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Sequence data deposition:

Sequence data deposition: 16S rRNA sequence data were deposited at NCBI GenBank: *Candidatus* Sulfurimonas marisnigri strain SoZ1 (accession number: HUE87_02100), *Candidatus* Sulfurimonas baltica strain GD2 (accession number: HUE_08945)
Genome sequence data were deposited at NCBI GenBank: *Candidatus* Sulfurimonas marisnigri strain SoZ1 (accession number: CP054493), *Candidatus* Sulfurimonas baltica strain GD2 (accession number: CP054492)
Sequences will be publicly available at the time of publication.

Abstract:

Species of the genus *Sulfurimonas* are reported and isolated from terrestrial habitats and marine sediments and water columns with steep redox gradients. Here we report on the isolation of strains SoZ1 and GD2 from the pelagic redoxcline of the Black Sea and the Baltic Sea, respectively. Both strains are gram-stain-negative and appear as short and slightly curved motile rods. The autecological preferences for growth of strain SoZ1 were 0-25 °C (optimum 20 °C), pH 6.5-9.0 (optimum pH 7.5-8.0) and salinity 10-40 g L^{-1} (optimum 25 g L^{-1}). Preferences for growth of strain GD2 were 0-20 °C (optimum 15 °C), pH 7.0-8.0 (optimum pH 7.0-7.5) and salinity 5–40 g L^{-1} (optimum 21 g L^{-1}). Strain SoZ1 grew chemolithoautotrophically, while strain GD2 also showed heterotrophic growth with short

chained fatty acids as carbon source. Both species utilized hydrogen (H_2), sulfide (H_2S here taken as the sum of H_2S, HS^- and S^{2-}), elemental sulfur (S^0) and thiosulfate ($S_2O_3^{2-}$) as electron donors and nitrate (NO_3^-), oxygen (O_2) and particulate manganese oxide (MnO_2) as electron acceptors. Based on 16S rRNA gene sequence similarity, both strains cluster within the genus *Sulfurimonas* with *Sulfurimonas gotlandica* GD1^T as the closest cultured relative species with a sequence similarity of 96.74% and 96.41% for strain SoZ1 and strain GD2, respectively. Strains SoZ1 and GD2 share a ribosomal 16S sequence similarity of 99.27% and were demarcated based on average nucleotide identity and average amino acid identity of the whole genome sequence. These calculations have been applied to the whole genus. We propose the names *Candidatus* Sulfurimonas marisnigri sp. nov. and *Candidatus* Sulfurimonas baltica sp. nov. for the thiotrophic manganese reducing culture isolates from the Black Sea and Baltic Sea, respectively.

Keywords: *Sulfide oxidation; manganese oxide reduction; Sulfurimonas; pelagic redoxcline; dissimilatory energy metabolism; genomic relatedness indices; ANI; AAI; POCP*

Introduction:

The genus *Sulfurimonas* was first proposed by Inagaki et al. (2003) and belonged at the time of writing to the class Campylobacteria (formerly Epsilonproteobacteria) within the phylum of Epsilonbacteraeota (formerly Proteobacteria) [46]. Species of the genus are found ubiquitous e.g. at hydrothermal deep-sea vents, marine sediments, pelagic water column redoxclines and terrestrial soils [13]. They can be dominant in their respective habitat with a high contribution to primary production via chemoautotrophy [6,13], specifically in pelagic and sedimentary redoxclines [3,10,11,45]. Species of the genus share a high versatility of their energy metabolism. Hydrogen (H_2) and reduced sulfur compounds are commonly utilized as electron donors and nitrate (NO_3^-) and oxygen (O_2) are common electron acceptors [13]. Just recently, Henkel et al. [14] reported a manganese oxide (MnO_2) reducing isolated species from the pelagic redoxcline of the Black Sea named '*S. marisnigri*' here proposed as *Candidatus* Sulfurimonas marisnigri SoZ1. The genus *Sulfurimonas* currently includes 8 validly published species with *S. autotrophica* OK10^T as type species. *S. autotrophica* OK10^T, *S. denitrificans*, *S. hongkongensis* AST-10, *S. xiamensis* 1-1N^T and *S. lithotrophica* GYSZ_1^T were isolated from coastal or deep-sea marine sediments, *S. paralvinellae* GO25^T from a deep-sea polychaete nest, *S. crateris* SN118^T from a terrestrial mud volcano and *S. gotlandica* GD1^T from the pelagic redoxcline of the Baltic Sea [5,17,23,42,43,47]. In this

study we report two novel chemolithoautotrophic MnO_2 reducing species of the genus *Sulfurimonas*, one from the pelagic redoxcline of the Black Sea and one from the pelagic redoxcline of the Baltic Sea. In calculations of genome relatedness indices with species of the genus we identified high similarities between *S.* sp. strain CVO and *S. xiamensis* 1-1N^T and propose to rename *S.* sp. strain CVO as *S. xiamensis* strain CVO.

Material and Methods:

Sampling, enrichment and isolation

The initial water sample for the isolation of strain SoZ1 was taken at the 21st of November in 2013 from the Black Sea at the lower boundary of the suboxic zone where sulfide (here taken as the sum of H_2S, HS^- and S^{2-}) was just detectable (water density σ_θ = 16.145 – [14]). The initial water sample for the isolation of strain GD2 was taken at the 14th of June in 2016 in the Gotland Deep of the Baltic Sea at a depth of 225m where anoxic but not sulfidic bottom waters were present after a salt water inflow event from the North Sea (major Baltic inflow event - MBI) in December 2014 [7,28]. The water samples were filled into gas-tight glass serum bottles and closed with butyl rubber stoppers. We added MnO_2 as sole electron acceptor to the water samples and H_2S regularly in daily small additions (~ 20 µM final concentration) for several weeks as electron donor to pre-enrich the organisms with the metabolism of interest. H_2S was added in this manner to minimize the loss of energy to the abiotic reaction of MnO_2 and H_2S (Equation 1) and to avoid H_2S toxification, as Grote et al. (2012) showed inhibitory effects of H_2S concentration above 20 µM for *Sulfurimonas gotlandica* GD1^T.

(1) $MnO_2 + H_2S + 2\,H^+ \longrightarrow S^0 + Mn^{2+} + 2\,H_2O$

After this pre-enrichment, we performed batch type cultivation with thiosulfate ($S_2O_3^{2-}$) as electron donor instead of H_2S, as it is not reacting with the MnO_2 used in this study [14], nontoxic even at high concentrations and can be typically utilized as electron donor by H_2S oxidizing bacteria. Both strains showed positive growth with MnO_2 as electron acceptor and $S_2O_3^{2-}$ as electron donor. The strains were isolated by dilution to extinction method with some specific adjustments, because DAPI stained samples of the enrichments showed cellular attachment to MnO_2 particles. It was concluded that the isolation of single cells would be difficult as the colonized MnO_2 particles harbored tens to hundreds of cells. Therefore, a subsample was shortly centrifuged with a benchtop centrifuge and the supernatant was serially diluted and re-cultivated in artificial culture medium. This procedure was performed for several months with re-cultivations every 2-3 weeks out of the highest dilution showing

positive growth indicated by a color change (Fig. A.1) from Black (MnO_2) to brownish-grey (Ca-rich $MnCO_3$) indicating complete reduction of MnO_2 [14,15]. Both strains were cryopreserved at -80 °C in glycerol (50% final concentration) for long-term storage. After cryopreservation cultures frequently showed a prolonged lag phase before resuming growth. We suggest that the movement and attachment to the particulate electron acceptor are energy consuming processes and just a small proportion of the cells are capable to re-start growth.

Medium preparation

Anoxic medium for standard cultivation with salinity of 21 (strain SoZ1), 14 (strain GD2) or 10 (*Sulfurimonas gotlandica* GD1^T) for cultivation was prepared with the following salts dissolved in MilliQ water (g L^{-1}): NaCl, 17.7,11.8 or 8.5; $MgCl_2$ * 6 H_2O, 3.2, 2.2 or 1.5; $CaCl_2$ * 2 H_2O, 0.4, 0.3 or 0.2; KCl, 0.7, 0.4 or 0.3. Addition of sulfate was omitted in the culturing media to simplify putative sulfate analysis. The artificial seawater was filled into glass vessels for cultivation [48], closed with screw caps and autoclaved. After sterilization, the artificial seawater was cooled under a constant flow of N_2 with 20% CO_2. The following solutions were prepared sterile under N_2 or Ar atmosphere in MilliQ water and added to the cooled artificial seawater (in mL L^{-1}): 1 M $NaHCO_3$, 30; 100 mM NH_4Cl, 0.2; 100 mM Na_2HPO_4, 0.1; trace elements solution SL7 [49], 1; vitamin solution, 0.42. The sterile filtered vitamin solution consisted of the following ingredients (g L^{-1}): B12, 0.1; inositol, 0.1; folic acid, 0.1; 4-aminobenzoic acid, 1; nicotinic acid, 10; d-pantothenate, 10; thiamine, 20. After all additions were made the glass vessel was closed and an overpressure was established to lower the pH. The medium was filled into gas-tight serum bottles as soon as the pH reached 7.5. A small headspace in the serum bottles was flushed with the N_2/CO_2 mixture for about 30 seconds and bottles were closed with a butyl rubber stopper.

The addition of the redox indicator resazurin was omitted because it had negative effects on growth of strains SoZ1 and GD2. We tried to avoid oxygen contaminations in the preparation of the culture medium as good as possible. Oxygen contaminations were measured with piercing O_2 microsensors (Unisense, Denmark) and were always below 10 µM. Due to these small oxygen contaminations the strains showed little growth in the absence of an additional electron acceptor which could be eliminated by the chemical removal of oxygen with cysteine prior to inoculation. Because cysteine could have interfered with the scope of some experiments (e.g. screening for putative electron donors) it was avoided in all cultivations if not mentioned otherwise. Regular culturing and experiments were performed in the dark at 10 °C.

Preparation of stock solutions

Stock solutions were prepared by dissolving the respective chemicals in MilliQ water. Solutions were filled in serum bottles, closed with butyl rubber stoppers and bubbled for at least 1 hour with N_2 or Ar-gas using two cannulas. Anoxic stock solutions were heat sterilized in closed bottles. Colloidal elemental sulfur was prepared after Blumentals et al. [2]. Goethite and amorphous FeOOH was prepared after Lovley and Phillips [27]. MnO_2 used in this study was purchased from Merck and was additionally ground with an agate ball mill. To test for H_2 as electron donor the bottle headspace was flushed for 30s with 0.2 µm filtered H_2-gas generated by a H_2-generator.

Autecological preferences

Effects of temperature, salinity, pH and O_2 concentration on growth of *Candidatus* Sulfurimonas marisnigri, *Candidatus* Sulfurimonas baltica and *Sulfurimonas gotlandica* GD1^T were investigated. Temperature was tested at 0, 4, 10, 15, 20, 25, 30, 35 and 40°C in temperature controlled rooms or incubators without replicates. Salinity was tested with at 5, 10, 14, 21, 25, 30, 40 and 60 PSU. Respective salinities were adjusted by lowering or raising the addition of all salt components listed above. Salt concentration was checked with a hand-held refractometer. N_2 with 10% CO_2 was used for the salinities 30, 40 and 60, because we observed precipitates with 20% CO_2 in the gas mixture. Growth was tested at pH 4, 5, 6, 6.5, 7, 7.5, 8 and 9 in triplicates and pH was adjusted as described by Inagaki et al. [17]. N_2 instead of N_2 with 20% CO_2 was used in the preparation of the medium and 2.5 mM HCO_3^- was added as C-source for autotrophic growth. To test for O_2 tolerance, 150 mL serum bottles were filled with 50 mL of anoxic medium as described above. The headspace was flushed with N_2 containing 20% CO_2 for at least 1 minute. 5 or 20 mL of the headspace volume was exchanged with sterile filtered atmospheric air with syringes to generate an O_2 concentration of 1% or 4%, respectively. No volume was exchanged for 0% O_2 treatments. The headspace was flushed for 1 minute with sterile atmospheric air for the 21% O_2 treatment. Growth was monitored by direct cell counting with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI)-staining following the protocol of Henkel et al. [14] or flow-cytometry with SYBR Green I (Molecular probes) as described in Henkel, J. V. - Dissertation [15].

DNA isolation, sequencing and processing

About 300 mL of pre-grown cultures of strains SoZ1 and GD2 were filled in 50 mL centrifugation tubes and centrifuged at 10000 x g for 30 min at 4 °C. The material was narrowed down into 2 mL microcentrifugation tubes and pelleted at 20000 x g. We added 700 µL of TSE-buffer (1 mM EDTA; 50 mM Tris; 6.7% sucrose; pH 8) to the pelleted cells and subsequently 19 µL of lysozyme. Tubes were incubated for 1 h at 37 °C and 1000 rpm in a

thermal shaker. We added 74 µL Tris-EDTA (250 mM EDTA; 50 mM Tris; pH 8) and 44 µL SDS-Tris-EDTA (20 mM EDTA, 50 mM Tris; 20% SDS; pH 8) and incubated for 1 h at 50 °C at 1000 rpm in a thermal shaker. Tubes were centrifuged at 8000x g at room temperature for 10 min. The supernatant was transferred in a new microcentrifugation tube (about 800 µL). We added 80 µL 5M NaCl and 800 µL phenol:chloroform (1:1) solution and centrifuged the samples at 8000x g at room temperature for 10 min. The clear upper phase was transferred into a new microcentrifugation tube and 800 µL isopropanol were added. The tubes were kept at -20 °C over night to precipitate the DNA. At the next day, the tubes were centrifuged at 10000x g at 4 °C for 30 min. The supernatant was discarded. The DNA was washed three times with ethanol. We added 500 µL of 70% ethanol to the pellet and centrifuged the tubes at 10000x g at 4 °C for 10 min. The supernatant was discarded. The final pelleted DNA was dried under a sterile working bench and eluted in TE-buffer (1 mM EDTA; 10 mM Tris; pH 8). For sequencing, the DNA was sent to the German collection of microorganisms and cell cultures (DSMZ, Braunschweig, Germany). At the DSMZ, the DNA was treated with Proteinase K (50µg/µl) and RNase A (5µg/µl) and purified applying DNA Clean & Concentrator-25 columns from Zymo Research (Freiburg, Germany) according to the manufacturer´s instructions.

In order to achieve circular genomes with high quality, DNA long and short reads were sequenced and processed bioinformatically. For long sequence reads, SMRTbell™ template libraries were prepared according to the instructions from Pacific Biosciences (Menlo Park, CA, USA) following the Procedure & Checklist – Preparing Multiplexed Microbial Libraries Using SMRTbell® Express Template Prep Kit 2.0. Briefly, for preparation of 10kb libraries 1µg genomic DNA was end-repaired and ligated to barcoded adapters applying components from the SMRTbell® Express Template Prep Kit 2.0 from Pacific Biosciences. Reactions were carried out according to the manufacturer´s instructions. Samples were pooled according to the calculations provided by the Microbial Multiplexing Calculator. Conditions for annealing of sequencing primers and binding of polymerase to purified SMRTbell™ template were assessed with the calculator in SMRT®link. Both samples were multiplexed according to their expected genome sizes together with 14 others on a single SMRT cell, which was sequenced on the Sequel *II* (PacificBiosciences, Menlo Park, CA, USA) taking one 15h movie.

For short sequence reads, a library for sequencing on Illumina platform was prepared from DNA of *Candidatus* Sulfurimonas baltica strain GD2 applying Nextera XT DNA Library

Preparation Kit (Illumina, San Diego, USA) with modifications according to Baym et al. (2015). Samples were sequenced on NextSeq™ 500.

Short reads for *Candidatus* Sulfurimonas marisnigri SoZ1 were generated at BaseClear (Leiden, Netherlands) using the Illumina HiSeq2500 system. FASTQ sequence files were generated using bcl2fastq2 version 2.18. Initial quality assessment was based on data passing the Illumina Chastity filtering. Subsequently, reads containing PhiX control signal were removed using an in-house filtering protocol. In addition, reads containing (partial) adapters were clipped up to minimum read length of 50bp. The second quality assessment was based on the remaining reads using the FASTQC quality control tool version 0.11.5.

Genome assemblies were performed with the Microbial Assembly protocol included in SMRT®link version 2.3.0 applying a target genome size of 2.2 Mbp in both cases. Both assemblies revealed a single circular chromosomal contig of 2.4 and 2.7 Mbp, respectively. Both chromosomal contigs were rotated to the *dnaA* gen. Error-correction was performed by a mapping of Illumina short reads onto finished circular chromosomes using Burrows-Wheeler Alignment bwa 0.6.2 [25] with subsequent variant and consensus calling using VarScan 2.3.6 [21]. Genome annotation was based on Prokka [39] with subsequent manual curation. 16S rRNA sequences were extracted from the circular genome sequence.

A phylogenetic tree based on 16S rRNA sequence data was constructed with SILVA ACT. SINA v. 1.2.11 [35] was used to align rRNA gene sequences, and FastTree v. 2.1.10 [34] was used to construct the phylogenetic tree with the GTR/GAMMA model. Ribosomal 16S RNA sequences from strains SoZ1, GD2 and CVO were identified using Prokka v1.8 [39] from the respective genome sequence. All other 16S rRNA sequence data were obtained from NCBI (accession numbers are given in respective figure). Average nucleotide identity (ANI) was calculated with FastANI v. 1.32 [18], average amino acid identity (AAI) was calculated with compareM v. 0.0.1 [31] and percentage of conserved proteins (POCP) was calculated with proteinortho v. 6.0.23 after [36]. Accession numbers for genome and 16S rRNA sequences are available in an extra file.

Visualization:

Scanning electron microscopy (SEM)

Imaging was performed with a Merlin Compact SEM (variable pressure, in-lens SE and HE-SE2 detectors) from Zeiss. Glutaraldehyde fixed samples (2.5 % final concentration) were filtered onto 0.2 µm polycarbonate filter material, washed with MilliQ-water and dried at 60 °C. Filters were sputter coated with a 10 nm chromium layer for imaging of bacterial cells.

Further information is given in the respective SEM images. Images were recorded with 2048 x 1536 px and line averaging of 8.

Secondary ion mass spectroscopy (nanoSIMS)

Samples for NanoSIMS analysis were prepared as described for SEM. Dried filters were sputter coated with a 30 nm gold layer for electrical conductivity. SIMS imaging was performed with a NanoSIMS 50 L from Cameca (Gennevilliers, France). Samples were implemented for 200 s with a $^{133}Cs^+$ ion beam at a beam current of 600 pA. Secondary ions were detected using mass detectors equipped with electron multipliers (Hamamatsu, Hamamatsu City, Japan). For analysis, the secondary ions $^{12}C^{14}N^-$ and $^{55}Mn^{16}O^-$ were generated with a $^{133}Cs^+$ ion beam and the secondary ion $^{40}Ca^+$ was generated with an $^{16}O^{2+}$ ion beam at a beam current of 1 pA and a pixel dwell time of 125 µs px^{-1}, each. SIMS analysis was done with a resolution 1024 x 1024 px in varying sample areas. 30 planes were recorded for each analysis. Data were evaluated with Look@NanoSIMS software [33]. Recorded planes were drift-corrected and accumulated. To assemble SIMS analyses from both ion sources, images from $^{16}O^{2+}$ were aligned on $^{133}Cs^+$ images. Thereby, overlays are cropped to the lower size of the $^{16}O^{2+}$ derived images.

Confocal laser scanning microscopy (CLSM)

Bacterial colonisation of particles in hydrated state was examined by CLSM using a TCS SP5X (Leica). The upright microscope setup was equipped with a super continuum light source and controlled by the LAS AF software version 2.4.1. For imaging a number of direct staining approaches were tested. The image presented was stained with the membrane probe FM1-43 (Molecular Probes, Invitrogen, Thermo-Fisher Scientific) according to the suppliers' protocol. The sample was stained and mounted in a CoverWell chamber with 0.2 mm spacer. Three-dimensional data sets were recorded using a 63x NA 1.2 water immersion objective lens and a step size of 0.25 µm. Maximum intensity projection of 80 optical sections from a 20 µm thick location. Excitation was at 480 nm, emission was recorded from 470-490 nm (reflection) and 580-650 nm (FM1-43). Resolution and contrast of the raw data set was improved by blind deconvolution using Huygens version 19.10 (SVI). Image data sets were projected with Imaris version 9.5.1 (Bitplane).

Results and Discussion:

Morphology

Cells of both strains appeared as short, slightly curved rods to vibrio-like forms comparable to the morphology of *S. gotlandica* $GD1^T$ [23] with one polar flagellum (Fig. 1). The cell

length was more variable than the diameter and differed within growth phases. Cells of strain GD2 had a length of 1.41 µm ± 0.41 µm and a width of 0.44 µm ± 0.08 µm (mean ± SD, N = 94) at the end of the exponential growth phase and typically polyphosphate (PolyP) inclusions at one or both cell poles were detectable by a yellow fluorescence in 4'6-Diamidino-2-Phenylindole (DAPI) stained filter samples. Cells of strain SoZ1 were slightly larger with a length of 1.76 µm ± 0.57 µm and a width of 0.46 µm ± 0.09 µm (mean ± SD, N = 79) at the end of the exponential growth phase (Fig. 1 A and B). When cultivated with the particulate electron acceptor MnO_2, cells were found mostly but not exclusively at the Mn-particle surface (Fig. 1). Attachment of strain GD2 onto Mn-particles was similar to strain SoZ1. Thus, cell to Mn-particle attachment shown in Fig. 1 (D – G) of strain SoZ1 is representative for both strains. At later stages of growth, cells frequently appeared in coccoid shape and tended to form aggregates as it was also observed for *S. gotlandica* GD1^T (Matthias Labrenz, personal communication).

Phylogeny

Based on the complete 16S rRNA gene sequence, strain SoZ1 and strain GD2 cluster within the genus *Sulfurimonas* with *S. gotlandica* GD1^T as the closest validly described species (Fig. 2, Table A.1) with a sequence similarity of 99.27%. This is confirmed by the percentage of conserved proteins (POCP) in an all-against-all calculation of species of the genus *Sulfurimonas* including *Candidatus* Sulfurimonas marisnigri SoZ1 and *Candidatus* Sulfurimonas baltica GD2 (same genus if ≥50% [36], see Tab. 1). Because the two isolates reported here share a high similarity in the complete 16S rRNA gene sequence, the genome based average nucleotide identity (ANI) and average amino acid identity (AAI) was calculated and expanded to all species of the genus again in an all-against-all comparison (Tab. 1). By this, *Ca.* S. marisnigri SoZ1 and *Ca.* S. baltica GD2 were identify as different species (ANI: 88.55%; AAI: 90.26% - same species if ≥96% [9] and ≥95% [22], respectively). We identified high sequence similarities between *S. xiamensis* 1-1N^T and *S.* sp. strain CVO [24] (16S rRNA sequence similarity: 99.70%; ANI: 98.61%; AAI: 98.58%, Tab. A.1, Tab. 1) and propose to rename *S.* sp. strain CVO as *S. xiamensis* strain CVO. Based on POCP values among all tested genomes (Tab. 1) show that the genus *Sulfurimonas* is consistent (i.e. in an all-against-all POCP analysis values were ≥50%).

The circular genomes of strain SoZ1 and GD2 had a size of 2.49 mpb (mean coverage 310x) and 2.74 mpb (mean coverage 729x) with a G+C content of 33.5 mol% and 33.8 mol%, respectively. No plasmids were detected.

Physiology

For the physiological characterization of strains SoZ1 and GD2, we cultivated the close relative *S. gotlandica* GD1^T along in most of the experiments as control which is summarized in comparison to

other cultured *Sulfurimonas* species in Tab. 2. Anoxic medium was prepared without the addition of chemical reductants, thus small oxygen contaminations (< 10 µM) could not be eliminated (see Material and Methods for details). Abiotic factors influencing growth were examined under regular culturing conditions with $S_2O_3^{2-}$ as electron donor and MnO_2 as electron acceptor for strains SoZ1 and GD2 and NO_3^- for *S. gotlandica* GD1^T. Growth of strain SoZ1 was observed from salinity 10-40 g L^{-1} (optimum at 25 g L^{-1}), in the temperature range of 0-25°C (optimum at 20°C) and at pH from 6.5 to 9 (optimum at 7.5 to 8). Growth of strain GD2 was observed from salinity 5-40 g L^{-1} (optimum at 21 g L^{-1}), in the temperature range of 0-20°C (optimum at 15 °C) and between pH 7 to 8 (optimum at 7.0 to 7.5). Overall lower growth yield of strain SoZ1 and GD2 have been observed in medium buffered with acetate, MES, HEPES and TRIS in comparison to medium buffered with HCO_3^- but not with *S. gotlandica* GD1^T. No major difference in the growth of *S. gotlandica* GD1^T in comparison to Labrenz et al. (2013) was observed (Fig A.2-4). Molecular hydrogen (H_2), H_2S, S^0 and $S_2O_3^{2-}$ served as electron donors and MnO_2, NO_3^- and O_2 as terminal electron acceptors for the growth of strain SoZ1 and GD2. No organotrophic growth with acetate, propionate and succinate with MnO_2 as electron acceptor was detectable with both strains. *S. gotlandica* GD1^T was unable to utilize MnO_2 as electron acceptor but showed growth with sulfite (SO_3^{2-}), which was not observed with strain SoZ1 and GD2. In electron donor limited cultivations with strain SoZ1 and GD2 with combinations of H_2S, S^0 or $S_2O_3^{2-}$ as electron donor and MnO_2, NO_3^- or O_2 added as electron acceptor, growth efficiencies were highest with MnO_2, followed by NO_3^- and O_2, indicating that MnO_2 is the primary electron acceptor for growth of both new isolates (Tab. A.1). With respect to the electron donor, growth efficiencies were highest with H_2S followed by $S_2O_3^{2-}$ and S^0 (Tab. A.1). Strains SoZ1 and GD2 showed positive growth with O_2 as sole electron acceptor at an O_2 partial pressure of 1% and 4% in bottle headspaces but not at atmospheric O_2 concentrations (~21%) (Fig. A.5). When MnO_2 was supplied as terminal electron acceptor in the presence of O_2, strain SoZ1 showed positive growth even at atmospheric oxygen concentrations while strain GD2 was only able to tolerate O_2 at 4% partial pressure but not at atmospheric concentrations (Fig. A.6). *S. gotlandica* GD1^T showed positive growth in the presence of O_2 with and without NO_3^- added as electron

acceptor up to atmospheric concentrations (Fig. A.5 & Fig. A.6). This is in contrast to the findings by Labrenz et al. (2013) which did not report positive growth of *S. gotlandica* GD1^T with O_2 as electron acceptor. This was surprising, because *S. gotlandica* GD1^T possess, like all other so far sequenced species of the genus, a *ccoNOQP* operon encoding a cbb3-type cytochrome c oxidase responsible for the reduction of O_2 to H_2O [12,13] thereby generating a proton gradient [32]. We therefore measured the dissolved O_2 concentration in the liquid phase after complete growth of all strains and calculated the difference to sterile controls with the same pO_2 in the headspace and liquid to gas phase ratio. We observed lower O_2 concentrations when positive growth was observed and unchanged concentrations when no growth was observed (Fig. A.5 & A.6 and Tab. A.2 & A.3). Lower O_2 concentrations were also observed in the presence of either MnO_2 or NO_3^- added as additional electron acceptors, but the difference to sterile controls was not as pronounced as in cultivations solely with O_2 as electron acceptor. In cultivations with *S. gotlandica* GD1^T the concentration of NO_3^- after complete growth where higher in parallels with elevated pO_2 indicating the simultaneous reduction of NO_3^- and O_2 and a pronounced utilization of O_2 when pO_2 was high (Tab. A.3). It cannot be ruled out that the agitation once per day was too little to ensure the presence of O_2 at all times throughout the liquid phase and that reduction of NO_3^- and O_2 was therefore separated spatially and not simultaneously. Nevertheless, this proves the ability of strains SoZ1, GD2 and *S. gotlandica* GD1^T to utilize O_2 as terminal electron acceptor to support growth.

Strains GD2 and SoZ1 showed positive growth with NO_3^- as electron acceptor with both $S_2O_3^{2-}$ and H_2S as electron donors. S^0 and SO_3^- have not been tested. Strain SoZ1 reduced NO_3^- stoichiometrically to NO_2^- [15]. Based on the comparable growth yield of both strains with NO_3^- as electron acceptor and all other electron acceptor and donor combinations tested, we strongly suggest that strain GD2 is also reducing NO_3^- to NO_2^-. This is supported by a lack of an overpressure in the serum bottles after growth, which would have indicated the production of N_2 in cultivation bottles, which was observed in cultivations with the complete denitrifier *S. gotlandica* GD1^T. This does not exclude N_2O as potential end product for NO_3^- reduction by strain GD2.

Often mentioned organotrophic Mn-reducing bacteria such as *Shewanella* spp. or *Geobacter* spp. reduce iron oxides amongst others next to MnO_2 [26,30]. We therefore tested $FeCl_3$, Fe_2O_3, goethite (alpha-FeOOH) and amorphous FeOOH as potential terminal electron acceptors for growth of strains SoZ1 and GD2. We observed small growth which was attributed to small oxygen contaminations rather than the reduction of iron oxides. The small

growth which we observed was comparable to controls without Fe-oxides and growth was absent when oxygen was eliminated chemically in these controls by the addition of cysteine prior to inoculation. We were further unable to identify cellular attachment to the tested Fe-oxides with microscopic inspection as it was visible with MnO_2. Further, no dissolved Fe could be identified with optical emission spectrometry with inductively coupled plasma (ICP-OES). We therefore suggest that strains SoZ1 and GD2 lack the ability to reduce $FeCl_3$, Fe_2O_3, goethite and amorphous FeOOH indicating a different mode of electron transfer in comparison to the organotrophic Mn reducers of the genera *Geobacter* and *Shewanella*. No organotrophic growth of strains SoZ1 and GD2 was observed with acetate, succinate and propionate as electron donors for MnO_2 reduction.

Disproportionation of colloidal S^0 and $S_2O_3^{2-}$ was examined in more detail, because both compounds might be generated chemically by the oxidation of H_2S with MnO_2 [4,16,50]. After about 10 days of experiment with S^0 or $S_2O_3^{2-}$ added as sole sources for energy generation cell numbers increased to a level which could have been expected due to oxygen contaminations (as described above). No smell of sulfide was observed after bottles were opened and no H_2S was detectable with H_2S microsensors in active cultivations of *Ca.* S. marisnigri SoZ1 solely on $S_2O_3^{2-}$. The absence of $S_2O_3^{2-}$ disproportionation was also supported by the inability of both strains to grow with iron oxides as electron acceptor. Iron oxides would have reacted spontaneously with the H_2S generated by $S_2O_3^{2-}$ disproportionation (Equation 2 – Finster er al. [8]) which should support growth due to the removal of the end product H_2S and result in the precipitation of FeS (Equation 3). This was not observed.

(2) $S_2O_3^{2-} + H_2O \longrightarrow SO_4^{2-} + HS^- + H^+$

(3) $Fe^{2+} + HS^- \longrightarrow FeS + H^+$

To exclude that disproportionation might not have been detectable due to low initial cell number we cultivated strain SoZ1 with MnO_2 and a surplus of $S_2O_3^{2-}$. The concentration of $S_2O_3^{2-}$ remained constant in the presence of 2.5 x 10^7 cells mL^{-1} after MnO_2 was depleted (Fig. A.7). We therefore suggest that SoZ1 and GD2 were unable to disproportionate S^0 and $S_2O_3^{2-}$ under the tested culturing conditions.

Strain SoZ1 was observed as obligate carbon-autotrophic while strain GD2 showed growth also with a mixture of the short chain fatty acids acetate, succinate and propionate in the absence of inorganic carbon sources. To test for the ability to fix molecular nitrogen (N_2) we

omitted the addition of NH_4^+ as source for nitrogen. Surprisingly, cells showed growth even after three consecutive re-cultivations, each with N_2 as the sole source for nitrogen. The growth yields of both strains under these culturing conditions were slightly smaller compared to the respective positive controls (Fig. A.8). The ability to fix N_2 was not observed in the genus *Sulfurimonas* before and to our knowledge, only *S. autotrophica* $OK10^T$ has been tested for N_2 fixation so far. Interestingly, Kirkpatrick et al. (2018) found that the *nifH* gene, a key enzyme for N_2 fixation, is actively expressed by *Sulfurimonas* spp. in the Black Sea redoxcline, where strain SoZ1 was isolated from, which may be confirmed with the two new isolates in future studies.

Conclusion:

The oxidation of reduced sulfur compounds coupled to the reduction of manganese oxide was suggested since the late 1980s and described by Henkel et al. in 2019. Based on the data we showed here and our general experience with the cultivation of the strains in the last 7 years we suggest that a few crucial details led to the successful isolation of these two strains. We pre-enriched the organisms by providing commercially available manganese(IV)-oxide as electron acceptor in the original water samples and added small amounts of H_2S daily over several weeks. In the natural system the prevailing electron donor H_2S is present in low concentrations but with constant supply from upward fluxes from deeper waters [38]. The abiotic reaction of H_2S and MnO_2 (Eq. 1) reduces the potential energy for H_2S-oxidizing and MnO_2-reducing bacteria. The rate of the abiotic reaction is hampered by low concentrations of both reactants [51] and the mineralogical structure of the added MnO_2 [16]. The use of commercially available MnO_2 in this study might have been a crucial detail, because it appeared to be less reactive than freshly precipitated MnO_2 (after Murray et al. [29]). The MnO_2 used in this study, which had a black rather than a brownish-black color and appeared in SEM micrographs in needle shape rather than in aggregates of spherical particles [29], was not reacting chemically with $S_2O_3^{2-}$ (see also Henkel et al. [14]), allowing batch type cultivation and simplified the dilution to extinction procedure.

Next to technical MnO_2, also the pH buffering had likely a large effect on the successful isolation of strains GD2 and SoZ1. The medium was buffered with bi-carbonate (HCO_3^- supplied as $NaHCO_3$) and the pH was adjusted with N_2 gas containing 20% CO_2 which was used also for the deoxygenation in the preparation of media and the bottle headspaces. As described by Henkel et al. [14], the Mn reducing activity of *Ca.* S. marisnigri strain SoZ1

raises the pH and the concentration of dissolved reduced Mn species, resulting in the formation of a Ca-rich Mn(II)-carbonate, which was also observed with strain GD2. This carbonate authigenesis scavenged almost the complete amount of Mn added to the medium [14] and consequently an even larger amount of inorganic C (CO_2 and its dissociation products in water). Therefore, we experienced lower growth yields in cultivations with the pH buffer ingredients acetate, MES, HEPES and TRIS used in experiments for the pH preferences. In these experiments, only 2-3 mM HCO_3^- were added as source for inorganic C instead of ~ 30 mM HCO_3^- added as pH buffer and N_2 without CO_2 was used for deoxygenation. In these experiments, no color change from black to brownish-gray was observed, which typically showed the complete reduction of MnO_2 to dissolved $Mn^{2+/3+}$ followed by the precipitation of a Ca-rich Mn(II)-carbonate [14]. However, Mn(II)-carbonates still precipitated and were found in SEM-EDX analysis, but the major part of the particles remained as unchanged MnO_2. The precipitation of Mn(II)-carbonate most probably scavenged inorganic C and autotrophic growth could not proceed, leading to incomplete reduction of MnO_2 and overall lower cell yields in these experiments even though the same amount of electron donor and acceptor was available. We conclude that the technical MnO_2 used in this study and the elevated inorganic carbon content in cultivations were probably the main reasons for a successful isolation of *Ca.* S. marisnigri SoZ1 and *Ca.* S. baltica GD2, whereas earlier attempts to cultivate bacteria with this physiology from the Black Sea [19] have failed. With the description of chemolithoautotrophy via Mn(IV) reduction, the availability and characterization of the two isolated species including the complete genome sequence reported here, the basis for detailed research is established.

Möglich: SoZ1 und GD2 Abgrenzung durch Habitat: Ostsee – Black Sea, pure pelagic – eventually also sedimentary, autotroph – mixotroph. Damit nicht alles begründet wird auf Sequenzunterschiede

Ca. S. marisnigri SoZ1 and *Ca.* S. baltica GD2 were identified as separate species based on the genome relatedness indices ANI and AAI. Both isolates belong to the genus *Sulfurimonas* based on POCP values with *S. gotlandica* $GD1^T$ as the closest validly described species based on 16S rRNA sequence similarity, ANI and AAI. *Ca.* S. marisnigri SoZ1 and *Ca.* S. baltica GD2 are proposed as *Candidatus* species because the deposition in culture collections takes longer that we have had expected. Until the strains are listed in culture collections we are willing to distribute culture samples upon request to interested colleagues.

Description of *Candidatus* Sulfurimonas marisnigri sp. nov.

Genus name	*Sulfurimonas*
Species name	*Candidatus* Sulfurimonas marisnigri
Specific epithet	marisnigri
Species status	sp. nov.
Species etymology	L. n. *mare -is*, the sea; L. masc. adj. *niger -gra -grum*, black; N.L. gen. n. *marisnigri*, of the Black Sea
Description of the new taxon and diagnostic traits	Cells are motile, slightly curved rods, an in size variable morphology with a length of 1.76 µm ± 0.57 µm and a width of 0.46 µm ± 0.09 µm (mean ± SD) observed in the late exponential phase, gram-staining-negative and motile by means of a polar flagellum with up to two polyphosphate inclusions located at the cell poles. Cells can appear coccoid and in aggregates in late growth stages. Growth is chemolithoautotrophic with manganese oxide (optimum), nitrate and oxygen as electron acceptors and hydrogen, sulfide (optimum), elemental sulfur and thiosulfate as electron donors. Can tolerate oxygen to atmospheric conditions when manganese oxide is provided as electron acceptor but does not show growth with oxygen as electron acceptor at an atmospheric partial pressure. Positive growth can be seen at 0 - 25 °C (optimum 20 °C - psychrotolerant), salinity from 10 - 40 g L^{-1} (optimum 25 g L^{-1}) and pH 6.5 – 9.0 (optimum 7.5 – 8.0). Ammonium and N_2 can serve as nitrogen sources. For details see Henkel, J. V. - Dissertation[15].
Country of origin	Ukraine
Region of origin	Black Sea
Source of isolation	Chemocline central Black Sea
Sampling date (dd/mm/yyyy)	21/11/2013
Latitude (xx°xx′xx″N/S)	44°16’45”N
Longitude (xx°xx′xx″E/W)	36°18’57”E
16S rRNA gene accession nr.	NCBI: HUE87_02100
Genome accession number [RefSeq; EMBL; …]	NCBI: CP054493
Genome status	Complete
Genome size	2,488.728
GC mol%	33.5
Number of strains in study	1
Information related to the Nagoya Protocol	Does not apply
Designation of the Type Strain	SoZ1

Description of *Candidatus* Sulfurimonas baltica sp. nov.

Genus name	*Sulfurimonas*
Species name	*Candidatus* Sulfurimonas baltica
Specific epithet	baltica
Species status	sp. nov.
Species etymology	M.L. fem. adj. *baltica*, pertaining to the Baltic Sea

Description of the new taxon and diagnostic traits	Cells are motile, slightly curved rods, an in size variable morphology with a length of 1.41 µm ± 0.41 µm and a width of 0.44 µm ± 0.08 µm (mean ± SD) observed in the late exponential phase, gram-staining-negative and motile by means of a polar flagellum with up to two polyphosphate inclusions located at the cell poles. Cells can appear coccoid and in aggregates in late growth stages. Growth is chemolithoautotrophic or heterotrophic with manganese oxide (optimum), nitrate and oxygen as electron acceptors and hydrogen, sulfide (optimum), elemental sulfur and thiosulfate as electron donors. Can tolerate oxygen at 4% partial pressure but not at atmospheric conditions. Positive growth can be seen at 0 - 20 °C (optimum 15 °C - psychrotolerant), a salinity ranging from 4 - 40 g L^{-1} (optimum 14 g L^{-1}) and pH 7.0 – 8.0 (optimum 7.0 – 7.5). Ammonium and N_2 can serve as nitrogen sources. For details see Henkel, J. V. - Dissertation [15].
Country of origin	Latvia
Region of origin	Baltic Sea
Source of isolation	Anoxic bottom water after Major Baltic Inflow in 2014 [7,28]
Sampling date (dd/mm/yyyy)	14/06/2016
Latitude (xx°xx′xx″N/S)	57°19’12”N
Longitude (xx°xx′xx″E/W)	20°3’0”E
16S rRNA gene accession nr.	NCBI: HUE88_08945
Genome accession number [RefSeq; EMBL; …]	NCBI: CP054492
Genome status	complete
Genome size	2,737.757
GC mol%	33.8
Number of strains in study	1
Information related to the Nagoya Protocol	Does not apply
Designation of the Type Strain	GD2

Author statement:

Henkel, J. V.: Conceptualization, methodology, validation, investigation, writing – original draft, visualization, project administration **Vogts, A.**: Investigation, writing – review & editing **Werner, J.**: Software, writing – review & editing **Neu, T. R.**: Investigation, writing – review & editing **Spröer, C.**: Resources, writing – review & editing **Bunk, B.**: Software, writing – review & editing **Schulz-Vogt, Heide N.**: Conceptualization, supervision, funding acquisition, writing – review & editing

Declaration of interests:

None

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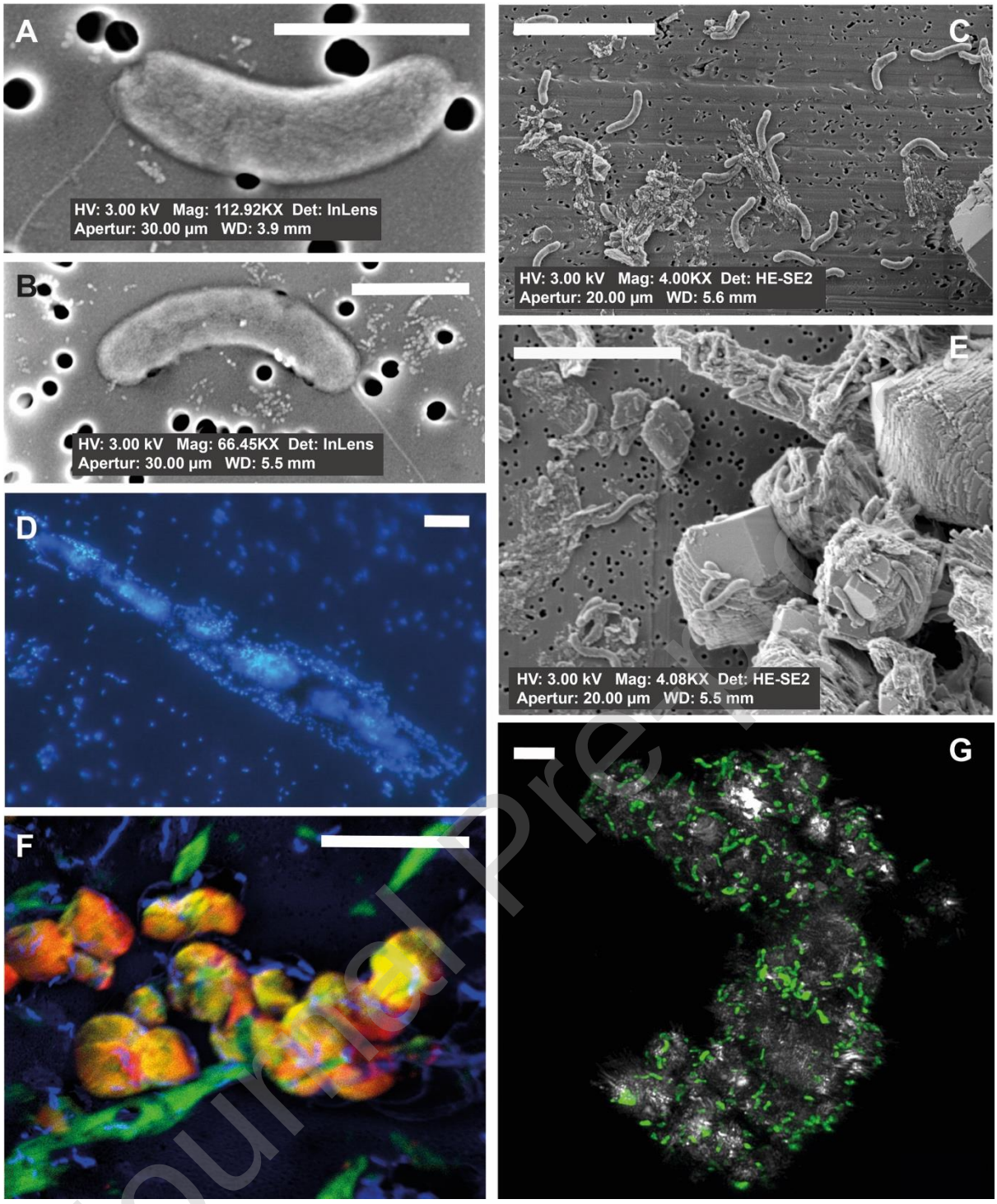

Fig. 1: Morphology of strain GD2 (A) and SoZ1 (B-G). Cells of both strains appeared as slightly curved rods, sometimes vibrio-like (A and B). Cells had one polar flagellum but depending on cellular attachment to Mn-particles and growth phase, cells without a flagellum were present. Cells showed attachment to particles (D – DAPI stained epifluorescence microscopy, E – scanning electron microscorpy, F – nanosclae secondary ion mass spectroscopy (NanoSIMS) and G – confocal laser scanning microscopy (CLSM) of a colonized particle stained with the membrane dye FM1-43 (green; white = reflection). With NanoSIMS analysis (F) particles were identified as Mn-particles (Elements were color coded: $^{12}C^{14}N^-$ as reference for bacterial cells and biomass, blue; $^{55}Mn^+$, green; $^{40}Ca^+$, red), where manganese(IV)-oxides appeared as green needle shaped particles and calcium-rich manganese(II)-carbonates as a mixture of green and red rhombohedral shaped particles – see also Henkel et al. (2019). Scale bars: A and B, 1 µm; C, E and G, 5 µm; D and F, 10 µm.

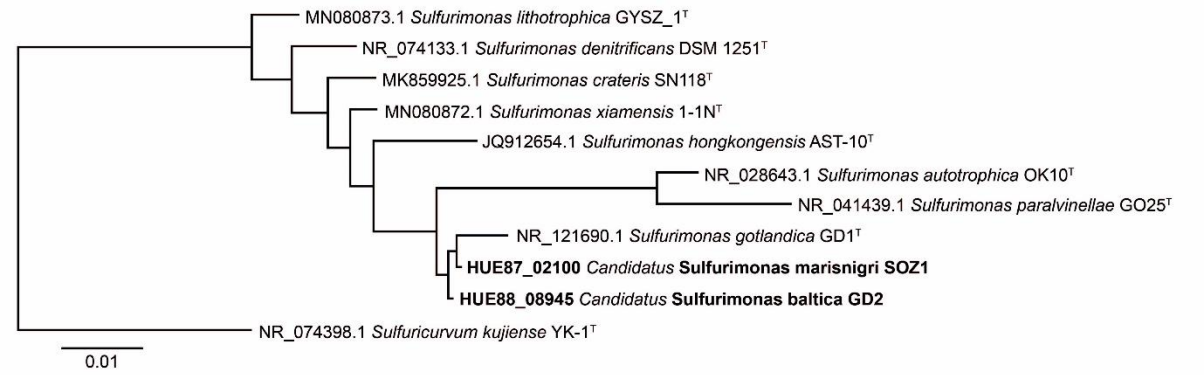

Figure 2: Phylogenetic tree based on 16S rRNA gene sequence of the genus *Sulfurimonas* with the new isolates *Candidatus* Sulfurimonas marisnigri SoZ1 and *Candidatus* Sulfurimonas baltica GD2. Accession number for the respective sequence data is provided before the species names. Scale bar in substitutions per nucleotide position.

Table 1: Average nucleotide identity (ANI), average amino acid identity (AAI) and percentage of conserved protein (POCP) analysis of available genome sequences of species of the genus *Sulfurimonas*. ANI and AAI were calculated for the demarcation of species and POCP was calculated to verify the consistency of the genus. Note that no genomic sequence for *S. paralvinellae* GO25^T was available. Values reaching the cut-off value are written in bold. Metagenome annotated genome (MAG) of *Ca.* S. ponti was taken from [45].

Average nucleotide identity (ANI) – same species if ≥96 % [9]												
	S.	*Ca.* S.	*S.*	*S.*	*S.*	*S.*	*S.*	*Ca.* S.	*S.*	*S.*	*S.* sp.	*Ca.*
S.	-	-	-	-	-	-	-	-	-	-	-	-
Ca. S. baltica	75.90	-	-	-	-	-	-	-	-	-	-	-
S. crateris	75.48	76.39	-	-	-	-	-	-	-	-	-	-
S.	75.59	76.84	77.57	-	-	-	-	-	-	-	-	-
S. gotlandica	76.16	77.48	75.89	76.53	-	-	-	-	-	-	-	-
S.	75.63	76.16	75.87	76.97	77.88	-	-	-	-	-	-	-
S.	75.86	75.81	75.51	75.66	75.95	75.61	-	-	-	-	-	-
Ca. S.	75.96	88.55	76.33	77.01	77.82	76.31	75.94	-	-	-	-	-
S. xiamensis	76.11	76.99	79.97	78.13	76.43	76.33	75.97	77.19	-	-	-	-
S. sp. CVO	76.04	76.92	80.09	78.33	76.41	76.34	75.94	77.17	**98.61**	-	-	-
Ca. S. ponti	75.95	75.97	75.15	75.60	76.40	75.47	75.74	76.41	75.65	-	75.64	-
Average amino acid identity (AAI) – same species if ≥95 % [22]												
S.	-	-	-	-	-	-	-	-	-	-	-	-
Ca. S. baltica	70.16	-	-	-	-	-	-	-	-	-	-	-
S. crateris	70.00	75.21	-	-	-	-	-	-	-	-	-	-
S.	68.57	73.73	77.67	-	-	-	-	-	-	-	-	-
S. gotlandica	71.22	75.43	73.25	71.51	-	-	-	-	-	-	-	-
S.	70.09	72.44	71.97	71.28	77.42	-	-	-	-	-	-	-
S.	69.45	70.57	70.24	69.09	71.08	70.21	-	-	-	-	-	-
Ca. S.	70.39	90.26	75.25	73.75	75.32	72.39	70.80	-	-	-	-	-
S. xiamensis	70.73	76.29	81.90	78.06	74.23	72.70	71.20	76.47	-	-	-	-
S. sp. CVO	70.66	76.02	81.85	78.04	74.11	72.77	70.99	76.27	**98.58**	-	-	-
Ca. S. ponti	71.16	72.04	71.21	69.82	72.81	71.15	70.09	72.23	71.76	-	71.67	-
Percentage of conserved proteins (POCP) – same genus if ≥50% [36]												
S.	-	-	-	-	-	-	-	-	-	-	-	-
Ca. S. baltica	**63.73**	-	-	-	-	-	-	-	-	-	-	-
S. crateris	**67.45**	**65.59**	-	-	-	-	-	-	-	-	-	-
S.	**66.94**	**64.90**	**74.98**	-	-	-	-	-	-	-	-	-
S. gotlandica	**66.57**	**66.50**	**66.49**	**65.99**	-	-	-	-	-	-	-	-
S.	**68.49**	**63.63**	**71.05**	**73.95**	**70.81**	-	-	-	-	-	-	-
S.	**67.89**	**65.00**	**68.30**	**67.87**	**69.38**	**69.21**	-	-	-	-	-	-
Ca. S.	**66.52**	**80.65**	**67.60**	**67.40**	**69.74**	**66.02**	**67.65**	-	-	-	-	-
S. xiamensis	**65.52**	**62.27**	**73.34**	**71.46**	**60.89**	**68.47**	**66.10**	**65.07**	-	-	-	-
S. sp. CVO	**65.62**	**63.03**	**73.11**	**72.29**	**61.00**	**68.70**	**66.15**	**65.42**	**91.60**	-	-	-
Ca. S. ponti	**65.76**	**62.43**	**62.51**	**61.15**	**64.86**	**62.84**	**64.71**	**68.15**	**60.38**	-	**59.91**	-

Table 2: Phenotypic characterization of *Candidatus* Sulfurimonas marisnigri SoZ1 and *Candidatus* Sulfurimonas baltica GD2 and other cultured species of the genus *Sulfurimonas*. § = In this study (see *S. gotlandica* GD1^T). ( ) = uncertain. * = to NO_2^-. References: [5,12,44,47,13,17,23,37,40–43]

Characteristic	*Candidatus* S. marisnigri SoZ1	*Candidatus* S. baltica GD2	*S. xiamensis* 1-1N^T	*S. lithotrophica* GYSZ 1^T	*S. crateris* SN118^T	*S. denitrificans*	*S. paralvinellae* GO25^T	*S. autotrophica* OK10^T	'*S. hongkongensis*' AST-10	*S. gotlandica* GD1^T
Shape	Curved rods	Curved rods	Rods to slightly curved	Rods to slightly curved	Straight or curved rods	Rods to spirilla- like	Rods	Rods	Rods	Curved rods to spirilla-like
Origin	Water column redoxcline, Black Sea	Water column redoxcline, Baltic Sea	Coastal sediment, Guanyinshan beach,	Coastal sediment, Guanyinshan beach,	Terrestrial mud volcano, Taman Peninsula	Sediment, Dutch Wadden Sea	Polychaete nest, deep-sea hydrothermal	Hydrothermal sediment, Mid-Okinawa Trough	Coastal Sediment, Hong Kong	Water column redoxcline, Baltic Sea
Motility	+	+	-	+	+	-	+	+	ND	+
Doubling time under optimal growth conditions (h)	9	6	12	8	2.2	12	13-16	1.4	6.1	13; 9§
Temperature range (Optimum) (°C)	0-25 (20)	0-20 (15)	10-45 (30)	4-45 (33)	5-40 (30)	10-30 (22)	4-35 (30)	10-40 (23-26)	15-35 (30)	4-20 (15); 0-25 (15-20)§
pH range (Optimal pH)	6.5-9.0 (7.5-8.0)	7.0-8.0 (7.0-7.5)	5.5-8.0 (7.0)	5.0-8.5 (6.5)	5.5-9.5 (8.0)	ND (7)	5.4-8.6 (6.1)	5.0-9.0 (6.5)	6.5-8.5 (7.0-7.5)	6.5-8.4 (6.7-8.0); 6.5-8.0 (7.5)§
NaCl requirement	ND	ND	-	+	+	-	+	+	+	+
Salinity tolerance (g L^{-1}) (Optimum)	10-40 (25)	5-40 (21)	0-90 (50)	5-90 (20)	5-80 (20-30)	ND	12-50 (30)	16-60 (40)	10-60 (30)	5-40 (14)§
Maximum O_2 concentration (%)	Atmospheric oxygen concentration	4	20	20	Atmospheric oxygen concentration	0.5	10	15	Obligate anaerobe	10<20, Atmospheric oxygen concentration§
Energy sources	H_2,HS^- ,S^0,$S_2O_3^{2-}$	H_2,HS^- ,S^0,$S_2O_3^{2-}$	H_2,S^0,$S_4O_6^{2-}$,$S_2O_3^{2-}$,HS^-	H_2,S^0,$S_2O_3^{2-}$,HS^-	S^0,$S_2O_3^{2-}$,HS^-	$S_2O_3^{2-}$,HS^-, H_2	H_2,S^0,$S_2O_3^{2-}$	S^0, $S_2O_3^{2-}$	H_2,HS^-,$S_2O_3^{2-}$	H_2,S^0,$S_2O_3^{2-}$,HS^-,SO_3^{2-}
Organic electron donors	-	-	-	-	-	Formate, fumarate, yeast extract alcohol mix	-	-	-	Formate, acetate, yeast extract, pyruvate,
Electron acceptors	MnO_2, NO_3^- *,O_2	MnO_2, NO_3^- (*),O_2	S^0, NO_3^-,O_2	S^0, NO_3^-,O_2	NO_3^-,NO_2^-,O_2	NO_3^-,NO_2^-,O_2	NO_3^-,O_2	O_2	NO_3^-	NO_3^-,NO_2^- ,$NO_2^{-(\$)}$,$O_2$§
S-disproportionation (S^0;$S_2O_3^{2-}$)	-;-	-;-	ND	ND	-;ND	ND	ND	ND	ND	ND, -;-§
C-source	CO_2/HCO_3^-	CO_2/HCO_3^-, acetate, propionate,	CO_2/HCO_3^-	CO_2/HCO_3^-	CO_2/HCO_3^-	CO_2/HCO_3^-	CO_2/HCO_3^-	CO_2/HCO_3^-	CO_2/HCO_3^-	CO_2/HCO_3^- ,(pyruvate)
N-fixation	+	+	ND	ND	ND	ND	ND	-	ND	ND
DNA G+C content (mol%)	33.5	33.8	34.5	33.2	38.8	36	37.6	35.2	34.9	33.6