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TAXONOMIC DESCRIPTION

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Polaribacter septentrionalilitoris sp. nov., isolated from the biofilm of a stone from the North Sea

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Abstract

A new member of the family *Flavobacteriaceae* was isolated from the biofilm of a stone at Nordstrand, a peninsula at the German North Sea shore. Phylogenetic analysis of the 16S rRNA gene sequence showed that strain ANORD1^T was most closely related to the validly described type strains *Polaribacter porphyrae* LNM-20^T (97.0%) and *Polaribacter reichenbachii* KMM 6386^T (96.9% 16S rRNA gene sequence similarity) and clustered with *Polaribacter gangjinensis* K17-16^T (96.0%). Strain ANORD1^T was determined to be mesophilic, Gram-negative, non-motile and strictly aerobic. Optimal growth was observed at 20–30 °C, within a salinity range of 2–7% sea salt and from pH 7–10. Like other type strains of the genus *Polaribacter*, ANORD1^T was tested negative for flexirubin-type pigments, while carotenoid-type pigments were detected. The DNA G+C content of strain ANORD1^T was 30.6 mol%. The sole respiratory quinone detected was menaquinone 6 (MK-6). The major fatty acids identified were $C_{15:0}$, iso-$C_{15:0}$, $C_{15:1}$ *ω6c* and iso-$C_{15:0}$ 3-OH. Based on the polyphasic approach, strain ANORD1^T represents a novel species in the genus *Polaribacter*, with the name *Polaribacter septentrionalilitoris* sp. nov. being proposed. The type strain is ANORD1^T (=DSM 110039^T=NCIMB 15081^T=MTCC 12685^T).

The genus *Polaribacter* (family *Flavobacteriaceae*, phylum *Bacteriodetes*) was originally described by John J. Gosink and members of the genus were initially proposed to be restricted to the polar regions, hence being assumed to be psychrophilic or psychrotrophic in nature [1]. The type species of this genus, *Polaribacter filamentus* 215^T and other pioneering members, *Polaribacter franzmanii* 301^T, *Polaribacter glomeratus* ATCC 43844^T and *Polaribacter irgensii* 23-P^T, were all isolated from either the Arctic region or Antarctica [1]. However, subsequent discoveries have shown members of this genus originating from diverse locations and exhibiting mesophilic character. For instance, *Polaribacter litorisediminis* OITF-11^T [2], *Polaribacter gangjinensis* K17-16^T [3], *Polaribacter porphyrae* LNM-20^T [4] and *Polaribacter reichenbachii* KMM 6386^T [5] were observed to be mesophilic in nature as opposed to their psychrophilic and psychrotrophic relatives. At the time of writing, the genus comprises 24 described species, all of which have been isolated from marine environments.

ISOLATION AND ECOLOGY

During a study by Saha *et al.* on the defense adaptation of invasive seaweeds to control bacterial epibionts, non-living substrata co-occurring with the red alga *Gracilaria vermiculophylla* were used as sources of bacterial strains [6]. Among these strains was ANORD1^T, which was isolated on 22 August 2013 from the biofilm of a stone collected at Nordstrand (North Sea, Germany, 54° 29.166′ N, 8° 48.746′ E), cultured on solid marine broth (MB) medium (Difco 2216, supplemented with Difco agar 15 g l^{-1}) and stored as described by Saha *et al.* [6]. Preliminary DNA extraction, 16S rRNA gene sequence amplification and sequencing revealed that the strain ANORD1^T was affiliated to the genus *Polaribacter*.

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Keywords: *Polaribacter septentrionalilitoris*; North Sea; polyphasic taxonomy.
Abbreviations: ANI, average nucleotide identity; EPS, extracellular polymeric substance; MB, marine broth 2216; ME, minimum-evolution; NJ, neighbour-joining.
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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the type strain ANORD1^T is MK260196. The DDBJ/ENA/GenBank accession numbers for whole genome sequences of strain ANORD1^T is VRMJ00000000.
Two supplementary figures are available with the online version of this article.

Hence, this current study aimed to develop a taxonomic characterization of ANORD1^T using a polyphasic approach.

To establish a comparison of the results for strain ANORD1^T, experiments were carried out as well with *P. gangjinensis* K17-16^T, *Polaribacter porphyrae* LNM-20^T and *Polaribacter reichenbachii* KMM 6386^T under the same conditions. These reference strains were obtained from the depositories of the Japan Collection of Microorganisms (JCM; Ibaraki, Japan; strain K17-16^T) and the Belgian Co-ordinated Collections of Microorganisms (BCCM/LMG; Gent, Belgium; strains LNM-20^T and KMM 6386^T).

16S rRNA GENE PHYLOGENY

In preparation for phylogenetic analysis, genomic DNA extraction was carried out on ANORD1^T using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. The 16S rRNA gene sequence was amplified using the primers Eub27F (5′-GAG TTT GAT CCT GGC TCA G-3′) [7] and Univ1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) [7] sequenced via Sanger sequencing [8] at the Institut für Klinische Molekularbiologie (IKMB, Kiel, Germany) with the primers 534R [9], 342F [10], 803F [10] and Univ1492R [7]. The sequenced contigs were assembled and the quality of the sequence was assessed using ChromasPro 2.1.8 (Technelysium). The partial 16S rRNA gene sequence comprised 1486 bp (accession number MK260196.2) and was 100% identical to the genome-based sequence with 1512 bp (MK260196.3). The genome-based sequence was loaded onto the NCBI Nucleotide BLAST algorithm to identify sequences of related type strains [11]. Sequences of strain ANORD1^T, all *Polaribacter* species type strains, *Polaribacter* sp. ZY113 and the outgroup sequences were aligned using CLUSTAL_W 2.1 [12]. The 16S rRNA gene sequences used in the tree reconstruction were obtained from the genome sequences, as far as available. Phylogenetic trees were constructed using the neighbour-joining (NJ) method [13] as well as the minimum-evolution (ME) method [14] to ensure the consistency of the tree topology. Both phylogenetic trees were reconstructed using MEGA 7.0.26 [15] by running the tree computation with a bootstrap replication of 1000 [16]. The resulting trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura–Nei method [17] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd +3rd +Noncoding. Phylogenetic analysis of the 16S rRNA gene sequence showed that the strain ANORD1^T belonged to the genus *Polaribacter* (Fig. 1). Applying the NJ method, strain ANORD1^T clustered with *P. gangjinensis* K17-16^T. This cluster branched with *Polaribacter* sp. ZY113. Calculation using the ME method revealed also a cluster composed by the three strains, but ANORD1^T clustered with *Polaribacter* sp. ZY113 and this cluster branched from *P. gangjinensis* K17-16^T (data not shown). The similarity of the 16S rRNA gene sequence of ANORD1^T to all known 24 type strains of the genus *Polaribacter* ranged from 93.8 to 97.0%. The closest related type strains of ANORD1^T were established to be *P. reichenbachii* KMM 6386^T (96.9 %) and *P. porphyrae* LNM-20^T (97.0%). There are four marine isolates exhibiting a sequence similarity ≥97.5% to strain ANORD1^T. *Polaribacter* sp. K20-5 from a seawater sample of the Republic of Korea (accession number HM010403.1) and *Polaribacter* sp. J3 from unknown origin (accession number JX854533) showed a similarity of 99.4 and 98.3%, respectively. *Polaribacter* sp. ZY113 (accession number MH000434.2), an isolate from a sea sediment sample, showed a similarity of 97.6%. *Polaribacter* sp. AG12 was derived from the surface of a stone collected at the shore of Gyeokpo adjacent to the Yellow Sea in the Republic of Korea (accession number KT121443.1) and represented 97.5% similarity.

GENOME FEATURES

The genomic relatedness between strain ANORD1^T and selected members of the genus *Polaribacter* was determined by whole genome sequence analysis of strain ANORD1^T, the closest related type strains *P. porphyrae* NBRC 108759^T (=LNM-20^T) and *P. reichenbachii* 6Alg 8^T (=KMM 6386^T) as well as *P. gangjinensis* KCTC 22729^T (=K17-16^T). The genome sequence of strain ANORD1^T was determined according to Imhoff *et al.* [18]. The general genomic features were determined using SPAdes 3.11 [19], Quast 4.1 [20], Prokka 1.3 [21] and CheckM [22] and are displayed in Table 1. The average nucleotide identity (ANI) values were calculated as described by Yoon *et al.* [23]. ANI values between strain ANORD1^T and the three reference strains were 78.6% (*P. porphyrae*), 79.4% (*P. reichenbachii*), and 76.2% (*P. gangjinensis*), respectively. Because of the relationship of strain ANORD1^T with *Polaribacter* sp. ZY113 (accession number GCA_003129485.1) in the whole genome based phylogenetic analysis (Fig. 2) the ANI value was also calculated and was accounted for 81.9%. The mean identities were all less than the suggested boundary (95–96%) for species delineation [24, 25], demonstrating that strain ANORD1^T represents a novel genomic species. DNA G+C content was 30.6 mol% (Table 1).

The whole genome-based phylogeny calculated with GTDBtk [26] showed strain ANORD1^T forming a clade with *Polaribacter* sp. ZY113, which was obtained from the red alga *Corallina officinalis* (Fig. 2). This clade branched from *P. porphyrae* NBRC 108759^T (=LNM-20^T), an isolate from the red alga *Porphyra yezoensis*. The group of all three strains was separated from the clade consisting of *P. reichenbachii* 6Alg 8^T (=KMM 6386^T), which was derived from the green alga *Ulva fenestrata* and two seawater isolates, i.e. *P. dokdenensis* DSW-5^T and *Polaribacter* sp. MED152. The differences in the 16S rRNA gene- and genome-based phylogenetic trees might be a result of the various target proteins used for the calculations, i.e. one 16S rRNA gene versus 120 single copy marker genes. Further, the different numbers of available sequences for the type strains, i.e. 24 versus 13, and the number of total sequences, i.e. 30 versus 23459, respectively may have led to divergent phylogenies. It is expected, that the comparison of phylogenetic trees will be more meaningful, when genomic

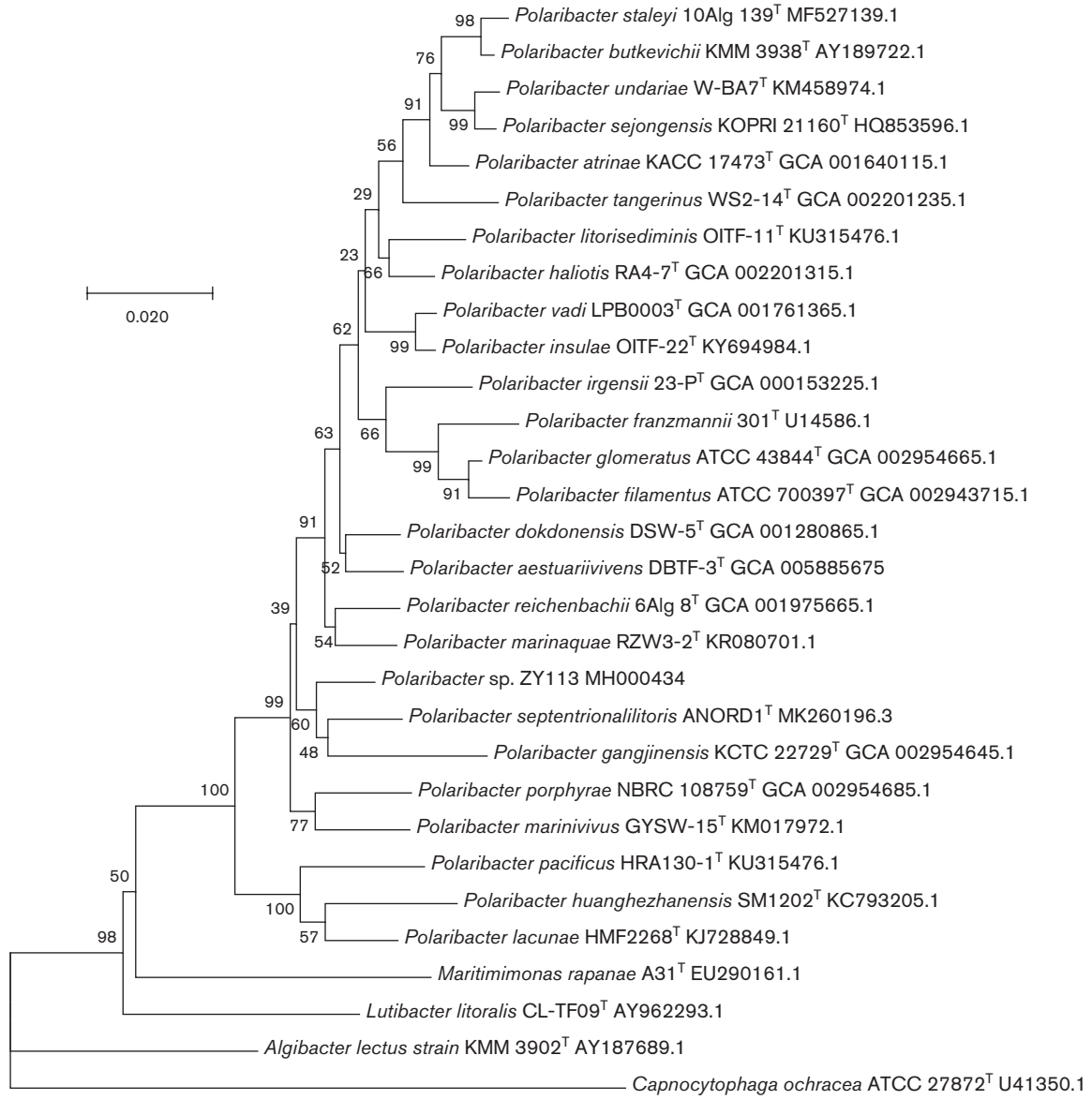

Fig. 1. Evolutionary relationships of taxa based on 16S rRNA gene sequences, inferred using the neighbour-joining method. As far as available, whole 16S rRNA sequences were derived from genome sequences applying ContEst16S [39]. Partial sequences of *P. butkevichii* and *P. sejongensis* were used, because the genome-based sequence comprised 1317 (versus 1433) nucleotides and there was no 16S rRNA sequence in the genome, respectively. Bootstrap samplings (≥50 %) based on 1000 replications are shown next to the branches. All positions with less than 95% site coverage were eliminated. There were a total of 1409 positions in the final dataset. The outgroups used were *Algibacter lectus* KMM 3902^T, *Capnocytophaga ochracea* ATCC 27872^T, *Lutibacter litoralis* CL-TF09^T and *Maritimimonas rapanae* A31^T. Bar, 0.02 substitutions per nucleotide position.

data will be available for all *Polaribacter* species type strains in the near future.

Genes coding for the utilization of D-xylose and L-rhamnose were found, among others, in the genome of strain ANORD1^T. These sugars are components of extracellular polymeric substances (EPSs), which are produced by a wide array of micro-organisms in marine habitats [27]. The secretion of exopolysaccharides supports the attachment of the producers to surfaces and contributes to the formation of biofilms [28]. Thus, it seemed likely, that the biofilm-derived strain ANORD1^T utilizes components of EPSs. Concerning the utilization of xylose, ANORD1^T featured a D-xylose proton-symporter XylE and the following enzymes: exo-1,4-β-xylosidase (EC 3.2.1.37) hydrolyzing (1->4)-β-D-xylans and cleaving off D-xylose monomers, endo-1,4-β-xylosidase (EC 3.2.1.8) and D-xylulose kinase (EC 2.7.1.17) catalyzing the formation of the intermediate in the pentose phosphate pathway D-xylulose-5-P from D-xylulose. The enzyme

Table 1. Comparison of the general genomic features of strain ANORD1^T and selected species of the genus *Polaribacter*

Strains: 1, ANORD1^T (GenBank accession no. VRMJ00000000); 2, *Polaribacter porphyrae* NBRC 108759^T=LNM-20^T (GCA_002954685); 3, *Polaribacter reichenbachii* 6Alg 8^T=KMM 6386^T (GCA_001975665); 4, *Polaribacter gangjinensis* KCTC 22729^T=K17-16^T (GCA_002954645).

Genome feature	1	2	3	4
Size (Mb)	3.45	3.89	4.13	2.94
G+C content (mol%)	30.6	29.05	29.51	31.48
N50 (bp)	401 k	346 k	4125 k	181 k
Completeness (%)	99.33	99.66	98.99	99.33
Contamination (%)	0.48	0.62	0.00	0.78
Number of:				
Contigs (>500 bp)	50	22	1	33
CDS	2967	3506	3491	2552
rRNA	4	3	9	3
tRNA	36	34	41	37

α-L-rhamnosidase (EC 3.2.1.40) cleaves off α-L-moieties from respective glycosides. Uptake of L-rhamnose into the cell is mediated by an L-rhamnose-proton symporter. The metabolism from L-rhamnose via L-rhamnulose and L-rhamnulose 1-phosphate to dihydroxyacetone phosphate is performed by L-rhamnose isomerase (EC 5.3.1.14), rhamnulokinase (EC 2.7.1.5), and rhamnulose-1-phosphate aldolase (EC 4.1.2.19), respectively.

PHYSIOLOGY AND CHEMOTAXONOMY

The morphological characteristics of strain ANORD1^T, *P. porphyrae* LNM-20^T, *P. reichenbachii* KMM 6386^T as well as *P. gangjinensis* K17-16^T were assessed using 5 day old cultures incubated on MB medium at 25 °C. Colony morphology and color was evaluated via observation with a loop, while cell morphology and motility were examined via light microscopy (Carl Zeiss Axiophot epifluorescence microscope). The results are depicted in Table 2. Gram-staining was performed using the bioMérieux Color Gram 2 Test Kit (bioMérieux) according to the manufacturer's instructions and showed strain ANORD1^T to be Gram-negative.

The physiological and biochemical characteristics were also studied. Salinity-dependent growth was determined with 0–7% (w/v) NaCl with intervals of 1, 0–7% (w/v) NaCl supplemented with 0.5% $MgCl_2{\cdot}6H_2O$ with intervals of 1, and 0–7% (w/v) Tropic Marin sea salt classic (Wartenberg) with intervals of 1% on marine agar (MA) medium (5.0 g BD bacto peptone, 1.0 g BD bacto yeast extract, 15.0 g BD bacto agar in 1 l deionized water) and incubation at 25 °C for 7 days. Additionally, growth with 8, 9, 10, 11 and 12% (w/v) Tropic Marin Sea Salt Classic was tested on strains ANORD1^T and *P. reichenbachii* KMM 6386^T. Temperature-dependent growth was assessed at 0–55 °C (intervals of 5 °C) on MB for 7 days. pH-dependent growth of the strains was assessed at 4.0–12.0 (intervals of one pH unit) on MB at 25 °C for 7 days, with the addition of 1 M NaOH and 1 M HCl solutions to adjust the pH level. The carbohydrate fermentation abilities were examined with the API 50 CH test kit from bioMérieux, using a salinity-adjusted inoculation medium of 2.5% (w/v) Tropic Marin sea salt classic. The API 50 CH test strips were incubated at 25 °C for 8 days. Further metabolic activities of the strains were assessed using the API 20 E and API 20 NE test kits (bioMérieux) according to the manufacturer's instructions, with inoculation solution adjusted to a salinity of 2.5% (w/v) Tropic Marin sea salt classic. Incubation of these test strips was conducted at 25 °C for 2 weeks. Specific enzymatic activities were studied using the semi-quantitative API ZYM test kit (bioMérieux) according to the manufacturer's instructions using 0.9% NaCl solution as an inoculum. The test strips were incubated for a period of 18 h at 25 °C in the dark. Catalase activity was determined by addition of 3% (v/v) hydrogen peroxide to colonies of the strains and the observation of gas bubbles formed. Oxidase activity was tested by smearing colonies onto a non-impregnated filter paper disc soaked with bioMérieux oxidase reagent (*N,N,N,N*-tetramethyl-1,4-phenylenediamine) and observing the development of a violet to purple coloration within 10–30 s according to the manufacturer's instructions. Oxygen requirements were assessed with the aerobic/anaerobic test tube method [29] using soft agar MB medium (7.48 g Difco marine broth 2216, 1.2 g BD bacto agar in 200 ml deionized water) and incubation at 25 °C for 1 week. The presence of pigments was also investigated. The KOH test was performed with 6 day old cultures of the strain ANORD1^T to detect the presence of flexirubin-type pigments according to the method of Bernardet *et al.* [30]. The presence of carotenoid-type pigments in ANORD1^T and the reference strains was assessed via the method described by Biebl and Drews [31] using bacterial colonies grown on solid MB media for 12 days at 25 °C. The detection of wavelengths corresponding to the absorbance values of carotenoids was carried out via HPLC with a VWR Hitachi LaChrom Elite

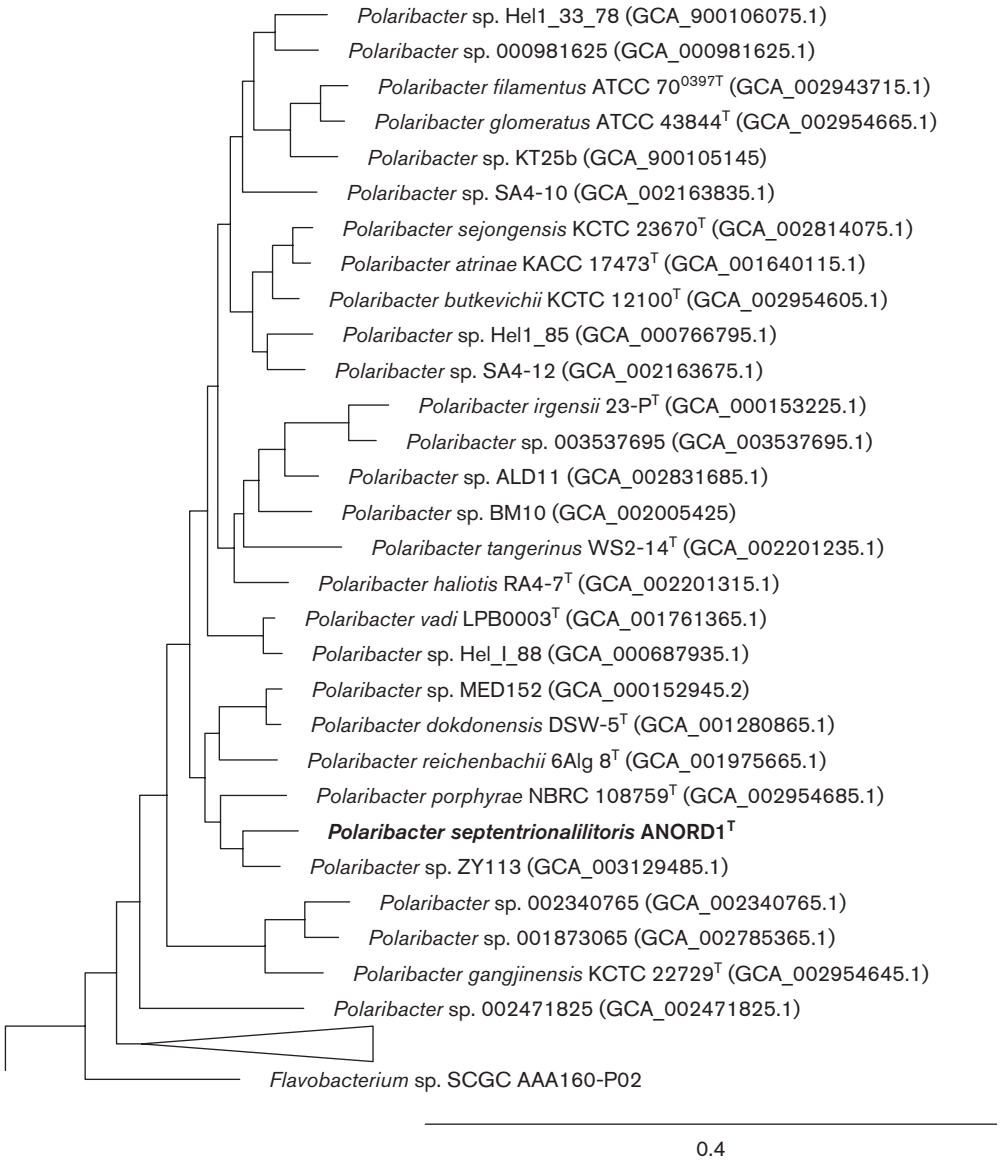

Fig. 2. Genome phylogeny was inferred using the GTDBtk pipeline [26]. The pipeline was used with its version 0.3.2. and is based on 23459 reference genomes. For bacterial genomes, the taxonomic identification is based on 120 single copy marker proteins. The pipeline employs FastTree [40, 41] to calculate the phylogenetic trees using the maximum-likelihood method. Bar, 0.4 substitutions per nucleotide position.

system (VWR International). For both respiratory quinone and polar lipid analyses, the pre-analysis preparation was carried out by freeze-drying material from a 5 day old culture of ANORD1^{T} incubated at 25 °C. Both analyses were carried out by the DSMZ according to Tindall [32]. For the cellular fatty acid analysis of strain ANORD5^{T} and the reference strains 5 day old cultures grown on MB medium at 25 °C were used. Cellular fatty acid analysis of the strains was carried out by the DSMZ, where fatty acid methyl esters were first obtained using the methods of Kuykendall *et al.* [33]. The fatty acid methyl mixtures were then separated using the Sherlock MIS (MIDI) system which consisted of an Agilent model 6890 N gas chromatograph fitted with a 5% phenyl-methyl silicone capillary column (0.2 mm×25 m), a flame ionization detector, Agilent model 7683A automatic sampler and an HP computer with the MIDI data base (Hewlett-Packard) using the TSBA40 method. Identification of cellular fatty acids and calculation of their percentage content was then carried out using the MIS Standard Software (Microbial ID). The susceptibility to a range of antibiotics was tested using the disc diffusion test carried out in reference to the methods by Briggs *et al.* [34]. The test was performed with Oxoid antimicrobial susceptibility test discs on MB medium, assessing the susceptibility of each strain to 29 different antibiotic compounds. Any presence of a zone of inhibition around the disc inferred the susceptibility of the particular strain to the antibiotic, while

Table 2. Differential characteristics between strain ANORD1^T, *Polaribacter porphyrae*, *Polaribacter reichenbachii* and *Polaribacter gangjinensis*

Strains: 1, ANORD1^T; 2, *Polaribacter porphyrae* LNM-20^T; 3, *Polaribacter reichenbachii* KMM 6386^T; 4, *Polaribacter gangjinensis* K17-16^T. All data were taken from this study, unless otherwise stated. All strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, cytochrome c oxidase, catalase, Voges–Proskauer test, gelatinase, esculin hydrolysis, D-glucose fermentation, potassium 5-ketogluconate fermentation and resistance to amikacin, gentamicin, kanamycin, neomycin, oxacillin, polymyxin B and streptomycin. BY, Bright yellow; PY, pale yellow; O, orange; PP, pinpoint; +, positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Colony size (mm)	1–2	1–6	1–3	PP–1
Colony colour	BY	BY	PY	O
Colony opacity	Translucent	Translucent	Opaque	Translucent
Growth on:				
1% Tropic Marin Sea Salt Classic	–	–	+	+
8% Tropic Marin Sea Salt Classic	+	–	+	–
10% Tropic Marin Sea Salt Classic	w	–	–	–
1% NaCl	w	–	–	+
3% NaCl	w	–	w	+
1% NaCl+0.5% $MgCl_2 \cdot 6H_2O$	w	w	+	+
5% NaCl+0.5% $MgCl_2 \cdot 6H_2O$	+	–	+	w
pH range for growth	4–10	6–10	6–10	5–10
Growth at 5 °C	+	–	+	w
Growth at 10 °C	+	w	+	w
Growth at 40 °C	–	+	w	+
α-Glucosidase	+	+	–	w
β-Glucosidase	+	+	w	–
N-Acetyl-β-glucosaminidase	–	+	+	+
Hydrolysis of:				
2-Nitrophenyl-β-D-galactopyranoside	–	–	–	+
4-Nitrophenyl-β-D-galactopyranoside	–	+	+	+
Oxidation and fermentation of:				
D-Glucose	–	–	+	+
D-Mannitol and L-rhamnose	–	–	+	–
Fermentation of:				
Maltose	–	+	–	w
Malic acid and phenylacetic acid	–	+	–	–
Starch, D-mannose and glycogen	+	–	+	+
Amygdalin, cellobiose, D-xylose and L-rhamnose	+	–	–	–
D-Galactose	w	–	–	+
Lactose	–	–	w	+
Trehalose and turanose	–	–	+	–
Potassium 2-ketogluconate	+	–	–	w
Resistance to bacitracin	+	+	–	+

Continued

Table 2. Continued

Characteristic	1	2	3	4
Resistance to nalidixic acid	–	–	w	+
Resistance to norfloxacin	+	–	–	+
Absorption maxima of pigments (nm)/ retention time (min):				
First detected pigment	425, 452, 479/36.3	421, 449, 473/41.6	336, 421, 449, 473/41.7	446, 472, 503/35.0
Second detected pigment	424, 446, 474/39.0	339, 420, 445, 472/42.9	341, 422, 446, 473/43.0	446, 472, 503/38.0

the diameter of the zone indicated its degree of susceptibility. The phenotypic results of ANORD1^T and the reference strains are displayed in Table 2, Fig. S1 (available in the online version of this article) and in the species description.

Like the reference strains, ANORD1^T exhibited the best growth with Tropic Marin Sea Salt MB medium with the densest growth observed in the 3–7% (w/v) Tropic Marin salinity range. In general, sparser growth was observed with ANORD1^T on this media when compared with the reference strains. Remarkably, ANORD1^T displayed minor growth even at 10% (w/v) Tropic Marin suggesting a possible salinity tolerance characteristic of this proposed novel species. In comparison, strain *P. reichenbachii* KMM 6386^T grew up to 8% (w/v) Tropic Marin while strains *P. porphyrae* LNM-20^T and *P. gangjinensis* K17-16^T grew up to 7% (w/v) Tropic Marin. No growth was observed at 1% Tropic Marin for both ANORD1^T and *P. porphyrae* LNM-20^T. On NaCl supplemented with 0.5% $MgCl_2{\cdot}6H_2O$ MB medium, no growth was observed with ANORD1^T at 0% and 1% NaCl, while sparse to dense growth was observed with *P. porphyrae* LNM-20^T, *P. reichenbachii* KMM 6386^T and *P. gangjinensis* K17-16^T. Strain ANORD1^T grew the poorest on NaCl containing MB media, with only sparse growth observed at 2–3% (w/v) NaCl. In contrast, *P. reichenbachii* KMM 6386^T displayed moderate to dense growth from 0–3% (w/v) NaCl. Decent growth was observed across ANORD1^T and the reference strains in the range of 15–30 °C. All strains exhibited high density growth at 25 °C and 30 °C. Strain ANORD1^T showed sparse growth at a lower limit of 0 °C and an upper limit of 45 °C. In comparison, growth was observed at an upper limit of 50 °C with the three reference strains. A high similarity in pH growth ranges was observed among ANORD1^T and the reference strains, where densest growth was observed from pH 7–10 across all strains. None of the strains displayed growth at pH 11. While the lower pH tolerance limit was pH 6 for both *P. porphyrae* LNM-20^T and *P. reichenbachii* KMM 6386^T and pH 5 for *P. gangjinensis* K17-16^T, one defining characteristic of strain ANORD1^T was its ability for growth at the lower limit of pH 4, highlighting the possibility of acid-adaptation strategies employed by this strain in coping with high proton concentrations [35]. Both catalase and oxidase activities were detected in ANORD1^T and the reference strains. These activities are also characteristics of most members of the genus *Polaribacter*. However, following the visual method of Iwase *et al.* [36], catalase activity (production of bubbles) was observed to be lower for ANORD1^T as compared to the reference strains. With the API ZYM profile, strikingly similar enzymatic reaction intensities were observed with 8 out of 11 positive assay tests, namely alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, α-chymotrypsin, and acid phosphatase. Additionally, across the current 24 described type strains (including ANORD1^T) in the genus *Polaribacter*, enzymatic activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, trypsin and naphthol-AS-BI-phosphohydrolase were detected for almost every strain. Displays sensitivity to the following antibiotics: ampicillin (10 µg), bacitracin (10 units), cefoxitin (30 µg), chloramphenicol (50 µg), ciprofloxacin (5 µg), erythromycin (15 µg), doripenem (10 µg), doxycycline (30 µg), imipenem (10 µg), lincomycin (15 µg), linezolid (30 µg), norfloxacin (10 µg), novobiocin (30 µg), ofloxacin (5 µg), oleandomycin (15 µg), penicillin G (10 units), rifampicin (30 µg), teicoplanin (30 µg), tetracycline (30 µg) and vancomycin (30 µg). Exhibits resistance to amikacin (30 µg), gentamicin (30 µg), kanamycin (30 µg), mupirocin (200 µg), nalidixic acid (30 µg), neomycin (30 µg), oxacillin (5 µg), polymyxin B (300 units), and streptomycin (25 µg). Results from the KOH test showed no colour changes in ANORD1^T and the reference strains, indicating the absence of flexirubin-type pigments. However, cells of strain ANORD1^T produced yellow pigments. Results from the HPLC analysis of ANORD1^T detected two pigments: the first with a retention time of 36.3 min and a maximum absorption wavelength peak of 452 nm and two more peaks at 425 nm and 479 nm (Table 2), and the second with a retention time of 39.0 min and a maximum absorption wavelength peak of 446 nm and two more peaks at 424 nm and 474 nm (Table 2). These two absorption spectra matched with that of the peak values of the carotenoids trans-β-carotene and trans-antheraxanthin (Fig. S2), respectively [37]. Wavelengths with absorption spectra similar to that of carotenoid-type pigments were also detected in the reference strains (Table 2.). The sole respiratory quinone detected in ANORD1^T and the reference strains was menaquinone MK-6, which was also the case in all other described members of the family

Flavobacteriaceae that are known so far. The DNA G+C content of the genome of ANORD1^T was determined to be 30.6 mol%, placing it at the lower end of the range in the genus *Polaribacter*, where values ranged from 29.1 mol% in the type strain *P. porphyrae* LNM-20^T to 36.4 mol% in *P. huanghezhanensis* SM 1202^T [38]. In comparison, the genomic G+C contents of the other two reference strains *P. gangjinensis* K17-16^T and *P. reichenbachii* KMM 6386^T were 31.5 mol% and 29.5 mol% respectively. The fatty acid composition of the four *Polaribacter* strains is shown in Table 3. Across the four strains, five common major fatty acids were detected, i.e. $C_{15:0}$, iso-$C_{13:0}$, iso-$C_{15:0}$, iso-$C_{15:1}$ G, $C_{15:1}$ ω6*c* and iso-$C_{15:0}$ 3-OH.

CONCLUSION

Phylogenetic analysis based on 16S rRNA gene sequences and on whole genomes demonstrated that strain ANORD1^T is a member of the genus *Polaribacter*. The DNA G+C content of ANORD1^T was also well within the range of the genus *Polaribacter*. High degrees of similarity in mechanisms of antibiotic sensitivity (especially resistance to aminoglycoside-class compounds), enzymatic activities, production of carotenoid-type pigments and other phenotypic traits were documented for ANORD1^T and the reference strains.

In contrast, strain ANORD1^T was distinct from other members of the genus *Polaribacter* based on its genomic features, 16S rRNA gene sequence similarity, unique DNA G+C content, tolerance of high salinity (10% w/v sea salt), tolerance of low pH (pH 4), unique absorption spectra of its pigments (in comparison with the reference strains) and other phenotypic traits. Therefore, strain ANORD1^T has been proposed to represent a novel species in the genus *Polaribacter*, for which the name *Polaribacter septentrionalilitoris* sp. nov. is proposed.

DESCRIPTION OF *POLARIBACTER SEPTENTRIONALILITORIS* SP. NOV.

Polaribacter septentrionalilitoris (sep.ten.tri.o.na.li.li´to.ris. L. masc. adj. *septentrionalis* northern; L. neut. n. *litus* beach; N.L. gen. n. *septentrionalis* of Nordstrand - North Beach, where the type strain was isolated).

Cells are Gram-negative, strictly aerobic, non-motile by gliding, cocci or rod-shaped, 0.4–1.0 µm wide and 0.4–2.1 µm long. Colonies are circular, raised, entire, bright yellow, translucent, butyrous, smooth and glistening, 1–2 mm in diameter and with an odour akin to soaked mushrooms. Growth occurs on 2–10% (w/v) sea salt (optimum 5–6%), 2–7% (w/v) NaCl supplemented with 0.5% $MgCl_2 \cdot 6H_2O$, 2–3% (w/v) NaCl, at 0–45 °C (optimum 25–30 °C) and at pH 4.0–10.0 (optimum pH 9.0). Strain is oxidase- and catalase-positive. Fermentation is observed with D-xylose, D-mannose, L-rhamnose, amygdalin, aesculin, cellobiose, maltose, sucrose, starch, glycogen, potassium 2-ketogluconate and potassium 5-ketogluconate, while weak fermentation was observed with erythritol, D-arabinose,

Table 3. Cellular fatty acid composition (%) of ANORD1^T and its closest phylogenetic relatives in the genus *Polaribacter*

Strains: 1, ANORD1^T; 2, *Polaribacter porphyrae* LNM-20^T; 3, *Polaribacter reichenbachii* KMM 6386^T; 4, *Polaribacter gangjinensis* K17-16^T. All data were derived from this study. Fatty acids amounting to <0.5% of the total in each strain are not shown; TR, trace (less than 1.0 %); –, not detected.

Fatty acid	1	2	3	4
Saturated:				
$C_{9:0}$	–	–	–	TR
$C_{11:0}$	–	–	–	TR
$C_{13:0}$	TR	TR	TR	TR
$C_{14:0}$	–	TR	TR	TR
$C_{15:0}$	9.5	7.8	13.1	19.8
$C_{16:0}$	TR	TR	TR	TR
$C_{18:0}$	TR	TR	TR	–
Branched:				
iso-$C_{12:0}$	–	–	–	–
iso-$C_{13:0}$	6.9	8.4	TR	TR
iso-$C_{14:0}$	TR	TR	TR	TR
iso-$C_{15:0}$	20.4	24.2	13.6	12.3
anteiso-$C_{15:0}$	–	–	1.6	0.9
iso-$C_{16:0}$	–	TR	–	TR
anteiso-$C_{15:1}$ A	–	–	–	TR
iso-$C_{15:1}$ G	8.3	10.0	10.8	5.2
iso-$C_{16:1}$ h	–	–	–	TR
iso-$C_{17:1}$ ω9*c*	–	–	–	TR
Unsaturated:				
$C_{15:1}$ ω6*c*	9.0	7.8	11.7	15.7
$C_{17:1}$ ω6*c*	1.2	tr	2.5	4.4
Hydroxy:				
$C_{13:0}$ 2-OH	–	–	–	TR
$C_{15:0}$ 2-OH	1.1	TR	1.0	4.5
$C_{15:0}$ 3-OH	3.4	3.9	5.6	6.0
$C_{16:0}$ 3-OH	1.1	1.5	2.2	1.6
$C_{17:0}$ 3-OH	TR	–	–	1.1
iso-$C_{15:0}$ 3-OH	20.9	14.2	16.3	9.2
iso-$C_{16:0}$ 3-OH	1.7	2.2	2.4	2.3
iso-$C_{17:0}$ 3-OH	5.8	7.3	3.7	3.8
Unknown:				
Unknown 11.543	TR	TR	TR	TR
Unknown 16.582	TR	TR	TR	TR

Continued

Table 3. Continued

Fatty acid	1	2	3	4
Summed feature 3*	7.6	8.5	8.6	7.6

*Summed features represent two or three fatty acids that could not separate by the Microbial Identification System. Summed feature 3 consisted of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

L-arabinose, D-ribose, D-galactose, D-glucose, D-fructose, inositol, D-mannitol, D-sorbitol, D-lyxose and D-fucose. Fermentation was negative for glycerol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, arbutin, salicin, lactose, melibiose, trehalose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-tagatose, L-fucose, D-arabitol, L-arabitol and potassium gluconate. Positive for ornithine decarboxylase, citrate utilization, acetoin production and gelatinase, but negative for ortho nitrophenyl-β-D-galactopyranosidase, lysine decarboxylase, H_2S production, urease, tryptophane deaminase, indole production, fermentation/oxidation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin, L-arabinose and nitrate reduction. Positive for aesculin and gelatin hydrolysis, but negative for arginine dihydrolase, para-nitrophenyl-β-D-galactopyranosidase, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase, but negative for α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Flexirubin-type pigments are absent, but two carotenoid-type pigments are present: first pigment with absorption peak maxima at 425 nm, 452 nm and 479 nm, and second pigment with absorption peak maxima at 424 nm, 446 nm and 474 nm. The major fatty acids (>5% of total composition) in ANORD1^T are $C_{15:0}$, iso-$C_{13:0}$, iso-$C_{15:0}$, iso-$C_{15:1}$ G, $C_{15:1}$ ω6c, iso-$C_{15:0}$ 3-OH and iso-$C_{17:0}$ 3-OH. The sole respiratory quinone detected is MK-6. Seven unidentified lipids and four unidentified aminolipids are detected. The DNA G+C content of ANORD1^T is 30.6 mol%.

The type strain, ANORD1^T (=DSM 110039^T=NCIMB 15081^T=MTCC 12685^T), was isolated from the biofilm of a stone collected at Nordstrand, along the German coast of the North Sea (54° 29.166′ N, 8° 48.746′ E).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the type strain ANORD1^T is MK260196. The DDBJ/ENA/GenBank accession numbers for whole genome sequences of strain ANORD1^T is VRMJ00000000.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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