Members of the class *Epsilonproteobacteria* are increasingly recognized as predominantly auto- to mixotrophic organisms globally ubiquitous in modern marine and terrestrial ecosystems (8). A number of studies have verified their significant role in biogeochemical cycles, particularly those that are sulfur-dependent, as demonstrated for deep-sea hydrothermal fields (8, 33, 35), sulfidic cave springs (11, 12), and autotrophic epiphytic associations (35, 40). Some studies have discovered the prevalence and diversity of *Epsilonproteobacteria* in pelagic marine redoxclines of the Black Sea (42) and the Cariaco Trench (31), two of the largest anoxic basins on this planet (8). Marine pelagic redoxclines, as the transition between oxic and anoxic milieus, are characterized by a gradient in the redox potential that is dependent on the activity of oxidizing versus reducing agents (16). Catalyzed reporter deposition and fluorescence in situ hybridization (CARD-FISH) analyses using gene probe EPS549 demonstrated maximal epsilonproteobacterial abundances of up to 30% of total cell counts in pelagic redoxclines of both basins (29), usually in zones where remarkable chemoheterotrophic activity had been previously detected (20, 29, 41).

The Baltic Sea itself is among the largest brackish basins of the world. The Baltic proper comprises a number of deep areas with anoxic bottom water, of which the Gotland Deep is the largest and the Landsort Deep, at 495 m, is the deepest. Pelagic redoxclines in the central Baltic Sea are also generally characterized by high carbon dioxide (CO₂) dark fixation rates, which may account for up to 30% of surface primary production (10). The simultaneous occurrence of high denitrification rates has already led to the conclusion that chemolithoautotrophic oxidation of sulfur compounds coupled to nitrate reduction likely plays an important role in these pelagic redoxclines (6). Furthermore, the epsilon-proteobacterium “uncultured *Helicobacteraceae* G138eps,” which is related to *Sulfurimonas denitrificans*, was identified as a potentially important chemolithoautotrophic sulfide oxidizer and nitrate reductase responsible for denitrification processes at the redoxcline of the Gotland Deep (5). Quantitative PCR (qPCR) (24) and calculations based on stimulation experiments (26) showed that the abundance of this organism within the redoxcline is as high as 5 × 10⁵ cells ml⁻¹. However, compared to the Black Sea (42) or Cariaco Trench (31), the Gotland Deep contains a lower epsilonproteobacterial diversity (5, 26), which raises the question whether the previously identified, uncultured autotrophic denitrifier dominates this class in Baltic redoxclines. In order to resolve this question, we determined the vertical distribution and in situ abundance of this strain in high-resolution profiles of different redoxclines of the central Baltic Sea by using a newly developed gene probe. The results were compared to the overall abundance of *Epsilonproteobacteria*.

### Materials and Methods

**Sampling.** The samples were obtained from the central Baltic Sea during research cruises onboard the RV *Alexander von Humboldt* in August 2003 (Baltic sea monitoring station 286, 58°0.00′ N; 19°54.00′ E, Farö Deep), the RV *Alkor*...
May 2005 (station 271, 57°19.2′N; 2°03′E, Gotland Deep), and the RV Maria S. Merian in February 2006 (station 271). Water samples from different depths around the chemocline, which we define as the shallowest appearance of sulfide, were collected in flow-through bottles attached to a conductivity, temperature, and depth rosette. Electrodes for salinity and temperature measurements were also attached to the conductivity, temperature, and depth rosette. Concentrations of inorganic nutrients, oxygen, and hydrogen sulfide were analyzed immediately after sampling, as described elsewhere (15).

CO₂ dark fixation. CO₂ dark fixation rates were determined using a modification of the method described by Steenbeek-Niemann (39). Accordingly, 100 µCi [1⁴C]bicarbonate in anoxic solution (specific activity, 53.0 mCi mmol⁻¹; Hartmann Analytic GmbH, Braunschweig, Germany) was added to 120-ml Winkler bottles containing the collected samples. After incubation at 15°C for 24 h in the dark, the samples were filtered through 0.2-µm filters and then exposed to HCl fumes. Radioactivity was counted in a scintillation counter (Packard).

Bacterioplankton preparation. For FISH and total cell counts, 100-ml portions of water samples were directly transferred to glass bottles and fixed with particle-free formaldehyde (2% final concentration) for 2 to 5 h at 4°C. Portions of 40 to 45 ml were filtered onto white polycarbonate membrane filters (type GFF, diameter 47 mm; Millipore). The filters were then rinsed with sterile seawater, air-dried, and stored at -80°C until further processing. For DNA extraction, 1 to 2 liters of water samples was filtered onto white Durapore filters (type GVWP; pore size, 0.22 µm; diameter, 47 mm; Millipore) and frozen at -80°C.

16S rRNA gene clone library construction and RFLP. A bacterial 16S rRNA gene clone library was prepared from a genomic DNA extract originating from the Faroè Deep (depth of 110 m). Nucleic acids were extracted by the procedure of Schauer et al. (36). To amplify nearly full-length 16S rRNA genes from the total community DNA, the bacterial primer system 27f (5'-AGAGTTTGATCCTTAGCTCAG-3') and 1492r (5'-GTTACCTTGTGACGACTT-3') (27) was used for PCR. PCR mixtures (50 µl) contained 1× PCR buffer, 250 µM of each deoxyribonucleotide triphosphate, 0.15 µM of each forward and reverse primer, and 1.25 U Tag polymerase (Fermentas). The reaction mixtures were incubated in a MyCycler (Biorad) under the following conditions: initial denaturation at 94°C for 5 min; 30 cycles, with 1 cycle consisting of 94°C for 1 min, 45°C for 45 s, and 72°C for 2 min; followed by one cycle of 72°C for 2 min. The PCR products were purified (MiniElute PCR purification kit; Qiagen) and then cloned following the manufacturer’s instructions using the pGEM-T Easy vector system (Promega) together with competent Escherichia coli JM109 cells. For restriction fragment length polymorphism (RFLP) and sequencing, the inserted fragment was PCR amplified with the vector-specific primers T7 and SP6. The unpurified PCR products were digested with the restriction enzymes Hinf I (Hha I) and Msp I (Hpi II) (Fermentas). Restricted fragments were analyzed by gel electrophoresis, and restriction patterns were compared visually. 16S rRNA gene clones with identical band patterns were merged into one RFLP group, and representative cloned fragments were sequenced by Seqlab (Gottingen, Germany) and JenaGen (Jena, Germany) using the primers 27f, 1492r, 533f [5'-GGTACCTTGTGACGACTT-3'] (27), and com2rpH (5'-GTGCCAGC(A/C)GC-3') (27), and com2rpH (5'-GTGCCAGC(A/C)GC-3') (27).

Phylogenetic analysis. 16S rRNA gene sequences were examined for accuracy using the software package SeqMan (DNAStar) and checked for chimeras using the Bellerophon program (19). Phylogenetic affiliations of the partial 16S rRNA sequences were initially estimated with the program BLAST (2). Sequences were aligned using the ARB software package (30). Sequences with similarity greater than 99% were grouped and named GD17. Phylogenetic trees were constructed based on sequences of approximately 1,400 nucleotides. These sequences were then clustered with 99% similarity using the Bellerophon program (19). Phylogenetic affiliations of the GD17 group.

In conformity with the protocol of Pernthaler et al. (34), filter sections em- derived in low-gelling-point agarose were permeabilized with lysosyme and then placed in 1.5-ml reaction vials containing 400 µl of hybridization buffer and 2 µl of probe working solution (50 pmmol µl⁻¹). For probe mix EU83381 to III, 600 µl of hybridization buffer and 3 µl of probe working solution were used. Hy- bridization was carried out at 35°C for 8 to 12 h on a rotary shaker in the dark. According to the procedure of Sekar et al. (38), filter sections were washed in prewarmed washing buffer and the tyramide signal was amplified with 5-(and 6)-carboxyfluorescein-labeled tyramides. The preparations were counterstained with a previously described mixture of 4',6-diamidino-2-phenylindole (DAPI), Citifluor, and VectaShield (34). Stained filter sections were examined with an epifluorescence microscope (Axioskop 2 mot plus; Zeiss) equipped with a 100× Plan Apochromat oil objective lens (Zeiss). Fluorescein isothiocyanate-stained

| Table 1. Probe sequence, appropriate target sites, and corresponding sequences in other bacteria, including reference organisms |
|---------------------------------------------------------------|------------------|
| **SUL90 probe**, a target, and bacteria | **No. of mismatched nucleotides** | **Sequence** |
| Probe | Target | SUL90 | Sulfuricurvum kuijensii YK-2 | Sulfuricurvum kuijensii YK-4 | Sulfurimonas denitrificans | Methylophaga marina |
| AUAACGUAIAACCCGCGCCGCG | 0 | 1 | 1 | 1 | 4 | ACGAGCGGCGGCGGAGGCGGA |
| AUAGAUGAAUGUGCCGGCCACG | 0 | 0 | 0 | 0 | 0 | ACGAGCGGCGGCGGAGGCGGA |
| Target/Reference organisms | | | | | | |
| Uncultured Helicobacteriae | 1 | | | | | |
| Clone GD17b | 0 | | | | | |
| Clone GD17a | 0 | 1 | | | | |
| Moraxella cuniculi | 1 | | | | | |
| Moraxella bovis | 1 | | | | | |
| Moraxella ovis | 1 | | | | | |
| Methylphaga sp. | 1 | | | | | |
| Sulfuricurvum kuijensii YK-1 | 1 | C- | | | | |
| Sulfuricurvum kuijensii YK-2 | 1 | | | | | |
| Legionella sp. | 1 | | | | | |
| Sulfurimonas denitrificans | 4 | GCG- | CCG- | | | |
| Methylophaga marina | 4 | ACG- | | | | |

a The 5'-Sul-0090-a-A-18 probe was named according to the Olignonucleotide Probe Database nomenclature (1) and is abbreviated as SUL90.

b The probe sequence is shown 3'-5', and the target sequence is shown 5'-3'. For the target/reference organisms, nucleotides identical to those in the target sequence (−) are indicated.

discrimination of non-target species was successful for the close relative Sulfurimonas denitrificans and for the less-related Methylphaga marina, whereas nonspecific binding with the negative-control strain Sulfuricurvum kuijensii was recorded at all formamide concentrations tested. However, the signal from this negative-control strain was always much weaker than that from the positive reference enrichment culture. We considered that the nonspecific binding with Sulfuricurvum kuijensii did not raise a problem in this study since the formamide concentration for the newly designed probe SUL90 was optimized (formamide concentrations tested, 55%, 60%, and 65%). A concentration of 10% formamide in the hybridization buffer was found to be optimal for probe specificity.

Discrimination of non-target organisms was successful for the close relative Sulfurimonas denitrificans and for the less-related Methylphaga marina, whereas nonspecific binding with the negative-control strain Sulfuricurvum kuijensii was recorded at all formamide concentrations tested. However, the signal from this negative-control strain was always much weaker than that from the positive reference enrichment culture. We considered that the nonspecific binding with Sulfuricurvum kuijensii did not raise a problem in this study, since Sulfuricurvum kuijensii, isolated from an underground crude oil storage cavity (23), was assumed to be not present in the Baltic Sea and has never been detected in this habitat before.

In conformity with the protocol of Pernthaler et al. (34), filter sections em- bedded in low-gelling-point agarose were permeabilized with lysosyme and then placed in 1.5-ml reaction vials containing 400 µl of hybridization buffer and 2 µl of probe working solution (50 pmmol µl⁻¹). For probe mix EU83381 to III, 600 µl of hybridization buffer and 3 µl of probe working solution were used. Hy- bridization was carried out at 35°C for 8 to 12 h on a rotary shaker in the dark. According to the procedure of Sekar et al. (38), filter sections were washed in prewarmed washing buffer and the tyramide signal was amplified with 5-(and 6)-carboxyfluorescein-labeled tyramides. The preparations were counterstained with a previously described mixture of 4',6-diamidino-2-phenylindole (DAPI), Citifluor, and VectaShield (34). Stained filter sections were examined with an epifluorescence microscope (Axioskop 2 mot plus; Zeiss) equipped with a 100× Plan Apochromat oil objective lens (Zeiss). Fluorescein isothiocyanate-stained...
cells were counted, followed by the determination of DAPI-stained cells as an indicator of total prokaryotic abundance. At least 1,000 DAPI-stained cells in randomly distributed microscopic fields were counted for each filter section. Negative-control counts with probe NonEUB averaged 0.11 to 0.28% for both the Faro¨ Deep and Gotland Deep in May 2005 and February 2006. Bacterial counting of both DAPI-stained samples and hybridized samples was usually done with a standard deviation of less than 7.5%.

Quantification with real-time PCR. An iCycler (Bio-Rad) and the iQ SYBR Green supermix were used for real-time PCR. A GD17-specific absolute DNA standard with a defined number of gene copies was prepared by amplifying inserted fragments of the GD17a clone (generated by the 27f/1492r primer pair [see above]) with the vector-specific primers T7 and SP6, resulting in a 1,661-bp PCR product. This was purified using the MinElute purification kit (Qiagen), and the PCR product concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The copy number nanogram⁻¹ was calculated based on the weight and length of the PCR product as described previously (24). Eventually, GD17 16S rRNA genes were quantified based on a nested PCR approach by using the specific primers OST 1F (5'-TCAGAGATGTGAAATCCATTGCTCA-3') and OST 1R (5'-CTTAGCGTCAGTTATGTTCCAGG-3'). Positions 803 to 825 as previously described (24). The conversion of environmental GD17 16S rRNA gene copy numbers into cell numbers was calculated based on the assumption of 2.3 16S rRNA gene copies per cell, which was previously reported as the average epsilonproteobacterial gene copy number (22). GD17 quantification was done with a standard deviation of less than 7.5%.

Statistical analyses. Spearman rank correlations were used to investigate the relations among different parameters at significance levels of 0.01.

Nucleotide sequence accession numbers. Sequences were deposited in the GenBank database under accession numbers EF405797 (GD17a) and EF405798 (GD17b).

RESULTS

Physicochemical features. The chemocline in the Faro¨ Deep in 2003 was located at a depth of 108 m (Fig. 1A). A small overlap of nitrate and sulfide was detected around the chemocline. In the Gotland Deep in May 2005, sulfide was first detected at a depth of 208 m (Fig. 2A). This water profile lacked a clear overlap of sulfide and nitrate. In 2006, the Gotland Deep chemocline was difficult to define because of the extended overlap of oxygen and sulfide at a depth of 121 to 127 m (Fig. 3A).

CO₂ dark fixation rates. In the oxic/sulfidic transition zone of the Gotland Deep, the CO₂ fixation rates increased in May 2005 from 0.12 μmol liter⁻¹ day⁻¹ at 206.5 m to 0.72 μmol liter⁻¹ day⁻¹ at 215.5 m, with a maximum in the sulfidic area (Fig. 2A). In February 2006, the CO₂ fixation rates increased from 0.1 μmol liter⁻¹ day⁻¹ at 123 m to the highest rate of 0.71 μmol liter⁻¹ day⁻¹ at a depth of 132 m in the sulfidic zone (Fig. 3A). No measurements were done in 2003.

16S rRNA gene clone library and probe design. RFLP pattern evaluation of 159 16S rRNA gene clones showed 24 visually distinguishable RFLP groups. Representative clones of all RFLP groups which occurred more than once in the clone library were sequenced, accounting for a total of 21 RFLP patterns. Sequencing confirmed 12 phylogenetically different
clones (see Table S1 in the supplemental material). The most abundant RFLP group (21%) consisted of clones phylogenetically closely related to the epsilonproteobacterium “uncultured Helicobacteraeae” (5) within the Sulfurimonas cluster and probably represented members of a new species (Fig. 4). The nearly identical full-length 16S rRNA gene sequences of two clones as well as the “uncultured Helicobacteraeae” sequence were pooled and named group GD17, and the group-specific rRNA-targeting oligonucleotide probe SUL90 was designed for the GD17 group. Probe details and number of mismatches with cultured organisms are presented in Table 1.

Quantification of bacteria by CARD-FISH. The total prokaryotic abundance in the Farö Deep redoxcline in August 2003 varied between $3.9 \times 10^5$ and $1.5 \times 10^6$ cells ml$^{-1}$, with maximal cell numbers within the redoxcline (Fig. 1B). Bacterial cell numbers followed the prokaryotic pattern, with 57% EUB338-positive cells. GD17 cells were absent at depths less than 104 m but increased in cell number, with a peak of 1.9 $\times 10^5$ cells ml$^{-1}$ at 110 m, i.e., shortly below the chemocline in February 2006. (A) Concentrations of H$_2$S, O$_2$, NO$_3^-$, and CO$_2$ dark fixation rates. (B) Vertical distributions of total cell numbers (DAPI), eubacteria (EUB), GD17, and epsilonproteobacterial (EPS) cell numbers. The broken horizontal line in panel B indicates the chemocline.

In February 2006, total prokaryotic abundance varied between $1.0 \times 10^5$ and $1.7 \times 10^5$ cells ml$^{-1}$ (Fig. 2B). Comparable to the situation in the Farö Deep, cell numbers were highest around the chemocline. Bacterial cell numbers followed the prokaryotic pattern, with 66% EUB338-positive cells. GD17 cells were detected from the suboxic zone to the sulfidic zone; their numbers varied between $1.9 \times 10^4$ and $1.5 \times 10^5$ cells ml$^{-1}$. The highest cell numbers were located directly at the chemocline (Fig. 2B), where they accounted for 9.1% of all DAPI-stained cells. The vertical distribution and abundance of Epsilonproteobacteria and GD17 cells were again very similar, with cell numbers ranging from $2.0 \times 10^4$ to $1.9 \times 10^5$ cells ml$^{-1}$ (Fig. 2B).

In February 2006, total prokaryotic abundance in the Gotland Deep redoxcline varied between $5.0 \times 10^5$ and $1.6 \times 10^6$ cells ml$^{-1}$. In contrast to the other two profiles, maximal cell numbers were reached below the chemocline (Fig. 3B). Bacterial cell numbers followed the prokaryotic pattern, with a proportion of 56% EUB338-positive cells. GD17 cells occurred from the oxic zone to the sulfidic zone; in this interval, cell numbers ranged between $9.4 \times 10^3$ and $2.0 \times 10^5$ cells ml$^{-1}$. The highest cell numbers were detected within an extended suboxic-sulfidic transition zone at a depth of 124 to 128 m (Fig. 3A and B). The highest GD17 cell abundance which accounted for up to 15.6% of all DAPI-stained cells appeared in the prolonged overlap of oxygen and sulfide where nitrate could not be detected anymore (Fig. 3A). The distribution and abundance of Epsilonproteobacteria closely resembled those of GD17 (Fig. 3B). Cell abundances according to the different zones of the water column are summarized in Table S2 in the supplemental material. Based on Spearman rank correlation with a level of $P \leq 0.01$, there was no significant correlation between GD17 cell numbers and CO$_2$ fixation rates in 2005 and 2006.

All GD17 cell numbers determined in the three profiles ($n =$...
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DISCUSSION

Specificity and applicability of probe SUL90. High-resolution in situ quantification of an already identified epsilonproteobacterium from Baltic Sea redoxclines (5, 18, 24) was obtained by designing a specific 16S rRNA-targeting oligonucleotide probe based on a culture-independent PCR clone library approach. Probe SUL90 was selected, since it was fully complementary to the three target sequences. Furthermore, the target region in the 16S rRNA of the probe was shown to be accessible for probe binding in Escherichia coli (13). There have already been two attempts to estimate absolute GD17-related cell numbers within pelagic redoxclines of the central Baltic Sea using methods independent of gene probes (24, 26). These attempts consisted of highly specific qPCR and calculations based on stimulation experiments. With maximal cell numbers of $1 \times 10^5$ to $5 \times 10^6$ cells ml$^{-1}$, these indirect methods revealed cell numbers for the redoxcline that were comparable to those determined by the CARD-FISH approach (Fig. 1B to 3B), a finding that further confirms the applicability of gene probe SUL90.

Potential ecological role of epsilonproteobacterium GD17 in Baltic Sea redoxclines. The vertical distribution of Epsilonproteobacteria in central Baltic Sea redoxclines was similar to the profiles described by Lin et al. (29) for redoxclines of the Black Sea and the Cariaco Basin. Analogous to these habitats, Epsilonproteobacteria in the Baltic Sea redoxclines were nearly absent in the upper oxygenated layer, reached relatively high abundances of 11 to 16% of total cell numbers around the chemocline, and were still present in significant numbers in the sulfidic zone. Even higher epsilonproteobacterial abundance of up to 30% were recorded for the Cariaco Basin and the Black Sea redoxclines (29). Nevertheless, absolute epsilonproteobacterial cell numbers were comparable because of generally higher total cell counts in Baltic Sea redoxclines (Fig. 1B to 3B).

The presence of specific epsilonproteobacterial taxa related to the Sulfurimonas group was also demonstrated for the Cariaco Basin by 16S rRNA gene clone libraries (31) and for the Black Sea by terminal RFLP fingerprinting (42). The results of both studies suggested that the Sulfurimonas group is globally distributed in this type of habitat. For the central Baltic Sea redoxclines, we were able to demonstrate that nearly all of the Epsilonproteobacteria (on average 94%) detectable by CARD-FISH belong to a single phylogenetic subgroup (Fig. 5). Special physicochemical conditions affect the availability of electron donors and acceptors in these pelagic redoxclines, and their availability may not be stable over time. Bacteria that are active along a suboxic-sulfidic transition zone should possess adaptations to react to changes in energy sources, and it seems that the best-adapted Epsilonproteobacteria in Baltic Sea redoxclines were members of group GD17.

Epsilonproteobacteria have been suggested to be strongly involved in the cycling of carbon, nitrogen and sulfur com-
pounds in sulfidic habitats, especially at oxic-anoxic interfaces (8). Additionally, the ecological significance of chemolithotrophic Epsilonproteobacteria concerning chemooautotrophic production fueled by reduced sulfur species at oxic-anoxic interfaces has been demonstrated (31, 41). Likewise, previous studies (5) pointed to a key role for group GD17 in catalyzing autotrophic denitrification, which is the main process for nitrogen removal in oxic-anoxic interfaces of the central Baltic Sea (17).

The group GD17 distribution, as determined by CARD-FISH (Fig. 1B to 3B) but especially by the qPCR data for the 2005 sampling (Fig. 2B), supported a restricted high-activity zone of this organism around the chemocline. If all CO2 fixation activity attributed to this area of the Baltic Sea was due to GD17 cells, maximal CO2 fixation rates would account for 2 to 11 fg of C day^-1 cell^-1. Based on an individual cell biomass of 20 fg (14), this would result in cell doubling times of 2 to 12 days, which is much longer than previously determined by stimulation experiments (5, 26). Due to substantially higher CO2 fixation rates in 2006 (Fig. 3A), analogous calculations for the chemocline yield maximal CO2 fixation of 3 to 20 fg C day^-1 cell^-1, corresponding to cell doubling times of 1 to 7 days. These differences in the potential CO2 fixation activity of GD17 cells could be due to different nutrient availabilities in situ. Advection water transport into depths of 100 to 150 m in the central Gotland Basin is a frequent phenomenon and also occurs in addition to the major inflow events into the Baltic Sea (32). Lateral intrusions as well as internal waves result in small-scale turbulence, which can potentially produce local mixing events (28) and, in turn, temporal mixing of H2S- and NO3- containing water. The frequency and magnitude of such mixing events, however, are presently unknown.

Interestingly, the CO2 fixation maxima were usually located several meters below the chemocline (Fig. 2 and 3), which is similar to findings from the Black Sea (20) and the Cariaco Trench (41). Moreover, for the Baltic Sea, it has been demonstrated that 20 to 40% of the total cell numbers within this area can consist of chemooautotrophic cells (21). GD17 cell numbers were still relatively high within these maxima (Fig. 2, 3, and 6), but it is not very probable that group GD17 was responsible for this deep peak in CO2 dark fixation based on autotrophic denitrification because nitrate does not occur in this layer. This could indicate other physiological capacities of GD17 cells as, e.g., microbial sulfur oxidation via reduction of particulate metal oxides, such as manganese oxide or iron oxide. However, it is unknown whether GD17 cells could be capable of using other inorganic electron donors and acceptors or even organic substances for gaining energy in these layers. So far, 16S rRNA gene and 16S rRNA (24) distribution data still indicate a more restricted activity zone of group GD17 closely around the chemocline. Still, our results support the previous proposed broader physiological capacity of this organism (25, 26).

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REFERENCES


