypothesized recycling route for the reutilization of diatom silica by sponge amoebocytes compared to the standard silicon uptake and production of siliceous spicules by the sclerocytes of demosponges.

the alternative or contemporary route, diatoms are phagocytosed and digested by an amoeboid cell, and within the digestive vesicle, silicases break down the frustules to nanospheres that are stored and probably transported to sclerocytes for later construction of the spicules (Fig. 8). In our observations, the digestion and storage of silica always occurred in amoeboid cells that were not actively making spicules (Figs 5, 6, 8) and hence they cannot be considered sclerocytes. However, there are no differential features between sclerocytes and such amoeboid cells at the microscopical level, and therefore we cannot discard that the sclerocytes are the ones phagocytosing and digesting the diatoms and later utilizing the silica nanospheres to build the spicules.

The level at which the strategy of silica reutilization is present among non-Antarctic sponges remains unknown to date. In most shallow-water areas of the modern ocean, characterized by relatively low DSi concentrations, the newly discovered diatom

reutilization pathway could well provide a mechanism for sponges to alleviate the overcompetition that diatoms, being most efficient DSi consumers, exert on the sponge communities. Likewise, the hypothesis that silicifiers other than sponges may actively recycle diatom silica may be worthy of further investigation.

REFERENCES

Altschul SF, **Gish W**, **Miller W**, **Myers EW**, **Lipman DJ. 1990.** Sequence homology searches done using BLAST. *Journal of Molecular Biology* **215:** 404–415.

Barthel D. 1995. Tissue composition of sponges from the Weddell Sea, Antarctica: not much meat on the bones. *Marine Ecology Progress Series* **123:** 149–153.

Bavestrello G, **Arillo A**, **Calcinai B**, **Cattaneo-Vietti R**, **Cerrano C**, **Gaino E**, **Penna A**, **Sara M. 2000.** Parasitic diatoms inside Antarctic sponges. *Biological Bulletin* **198:** 29–33.

Bhattacharyya P, **Volcani BE. 1980.** Sodium-dependent silicate transport in the apochlorotic marine diatom *Nitzschia alba*. *Proceedings of the National Academy of Sciences of the USA* **77:** 6386–6390.

Bolger AM, **Lohse M**, **Usadel B. 2014.** Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30:** 2114–2120.

Cardinal D, **Alleman L**, **Dehairs F**, **Savoye N**, **Trull T**, **André L. 2005.** Relevance of silicon isotopes to Si-nutrient utilization and Si-source assessment in Antarctic waters. *Global Biogeochemical Cycles* **19:** GB2007.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17:** 540–552.

Cattaneo-Vietti R, **Bavestrello G**, **Cerrano C**, **Gaino E**, **Mazzella L**, **Pansini M**, **Sarà M. 2000.** The role of sponges in the Terra Nova Bay ecosystem. In: Faranda FM, Guglielmo L, Ianora A, eds. *Ross Sea ecology: Italiantartide expeditions (1985–1995)*. Berlin, Heidelberg: Springer, 539–549.

Cerrano C, **Arillo A**, **Bavestrello G**, **Calcinai B**, **Cattaneo-Vietti R**, **Penna A**, **Sarà M**, **Totti C. 2000.** Diatom invasion in the Antarctic hexactinellid sponge *Scolymastra joubini*. *Polar Biology* **23:** 441–444.

Cerrano C, **Calcinai B**, **Cucchiari E**, **Camillo CD**, **Nigro M**, **Regoli F**, **Sarà A**, **Schiaparelli S**, **Totti C**, **Bavestrello G. 2004a.** Are diatoms a food source for Antarctic sponges? *Chemistry and Ecology* **20:** 57–64.

Cerrano C, **Calcinai B**, **Cucchiari E**, **Di Camillo C**, **Totti C**, **Bavestrello G. 2004b.** The diversity of relationships between Antarctic sponges and diatoms: the case of *Mycale acerata* Kirkpatrick, 1907 (Porifera, Demospongiae). *Polar Biology* **27:** 231–237.

Cha JN, **Shimizu K**, **Zhou Y**, **Christiansen SC**, **Chmelka BF**, **Stucky GD**, **Morse DE. 1999.** Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones *in vitro*. *Proceedings of the National Academy of Sciences of the USA* **96:** 361–365.

Chu JW, **Maldonado M**, **Yahel G**, **Leys SP. 2011.** Glass sponge reefs as a silicon sink. *Marine Ecology Progress Series* **441:** 1–14.

Conesa A, **Götz S**, **García-Gómez JM**, **Terol J**, **Talón M**, **Robles M. 2005.** Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21:** 3674–3676.

Conley DJ, **Frings PJ**, **Fontorbe G**, **Clymans W**, **Stadmark J**, **Hendry KR**, **Marron AO**, **De La Rocha CL. 2017.** Biosilicification drives a decline of dissolved Si in the oceans through geologic time. *Frontiers in Marine Science* **4:** 397.

Cox G, **Larkum A. 1983.** A diatom apparently living in symbiosis with a sponge. *Bulletin of Marine Science* **33:** 943–945.

Cui H, **Kaufman AJ**, **Xiao S**, **Peek S**, **Cao H**, **Min X**, **Cai Y**, **Siegel Z**, **Liu XM**, **Peng Y. 2016.** Environmental context for the terminal Ediacaran biomineralization of animals. *Geobiology* **14:** 344–363.

Dayton PK, **Robilliard GA**, **Paine RT**, **Dayton LB. 1974.** Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecological Monographs* **44:** 105–128.

De Goeij JM, **Van den Berg H**, **Van Oostveen MM**, **Epping EHG**, **Van Duyl FC. 2008.** Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series* **357:** 139–151.

De La Rocha CL. 2003. Silicon isotope fractionation by marine sponges and the reconstruction of the silicon isotope composition of ancient deep water. *Geology* **31:** 423–426.

Deheyn DD, **Gendreau P**, **Baldwin RJ**, **Latz MI. 2005.** Evidence for enhanced bioavailability of trace elements in the marine ecosystem of Deception Island, a volcano in Antarctica. *Marine Environmental Research* **60:** 1–33.

Ehrlich H. 2011. Silica biomineralization, sponges. In: Reitner J, Thiel V, eds. *Encyclopedia of geobiology*. Dordrecht: Springer Netherlands, 796–808.

Elderfield H. 1972. Effects of volcanism on water chemistry, Deception Island, Antarctica. *Marine Geology* **13:** M1–M6.

Fontorbe G, **Frings PJ**, **De La Rocha CL**, **Hendry KR**, **Conley DJ. 2016.** A silicon depleted North Atlantic since the Palaeogene: evidence from sponge and radiolarian silicon isotopes. *Earth and Planetary Science Letters* **453:** 67–77.

Frøhlich H, **Barthel D. 1997.** Silica uptake of the marine sponge *Halichondria panicea* in Kiel Bight. *Marine Biology* **128:** 115–125.

Frost TM. 1981. Analysis of ingested particles within a freshwater sponge. *Transactions of the American Microscopical Society* **1:** 271–277.

Gaino E, **Bavestrello G**, **Cattaneo-Vietti R**, **Sarà M. 1994.** Scanning electron microscope evidence for diatom uptake by two Antarctic sponges. *Polar Biology* **14:** 55–58.

Gaino E, **Rebora M. 2003.** Ability of mobile cells of the freshwater sponge *Ephydatia fluviatilis* (Porifera, Demospongiae) to digest diatoms. *Italian Journal of Zoology* **70:** 17–22.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29:** 644–652.

Gutt J, **Böhmer A**, **Dimmler W. 2013.** Antarctic sponge spicule mats shape macrobenthic diversity and act as a silicon trap. *Marine Ecology Progress Series* **480:** 57–71.

Hamilton PB, **Poulin M**, **Yang J-R**, **Klöser H. 1997.** A new diatom genus, *Porannulus* (Bacillariophyta), associated with marine sponges around King George Island, south Shetland Islands, Antarctica. *Diatom Research* **12:** 229–242.

Hendry KR, **Georg RB**, **Rickaby REM**, **Robinson LF**, **Halliday AN. 2010.** Deep ocean nutrients during the Last Glacial Maximum deduced from sponge silicon isotopic compositions. *Earth and Planetary Science Letters* **292:** 290–300.

Hewett-Emmett D. 2000. Evolution and distribution of the carbonic anhydrase gene families. In: Chegwidden WR, Carter ND, Edwards YH, eds. *The carbonic anhydrases: new horizons*. Basel: Birkhäuser Verlag, 29–76.

Hildebrand M, **Lerch SJL**, **Shrestha RP. 2018.** Understanding diatom cell wall silicification—moving forward. *Frontiers in Marine Science* **5:** 125.

Katoh K, **Standley DM. 2013.** MAFFT: multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30:** 772–780.

Krasko A, **Lorenz B**, **Batel R**, **Schröder HC**, **Müller IM**, **Müller WE. 2000.** Expression of silicatein and collagen genes in the marine sponge *Suberites domuncula* is controlled by silicate and myotrophin. *European Journal of Biochemistry* **267:** 4878–4887.

Langmead B, **Salzberg SL. 2012.** Fast gapped-read alignment with Bowtie 2. *Nature Methods* **9:** 357.

Leng MJ, **Barker PA. 2006.** A review of the oxygen isotope composition of lacustrine diatom silica for palaeoclimate reconstruction. *Earth-Science Reviews* **75:** 5–27.

Leng MJ, **Sloane HJ. 2008.** Combined oxygen and silicon isotope analysis of biogenic silica. *Journal of Quaternary Science* **23:** 313–319.

Lewin JC. 1961. The dissolution of silica from diatom walls. *Geochimica et Cosmochimica Acta* **21:** 182–198.

Li B, **Dewey CN. 2011.** RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12:** 323.

López-Acosta M, **Leynaert A**, **Maldonado M. 2016.** Silicon consumption in two shallow-water sponges with contrasting biological features. *Limnology and Oceanography* **61:** 2139–2150.

López-Acosta M, **Leynaert A**, **Coquille V**, **Maldonado M. 2018a.** Silicon utilization by sponges: an assessment of seasonal changes. *Marine Ecology Progress Series* **605:** 111–123.

López-Acosta M, **Leynaert A**, **Grall J**, **Maldonado M. 2018b.** Silicon consumption kinetics by marine sponges: an assessment of their role at the ecosystem level. *Limnology and Oceanography* **63:** 2508–2522.

Maldonado M, **Carmona MC**, **Uriz MJ**, **Cruzado A. 1999.** Decline in Mesozoic reef-building sponges explained by silicon limitation. *Nature* **401:** 785.

Maldonado M, **Carmona MC**, **Velásquez Z**, **Puig A**, **Cruzado A**, **López A**, **Young CM. 2005.** Siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. *Limnology and Oceanography* **50:** 799–809.

Maldonado M, **Riesgo A**, **Bucci A**, **Klaus R. 2010a.** Revisiting silicon budgets at a tropical continental shelf: silica standing stocks in sponges surpass those in diatoms. *Limnology and Oceanography* **55:** 2001–2010.

Maldonado M, **Zhang X**, **Cao X**, **Xue L**, **Cao H**, **Zhang W. 2010b.** Selective feeding by sponges on pathogenic microbes: a reassessment of potential for abatement of microbial pollution. *Marine Ecology Progress Series* **403:** 75–89.

Maldonado M, **Navarro L**, **Grasa A**, **Gonzalez A**, **Vaquerizo I. 2011.** Silicon uptake by sponges: a twist to understanding nutrient cycling on continental margins. *Scientific Reports* **1:** 30.

Maldonado M, **Ribes M**, **Van Duyl FC. 2012.** Nutrient fluxes through sponges: biology, budgets, and ecological implications. *Advances in Marine Biology* **62:** 113–182.

Maldonado M, **López-Acosta M**, **Sitjà C**, **García-Puig M**, **Galobart C**, **Ercilla G**, **Leynaert A. 2019.** Sponge skeletons as an important sink of silicon in the global oceans. *Nature Geoscience* **12:** 815–822.

Maliva RG, **Knoll AH**, **Siever R. 1989.** Secular change in chert distribution: a reflection of evolving biological participation in the silica cycle. *Palaios* **4:** 519–532.

Marron AO, **Alston MJ**, **Heavens D**, **Akam M**, **Caccamo M**, **Holland PW**, **Walker G. 2013.** A family of diatom-like silicon transporters in the siliceous loricate choanoflagellates. *Proceedings of the Royal Society, ser. B* **280:** 20122543.

McClintock JB, **Amsler CD**, **Baker BJ**, **Van Soest RW. 2005.** Ecology of Antarctic marine sponges: an overview. *Integrative and Comparative Biology* **45:** 359–368.

Müller WE, **Krasko A**, **Le Pennec G**, **Steffen R**, **Wiens M**, **Ammar MSA**, **Müller IM**, **Schröder HC. 2003.** Molecular mechanism of spicule formation in the demosponge *Suberites domuncula*: silicatein-collagen-myotrophin. In: Müller WEG, ed. *Silicon biomineralization, biology - biochemistry - molecular biology - biotechnology*. Berlin, Heidelberg: Springer, 195–221.

Núñez-Pons L, **Carbone M**, **Paris D**, **Melck D**, **Ríos P**, **Cristobo J**, **Castelluccio F**, **Gavagnin M**, **Avila C. 2012.** Chemo-ecological studies on hexactinellid sponges from the Southern Ocean. *Naturwissenschaften* **99:** 353–368.

Pile AJ, **Grant A**, **Hinde R**, **Borowitzka MA. 2003.** Heterotrophy on ultraplankton communities is an important source of nitrogen for a sponge–rhodophyte symbiosis. *Journal of Experimental Biology* **206:** 4533–4538.

R Core Team. **2013.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Ragueneau O, **Tréguer P**, **Leynaert A**, **Anderson R**, **Brzezinski M**, **DeMaster D**, **Dugdale R**, **Dymond J**,

Fischer G, **Francois R. 2000.** A review of the Si cycle in the modern ocean: recent progress and missing gaps in the application of biogenic opal as a paleoproductivity proxy. *Global and Planetary Change* **26:** 317–365.

Regoli F, **Nigro M**, **Chierici E**, **Cerrano C**, **Schiapparelli S**, **Totti C**, **Bavestrello G. 2004.** Variations of antioxidant efficiency and presence of endosymbiotic diatoms in the Antarctic porifera *Haliclona dancoi*. *Marine Environmental Research* **58:** 637–640.

Reiswig HM. 1971. Particle feeding in natural populations of three marine demosponges. *Biological Bulletin* **141:** 568–591.

Ribes M, **Coma R**, **Gili J-M. 1999.** Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series* **176:** 179–190.

Ronquist F, **Teslenko M**, **Van der Mark P**, **Ayres DL**, **Darling A**, **Höhna S**, **Larget B**, **Liu L**, **Suchard MA**, **Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61:** 539–542.

Sandford F. 2003. Physical and chemical analysis of the siliceous skeletons in six sponges of two groups (Demospongiae and Hexactinellida). *Microscopy Research and Technique* **62:** 336–355.

Schröer HC, **Krasko A**, **Le Pennec G**, **Adell T**, **Wiens M**, **Hassanein H**, **Müller IM**, **Müller WE. 2003.** Silicase, an enzyme which degrades biogenous amorphous silica: contribution to the metabolism of silica deposition in the demosponge *Suberites domuncula*. In: Müller WEG, ed. *Silicon biomineralization, biology - biochemistry - molecular biology - biotechnology*. Berlin, Heidelberg: Springer, 249–268.

Shimizu K, **Cha J**, **Stucky GD**, **Morse DE. 1998.** Silicatein α: cathepsin L-like protein in sponge biosilica. *Proceedings of the National Academy of Sciences of the USA* **95:** 6234–6238.

Shimizu K, **Amano T**, **Bari MR**, **Weaver JC**, **Arima J**, **Mori N. 2015.** Glassin, a histidine-rich protein from the siliceous skeletal system of the marine sponge *Euplectella*, directs silica polycondensation. *Proceedings of the National Academy of Sciences of the USA* **112:** 11449–11454.

Siever R. 1991. Silica in the oceans: biological-geochemical interplay. In: Schneider SS, Boston PJ, eds. *Scientists on Gaia*. Cambridge: MIT Press, 287–295.

Struyf E, **Smis A**, **Van Damme S**, **Meire P**, **Conley DJ. 2009.** The global biogeochemical silicon cycle. *Silicon* **1:** 207–213.

Sun M-K, **Alkon DL. 2002.** Carbonic anhydrase gating of attention: memory therapy and enhancement. *Trends in Pharmacological Sciences* **23:** 83–89.

Totti C, **Calcinai B**, **Cerrano C**, **Di Camillo C**, **Romagnoli T**, **Bavestrello G. 2005.** Diatom assemblages associated with *Sphaerotylus antarcticus* (Porifera: Demospongiae). *Journal of the Marine Biological Association of the United Kingdom* **85:** 795–800.

Treguer P, **Nelson DM**, **Van Bennekom AJ**, **DeMaster DJ**, **Leynaert A**, **Quéguiner B. 1995.** The silica balance in the world ocean: a reestimate. *Science* **268:** 375–379.

Tréguer PJ, **De La Rocha CL. 2013.** The world ocean silica cycle. *Annual Review of Marine Science* **5:** 477–501.

Turon X, **Galera J**, **Uriz MJ. 1997.** Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. *Journal of Experimental Zoology* **278:** 22–36.

Uriz MJ. 2006. Mineral skeletogenesis in sponges. *Canadian Journal of Zoology* **84:** 322–356.

Uriz MJ, **Turon X**, **Becerro MA. 2000.** Silica deposition in demosponges: spiculogenesis in *Crambe crambe*. *Cell and Tissue Research* **301:** 299–309.

Volcani BE. 1983. Aspects of silicification in biological systems. In: Lowenstam HA, Weiner S, Westbroek P, De Jong EW, eds. *Biomineralization and biological metal accumulation*. Dordrecht: Springer, 389–405.

Weaver JC, **Pietrasanta LI**, **Hedin N**, **Chmelka BF**, **Hansma PK**, **Morse DE. 2003.** Nanostructural features of demosponge biosilica. *Journal of Structural Biology* **144:** 271–281.

Wille M, **Sutton J**, **Ellwood MJ**, **Sambridge M**, **Maher W**, **Eggins S**, **Kelly M. 2010.** Silicon isotopic fractionation in marine sponges: a new model for understanding silicon isotopic variations in sponges. *Earth and Planetary Science Letters* **292:** 281–289.

Wolfrath B, **Barthel D. 1989.** Production of faecal pellets by the marine sponge *Halichondria panicea* Pallas (1766). *Journal of Experimental Marine Biology and Ecology* **129:** 81–94.

Yool A, **Tyrrell T. 2003.** Role of diatoms in regulating the ocean's silicon cycle. *Global Biogeochemical Cycles* **17:** 1103.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

File S1. Phylogenetic tree for silicase obtained using Bayesian Inference in MrBayes. Posterior probabilities are presented over the branches, and bootstrap values from Maximum Likelihood analysis under the branches. Sequences obtained in this study appear in bold letters.

File S2. Phylogenetic tree for silicatein obtained using Bayesian Inference in MrBayes. Posterior probabilities are presented over the branches. Sequences obtained in this study appear in bold letters.

File S3. Amino acid alignments (nexus) for both silicatein and silicase.