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Description of *Hymenobacter sediminicola* sp. nov., isolated from contaminated sediment

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Abstract A polyphasic taxonomic study was conducted on two Gram-negative, non-sporulating, non-motile bacterial strains, S2-20-2^T and S2-21-1, isolated from a contaminated freshwater sediment in China. Comparative 16S rRNA gene sequence studies revealed a clear affiliation of two strains with *Bacteroidetes*, which showed the highest pairwise sequence similarities with *Hymenobacter duratus* BT646^T (99.3%), *Hymenobacter psychrotolerans* Tibet-IIU11^T (99.3%), *Hymenobacter kanuolensis* T-3^T (97.6%), *Hymenobacter swuensis* DY53^T (96.9%), *Hymenobacter tenuis* POB6^T (96.8%), *Hymenobacter seoulensis* 16F7G^T (96.7%), and *Hymenobacter rigui* KCTC 12533^T (96.5%). The phylogenetic analysis based on 16S rRNA gene sequences showed that two strains formed a clear phylogenetic lineage with the genus *Hymenobacter*. Major fatty acids were identified as *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$, and summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c/t*) and summed feature 4 (*iso*-$C_{17:1}$ I and/or *anteiso*-$C_{17:1}$ B). Major cellular polar lipids were identified as phosphatidylethanolamine, three unidentified aminolipids, an unidentified aminophosopholipid and an unidentified lipid. The respiratory quinone was detected as MK-7 and the genomic DNA G+C content was determined to be 57.9% (genome) for type strain S2-20-2^T and 57.7 mol% (HPLC) for strain S2-21-1. The observed ANI and dDDH values between strain S2-20-2^T and its closely related strains were 75.7–91.4% and 21.2–43.9%, respectively. Based on physiological, biochemical, genetic and genomic characteristics, we propose that strains S2-20-2^T and S2-21-1 represent a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter sediminicola* sp. nov. is proposed. The type strain is S2-20-2^T (=CGMCC 1.18734^T=JCM 35801^T).

Keywords *Hymenobacter* · *Hymenobacter sediminicola* · S2-20-2 · S2-21-1

Tingting Ren and Chengxiao Zhang have contributed equally to this work.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains S2-20-2^T and S2-21-1 are MW073560 and MW073561, respectively. The GenBank accession number for the whole genome sequence of type strain S2-20-2^T is CP060202.

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Introduction

It's been over 20 years since the first species *Hymenobacter roseosalivarius* was proposed by Hirsch et al. (1998). At the time of writing, the genus *Hymenobacter* represents a member of the family *Hymenobacteraceae*, within the phylum *Bacteroidetes*, including 101 species with validly published names (https://lpsn.dsmz.de/genus/hymenobacter) (Parte 2020). *Hymenobacter* is of interest because of its wide range of natural habitats and geographical distribution. *Hymenobacter* spp. were isolated from soil, sediment, water, ice, snow, ore, rock, sand and air including extreme environments (Buczolits et al. 2006; Zhang et al. 2007; Dai et al. 2009; Klassen and Foght 2011; Jin et al. 2014; Subhash et al. 2014; Kojima et al. 2016; Sedláček et al. 2017; Sheu et al. 2017; Feng et al. 2019). Some of *Hymenobacters* are also unique in being radiation-resistant, UV-resistant or desiccation-resistant (Buczolits et al. , 1999; Collins et al. 2000; Zhang et al. 2007; Dai et al. 2009; Su et al. 2014; Maeng et al. 2020). *Hymenobacter* strains generate mucous colonies with pink to red and several color variants, and the bacterial pigmentation serves multiple purposes, including light-harvesting, UV-resistance or photo-protection in cell membranes, where they build an integral part of the complex membrane structure. Pigments from soil-derived bacteria exhibit biological properties, including antiviral, antibacterial, antitumor, and antifungal activities (Klassen and Foght 2011; Sedláček et al. 2019). These characteristics increase scientists' interest in this genus. The majority of *Hymenobacter* species are Gram-reaction-negative, non-motile, pink to red-pigmented and rod-shaped; contain MK-7 as the predominant menaquinone and phosphatidylethanolamine as the major polar lipid (Hirsch et al. 1998; Buczolits et al. 2006; Srinivasan et al. 2015; Ten et al. 2017; Han et al. 2018; Sedláček et al. 2019; Maeng et al. 2020).

Sediments are capable of interfering with vital ecosystems via deleterious or enriching effects, with the contaminants potentially resulting in significant ecological changes, including the transformation of benthic microbial communities. Heavy metals including arsenic (As), chromium (Cr), copper (Cu), cadmium (Cd), nickel (Ni), zinc (Zn), and lead (Pb) have been identified in the lake sediments of the Huaihe River, and the ecological risk of these sediments has been assessed (Zhang et al. 2016, Wu et al. 2022; Xu et al. 2023). During an investigation into the microbial diversity of contaminated sediment in a freshwater river, bacterial microorganisms were isolated from sediments (Prat et al. 2014; Boulanger et al. 2019; Jin et al. 2022). In this study, we describe two pink-pigmented aerobic bacterial strains, S2-20-2^T and S2-21-1, isolated from a contaminated freshwater sediment sample in China. Genomic analysis revealed that strain S2-20-2^T contained g for cold shock proteins, carbon storage-related genes, and glycogen-debranching genes, which assist the microorganism in adapting to cold environments during growth (Goordial et al. 2015). Additionally, membrane transporter genes encoding for metal-translocating P-type ATPases were identified, and these genes were confirmed to be responsible for metal transport/resistance (Nies 2003; Kaur et al. 2006). Phylogenetic analysis of 16S rRNA gene sequences revealed that two strains S2-20-2^T and S2-21-1 were closely related to members of the genus *Hymenobacter*. Based on a polyphasic approach, we propose strains S2-20-2^T and S2-21-1 as a new species *Hymenobacter sediminicola* sp. nov.

Materials and methods

Isolation, morphological and physiological characterization

Sediment samples were collected from aquaculture area of the Huaihe River at a water depth of 4 m in Jiangsu, China (33° 06′ 58″ N, 118° 30′ 51″ E) in October 2018. For the serial dilution, 1 g sediment sample was dispersed in 5 ml of sterile saline solution, and 100 µl of this suspension was spread by L-loop on the surface of a modified R2A agar (Jin et al. 2020) and cultivated at room temperature for up to 30 days. Two pink-pigmented isolates were selected for next characterization. For recovering pure cultures, the single colony was purified by repeated streaking on new R2A plates. Macromorphology for colony was examined after incubating for 2 days at 30 °C on R2A agar plates. The Gram staining was determined using a Gram stain kit (Difco) following the manufacturer's instructions. The cell morphology and motility were examined under a phase-contrast microscope (Nikon Eclipse 80i microscope, 1000×magnification) using the cells grown at 30 °C for 48 h. Different

temperatures range (4, 8, 10, 15, 20, 30, 37, and 42 °C) and pH range (pH 5–10 at intervals of 1 unit) of the cell growth were determined in R2A medium, and different buffer systems for the cell growth were applied as described previously by Yang et al. (2020). NaCl tolerance for cell growth was observed in R2A agar using different NaCl concentrations from 1 to 5% (w/v). The activity of oxidase was tested using 1% tetramethyl-*p*-phenylenediamine and catalase activity was determined by observing the production of O_2 bubbles after dropping 3% (w/v) H_2O_2 on a fresh culture grown for 48 h on R2A medium (Wu et al. 2020). Carbon source utilization, enzyme activities and additional physiological and biochemical characterization were performed using API 20NE, API ID 32GN and API ZYM kits (bioMérieux) following the manufacturer's instructions.

Chemotaxonomic characterization

For the comparative analysis of whole-cell fatty acid profiling, strains S2-20-2^T and S2-21-1 were cultured on R2A agar at 25 °C for 72 h. and the cell harvesting standardization was done following the method described by MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). We harvested the cell mass when the cell culture reached the late exponential phase for extracting the fatty acids. The fatty acids were analysed using GC (Hewlett Packard 6890) and identified in the TSBA 6 database provided in Sherlock software 6.1. Respiratory isoprenoid quinone was extracted following the method as described by Komagata and Suzuki (1988), and analysed using HPLC (Shimadzu) with an YMC-Pack ODS-A column. Polar lipids were extracted following the method described by Tindall, the biomass used for lipid extraction was obtained from cultures growing on R2A agar plates at 25 °C for 3 days. For visualizing the spots on the two-dimensional thin layer chromatography (TLC) on silica gel 60 F254 plates (Merck), the spraying reagents were applied as follow: molybdatophosphoric acid for total spots, ninhydrin for aminolipids, molybdenum blue for phospholipids and alphanaphthol solution for glycolipids.

Genomic and phylogenetic analyses

Whole genomic DNA was extracted using a FastDNA™ SPIN kit for soil (MPbio) the manufacturer's instructions. The purity of DNA was examined using a ND2000 spectrometer (Nanodrop Technologies, Inc.). The 16S rRNA genes of strains S2-20-2^T and S2-21-1 were amplified by PCR with the universal bacterial primer sets: 27F (5'-AGA GTT TGA TCM TGG CTC AG-3'; *Escherichia coli* position 8–27) and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3'; *E. coli* position 1492–1510) (Weisburg et al. 1991). The PCR cycling conditions were as follows: 95 °C for 5 min and 30 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min followed by a final extension step for 7 min at 72 °C. For the phylogenetic analysis, 16S rRNA gene sequences of strains S2-20-2^T, S2-21-1 and closely related species were aligned using CLUSTAL X (Thompson et al. 1997), and edited using BIOEDIT (Hall 1999) software. The neighbour-joining, maximum-parsimony and maximum-likelihood (Fitch 1971; Felsenstein 1981; Saitou and Nei 1987) algorithms were applied in the MEGA 7 software (Kumar et al. 2016) based on the aligned sequences. Bootstrap values were determined on 1000 resamplings of the sequences in each case (Felsenstein 1985).

The whole genome sequencing of type strain S2-20-2^T, was carried out suing the SMRT (Single Molecule, Real-Time) platform at Oxford Nanopore Technologies (Wuhan, PR China) together with Illumina next-generation sequencing technology. Illumina short-reads and Nanopore long-reads sequencing data were assembled in a hybrid assembler, Unicycler (version 0.4.4) (Wick et al. 2017) with SPAdes dependency (version 3.6.2). The genome of type strain S2-20-2^T was annotated in the RAST pipeline, and the genome comparison was made with the SEED Viewer (Aziz et al. 2008, 2012). For generating functional category, the CDSs (predicted protein coding sequences) were submitted to the COG (Clusters of Orthologous Groups) database (http://www.ncbi.nlm.nih.gov/COG/) (Tatusov et al. 1997, 2003). EasyFig software was used to map and compare the genomes of related species (Sullivan et al. 2011). The average nucleotide identity (ANI) values were determined in the Ortho ANI Tool from the EZBioCloud server (Lee et al. 2016), and the average amino acid identity (AAI) values were determined in a webserver developed by Kostas lab (http://enve-omics.ce.gatech.edu/aai/), which is considered sensitive over greater evolutionary distance. The digital DNA–DNA hybridization (dDDH) values were determined using

the GGDC 2.0 (genome-to-genome distance calculator) (Meier-Kolthoff et al. 2013). The phylognomic tree base on protein coding amino acid sequences was constructed using CVTree 4.0 with the default parameters (Qi et al. 2004; Zuo 2021). The genomic DNA G+C content (mol%) of strain S2-21-1 was analyzed using HPLC following the method described by Tamaoka and Komagata (1984), and non-methylated λ DNA (Sigma) was used as a standard.

Results and discussion

Strains S2-20-2^T and S2-21-1 were observed to form visible colonies within 48 h on an R2A agar when incubated at 25 °C. The cell growth was found to occur at temperatures ranging from 4 to 30 °C, but no growth was observed at 37 °C or above. Growth was found to occur at pH 5–8, but no growth was observed at pH 4 or 9. The colonies were observed to be red-pigmented, smooth, convex and circular with entire edges. The cells were found to be Gram-stain-negative, catalase-positive but oxidase-negative, non-motile and rod-shaped. The strains were found to be positive for the utilization of L-fucose, histidine, 3-hydroxy-butyrate, malate, maltose, D-mannose, D-sorbitol and D-sucrose; variable for L-alanine (positive for strain S2-20-2^T), D-glucose (positive for strain S2-20-2^T), D-monnitaol (positive for strain S2-20-2^T), phenyl acetate (positive for strain S2-21-1), rhamnose (positive for strain S2-20-2^T), L-serine (positive for strain S2-20-2^T), salicin (positive for strain S2-20-2^T) and D-melibiose (positive for strain S2-20-2^T); negative for acetate, *N*-acetyl-glucosamine, adipate, L-arabinose, caprate, citrate, gluconate, glycogen, 3-hydroxy-benzoate, 4-hydroxy-benzoate, inositol, itaconate, DL-lactate, 2-ketogluconate, 5-ketogluconate, malonate, L-proline, propionate, D-ribose, suberate and valerate. Positive for the following enzyme activities: alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphtol-AS-BI-phosphohydrolase and valine arylamidase; variable for *N*-acetyl-*β*-glucosaminidase (positive for strain S2-20-2^T) and acid phosphatase (positive for strain S2-20-2^T); but negative for the following enzyme activities: *α*-chymotrypsin, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase and trypsin (Table 1). Strains S2-20-2^T and S2-21-1 could be differentiated from the most close species *H. duratus*; by assimilating L-fucose, histidine, malate, D-sorbitol, and D-sucrose; by activities of oxidase, cystine arylamidase, esterase (C4), Naphthol-AS-BI-phosphohydrolase. Some physiological characteristics, including growth temperature range, carbon utilization, and enzyme activities, distinguished the two strains from their formal relatives.

The major fatty acids (> 10%) were identified as *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$, and summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c/t*) and summed feature 4 (*iso*-$C_{17:1}$ I and/or *anteiso*-$C_{17:1}$ B) (Supplementary Table S1). The major fatty acids in strains S2-20-2^T and S2-21-1 were consistent with the major fatty acid components in species from the genus *Hymenobacter*. However, some qualitative and quantitative differences, or the presence/absence of several components were observed (Supplementary Table S1). The predominant respiratory quinone was menaquinone-7 (MK-7). The polar lipids were composed of phosphatidylethanolamine (PE), three unidentified aminolipids (AL1, AL2, and AL3), an unidentified aminophosopholipid (APL) and an unidentified lipid (L) for type strain S2-20-2^T (Supplementary Fig. S1). The profile of polar lipids is similar to that of closely related species of genus *Hymenobacter* with major component of PE; however, strain S2-20-2^T contains three unidentified aminolipids that are absent in the closest relative *H. duratus* BT646^T, and strain S2-20-2^T does not contain glycolipids that are detected in *H. duratus* BT646^T.

Phylogenetic and whole genome sequence analysis

The 16S rRNA sequences of strains S2-20-2^T and S2-21-1 were determined and compared with related species in the EzTaxon-e server (Yoon et al. 2017), and both strains showed 97.6 − 99.3% similarities with *H. duratus* BT646^T, *H. psychrotolerans* Tibet-IIU11^T and *H. kanuolensis* T-3^T and less than 97% with all other species within the genus *Hymenobacter*. Strains S2-20-2^T and S2-21-1 shared 100% 16S rRNA gene sequence similarity. Based on the neighbor-joining phylogenetic analysis, strains S2-20-2^T and S2-21-1 clearly clustered with *H. duratus* BT646^T, *H. psychrotolerans* Tibet-IIU11^T, *H. kanuolensis* T-3^T and *H. guriensis* BT594^T, this dendrogram was also observed both in maximum-parsimony

Table 1 Comparative characteristics of strains S2-20-2^T and S2-21–1 from some close members of *Hymenobacter*

Characteristics	1	2	3	4	5	6
Isolation source	Sediment	Sediment	Soil	Permafrost sediment	Soil	Soil
Growth range (°C)	4–30	4–30	10–30	4–28	4–37	10–30
pH range	5–8	5–8	6–9	5–10	6–8	6–9
Oxidase/Catalase	−/+	−/+	+/+	+/+	−/+	+/+
Urease	−	−	−	−	−	+
Glucose acidification	−	−	−	+	−	−
Esculin hydrolysis	−	−	−	+	−	+
Gelatine hydrolysis	+	+	+	−	+	−
Enzyme activities:						
N-acetyl-*β*-glucosaminidase	+	−	−	−	+	+
Acid phospatase	+	−	−	+	+	+
Cystine arylamidase	+	+	−	+	+	−
Esterase (C4)	+	+	−	+	+	+
α-Glucosidase	−	−	−	−	−	+
β-Glucosidase	−	−	−	−	−	+
Naphthol-AS-BI-phosphohydrolase	+	+	−	+	+	+
Carbon utilization:						
L-Alanine	+	−	−	−	+	−
L-Fucose	+	+	−	−	−	−
D-Glucose	+	−	+	+	+	+
Glycogen	−	−	+	−	−	+
Histidine	+	+	−	−	+	−
3-Hydroxy-butyrate	+	+	+	−	−	−
Inositol	−	−	+	−	+	−
Malate	+	+	−	−	−	−
Maltose	+	+	+	−	+	−
D-Mannitol	+	−	−	−	−	−
D-Mannose	+	+	+	+	−	−
D-Melibiose	+	−	−	−	−	−
Phenyl acetate	−	+	−	−	−	−
L-Proline	−	−	−	−	+	−
Propionate	−	−	+	−	−	−
Rhamnose	+	−	+	−	+	−
Salicin	+	−	−	−	−	−
L-Serine	+	−	−	−	−	−
D-Sorbitol	+	+	−	−	−	−
D-Sucrose	+	+	−	+	−	−
DNA G+C content (mol%)	57.9	57.7	59.5[a]	60.8[b]	69.2[c]	59.8[a]

Strains: 1, S2-20-2^T; 2, S2-21-1; 3, *H. duratus* BT646^T; 4, *H. psychrotolerans* DSM 18569^T; 5, *H. kanuolensis* KCTC 32407^T; 6. *H. guriensis* BT594^T. All data were from this study, unless indicated. All strains were observed to be negative for activities of nitrate reduction, inlole production, arginine dihydrolase, *α*-chymotrypsin, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase lipase (C14), *α*-mannosidase, and trypsin; carbon assimilation of acetate, *N*-acetyl-glucosamine, adipate, L-arabinose, caprate, citrate, gluconate, 4-hydroxy-benzoate, itaconate, 2-ketogluconate, DL-lactate, malonate, D-ribose, suberate, and valerate. +, positive; –, negative

[a]Data taken from Damdin-togtokh et al. (2021)

[b]Zhang et al. (2008)

[c]Su et al. (2014)

and maximum-likelihood phylogenetic trees (Supplementary Fig. S2). The phylogenomic tree based on the genome-wide amino acid sequences also supported that type strain S2-20-2^T formed an evolutionary lineage clustered with *H. duratus* BT646^T and *H. psychrotolerans* Tibet-IIU11^T (Fig. 1). The topology of the phylogenetic and phylogenomic trees is in accordance with the sequence similarities for the 16S rRNA gene of the novel strains versus the type strains of the *Hymenobacter* species. The 16S rRNA gene similarity between these two isolates and *H. duratus* BT646^T and *H. psychrotolerans* Tibet-IIU11^T, which

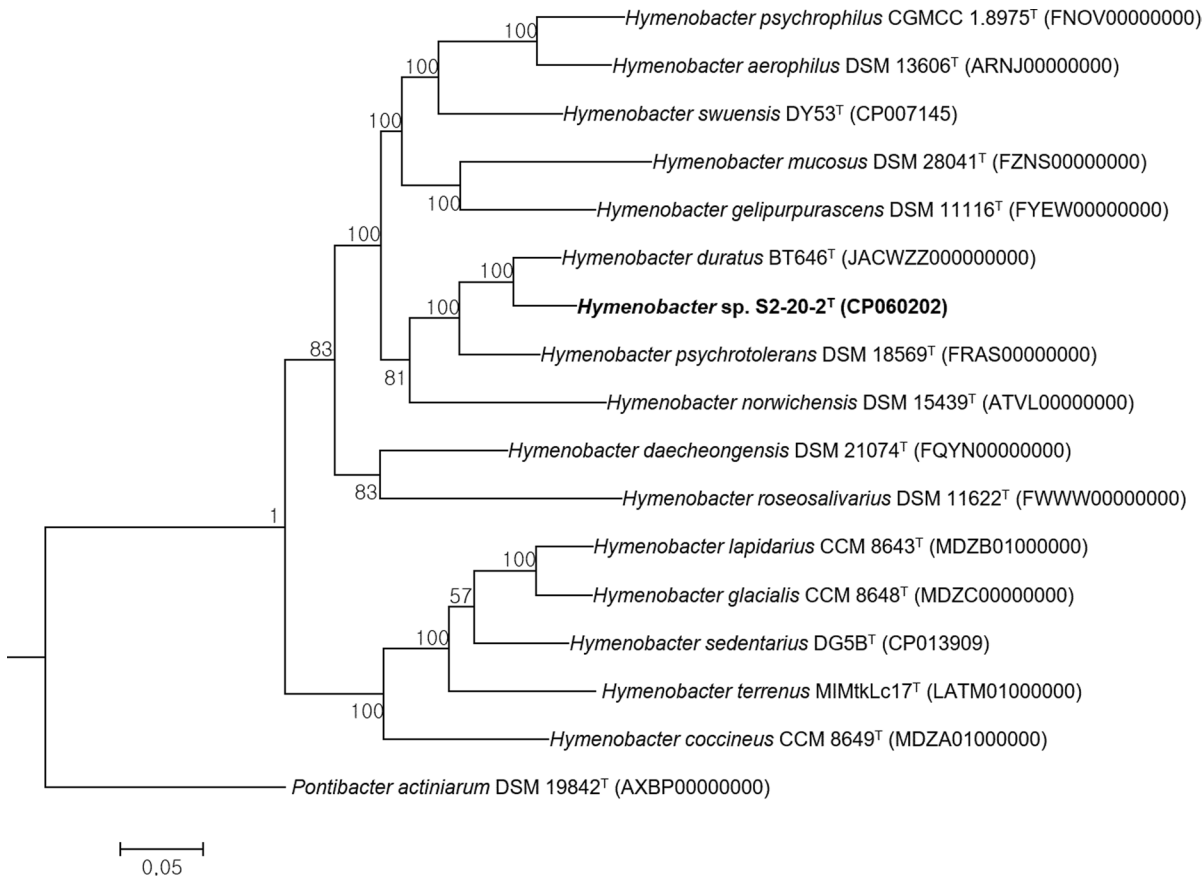

Fig. 1 A Phylogenomic tree reconstructed by core gene sets on the autoMLST web platform shows that the position of type strain S2-20-2^T among the type species within the genus *Hymenobacter*. The MLSA tree was reconstructed based on 85 different housekeeping genes. *Pontibacter actiniarum* DSM 19842^T was used as the outgroup. Bar, 5% nucleotide substitution

is much higher than the species discrimination value of 98.7% (Stackebrandt and Ebers 2006). For further characterization, ANI, AAI and dDDH analysis based on whole-genome sequences was applied. Table 2 shows that relative to the type strain S2-20-2^T, the ANI values of the named species varied between 75.7% (*H. wooponensis* JCM 19491^T) and 91.4% (*H. duratus* BT646^T), which is lower than the threshold value of 95–96% for bacterial species circumscription (Fig. 2). The AAI (dDDH) values obtained for all pairwise comparisons between type strain and related *Hymenobacter* species ranged from 71.7 to 94.6% (21.2 to 43.9%), which is also in borderline of 95% (ANI) and 70% (dDDH) species delineation (Goris et al. 2007; Richter and Rosselló-Móra 2009; Kim et al. 2014; Luo et al. 2014; Konstantinidis et al. 2017; Nicholson et al. 2020). The conclusion is also supported by genome alignment and comparison between S2-20-2^T and the two most closely related strains, *H. duratus* BT646^T and *H. psychrotolerans* DSM 18552^T (Fig. 3). According to analyses of the resulting architecture, the genomes of S2-20-2^T and *H. duratus* BT646^T were collinear and shared 91.4% of their content. Large structural differences between S2-20-2^T and *H. psychrotolerans* DSM 18552^T, however, consist of intra-chromosomal translocation and inversion. The complete circular chromosome of strain S2-20-2^T was of 4,511,071 bp with the GC content of 57.9%, and the genome encoded a total of 3,860 genes, including 3,785 protein-coding genes, 49 tRNA genes, 9 rRNA genes, 3 ncRNA genes and 14 pseudo genes (Supplementary Table S2). The circular genome map of strain S2-20-2^T was shown in Fig. 4. The genome sequence of strain S2-20-2^T

Table 2 General features and relationship of the genomes of type strain S2-20-2^T with the closely related species of the genus *Hymenoobacter*

No	Strains	16S rRNA	ANI	AAI	dDDH
1	*Hymenobacter aerophilus* DSM 13606^T (ARNJ00000000)	94.7	76.8	72.4	22.6
2	*Hymenobacter amundsenii* CCM 8682^T (NIRR00000000)	96.2	76.2	72.4	22.4
3	*Hymenobacter chitinivorans* DSM 11115^T (PGFA00000000)	94.7	75.9	72.4	21.2
4	*Hymenobacter daecheongensis* DSM 21074^T (FQYN00000000)	94.6	76.6	73.1	21.6
5	*Hymenobacter duratus* BT646^T (JACWZZ000000000)	99.3	91.4	94.6	43.9
6	*Hymenobacter gelipurpurascens* DSM 11116^T (FYEW00000000)	95.5	76.5	74.9	22.0
7	*Hymenobacter glacieicola* CGMCC 1.12990^T (BMGS00000000)	95.9	76.9	75.0	19.5
8	*Hymenobacter guriensis* BT594^T (JADWYK010000000)	96.7	76.7	72.8	22.4
9	*Hymenobacter mucosus* DSM 28041^T (FZNS00000000)	94.2	75.2	75.0	21.2
10	*Hymenobacter norwichensis* DSM 15439^T (ATVL00000000)	94.4	77.2	77.3	21.5
11	*Hymenobacter perfusus* LMG 26000^T (RWIU00000000)	96.5	77.7	75.5	23.8
12	*Hymenobacter persicinus* 1-3-3-3^T (SEWE00000000)	94.2	76.3	73.4	21.2
13	*Hymenobacter psychrophilus* CGMCC 1.8975^T (FNOV00000000)	95.1	76.0	71.7	22.2
14	*Hymenobacter psychrotolerans* Tibet-IIU11^T (FRAS00000000)	99.3	84.0	87.8	27.4
15	*Hymenobacter rigui* KCTC 12533^T (RWIT00000000)	96.5	77.2	74.7	23.5
16	*Hymenobacter rubripertinctus* CCM 8852^T (QYCN00000000)	95.0	76.7	73.1	22.8
17	*Hymenobacter sediminis* ELS1360^T (QKNS00000000)	95.1	76.4	74.8	22.1
18	*Hymenobacter swuensis* DY53^T (CP007145)	96.9	77.5	75.3	23.7
19	*Hymenobacter wooponensis* JCM 19491^T (SRKZ00000000)	95.0	75.7	74.4	22.3

was deposited at DDBJ/EMBL/GenBank with the accession number CP060202. Since members of the genus *Hymenobacter* demonstrated UV radiation resistance, we concentrated on identifying the genes responsible for its high tolerance to UV-light irradiation and ability to survive under harsh environmental circumstances. A total of 38 genes associated with DNA repair were observed. Based on their function, these genes were categorized into several groups: the bacterial MutL–MutS system (4), UvrABC system (5), bacterial photolyase (1), the DNA repair system including RecA and MutS (3), the bacterial RecFOR pathway (8), RecA and RecX (2), and other DNA (15). In addition, the S2-20-2^T strain possesses a large number of genes for stress tolerance (29) and resistance to heavy metals, antibiotics, and toxic compounds (15) (Fig. 4). Additionally, S2-20-2^T contained cold shock protein genes (GenBank accession numbers QNH62622, QNH63062, QNH63812, QNH61051, and QNH61773). Carbon storage-related genes and the glycogen-debranching gene (QNH62608) were also discovered in the genome, both of which assist the microorganism in adapting to frigid environments during growth. Strain S2-20-2^T possesses numerous genes responsible for metal transport/resistance, including copper-translocating P-type ATPase (QNH63542), cation-transporting P-type ATPase (QNH63944), cadmium-translocating P-type ATPase (QNH64153), and heavy metal translocating P-type ATPase (QNH61699).

Based on phenotypic and phylogenetic and phylogenomic characteristics, strains S2-20-2^T and S2-21-1 are considered to be members of the genus *Hymenobacter*. Some physiological evidences, like temperature growth range, carbon utilization, and enzyme activities, differentiated the two strains from their closely related species of *Hymenobacter*. Here, we propose that strains S2-20-2^T and S2-21-1 represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter sediminicola* sp. nov. is proposed.

Description of *Hymenobacter sediminicola* sp. nov.

Hymenobacter sediminicola (se.di.mi.ni'co.la. L. n. *sedimen*, *-inis* sediment; L. suff. *-cola* inhabitant, dweller; N.L. n. *sediminicola* sediment-dweller, referring to the source of the type strain).

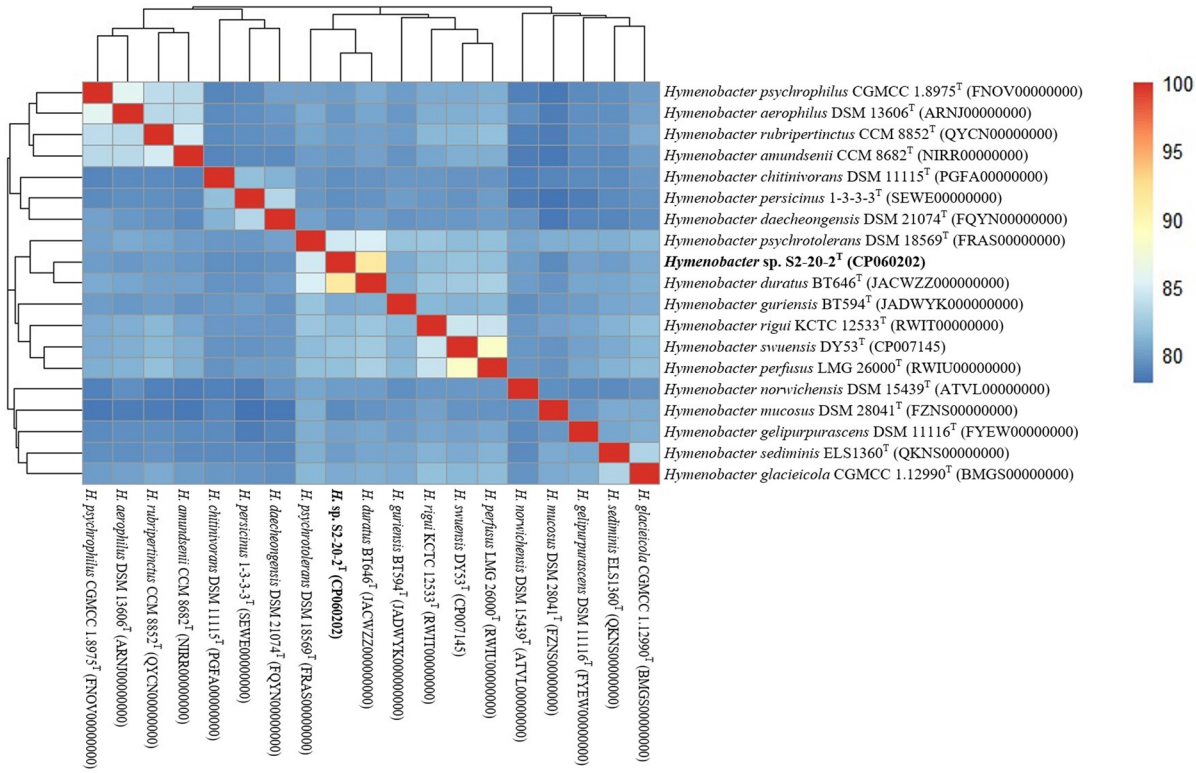

Fig. 2 Heatmap of ANI values for closely related *Hymenobacter* genomes from NCBI. Genomes are clustered using hierarchical clustering of ANI values, as implemented in the R package "pheatmap" (v1.0.12)

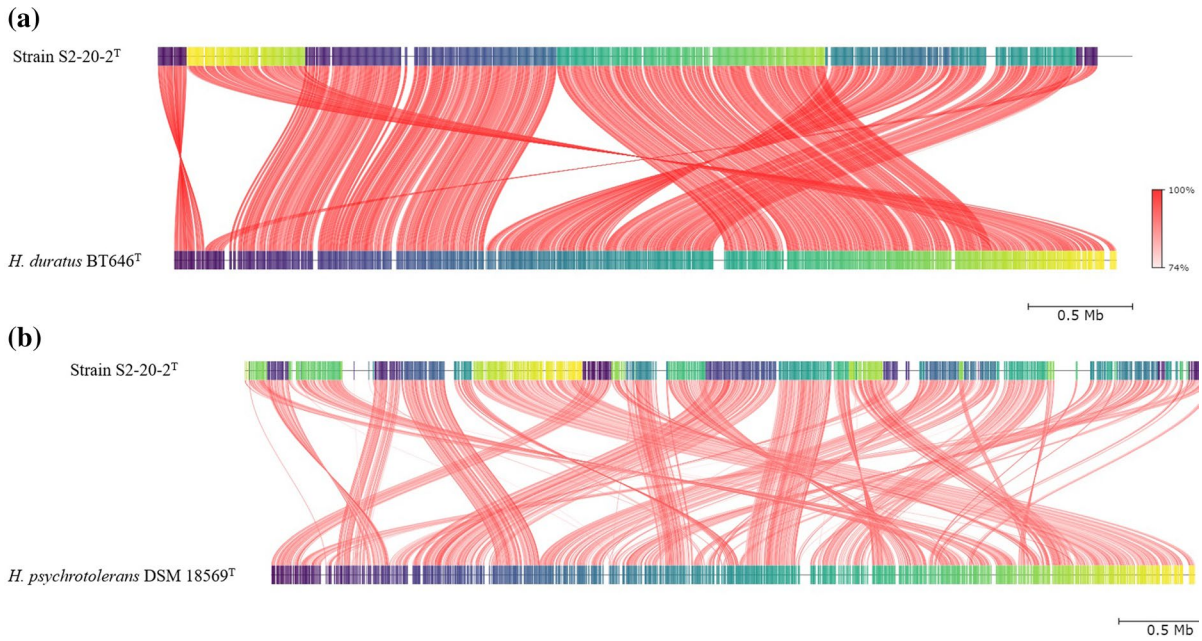

Fig. 3 The whole genome alignment and comparison between the genomes of S2-20-2^T, *H. duratus* BT646^T and *H. psychrotolerans* DSM18569^T

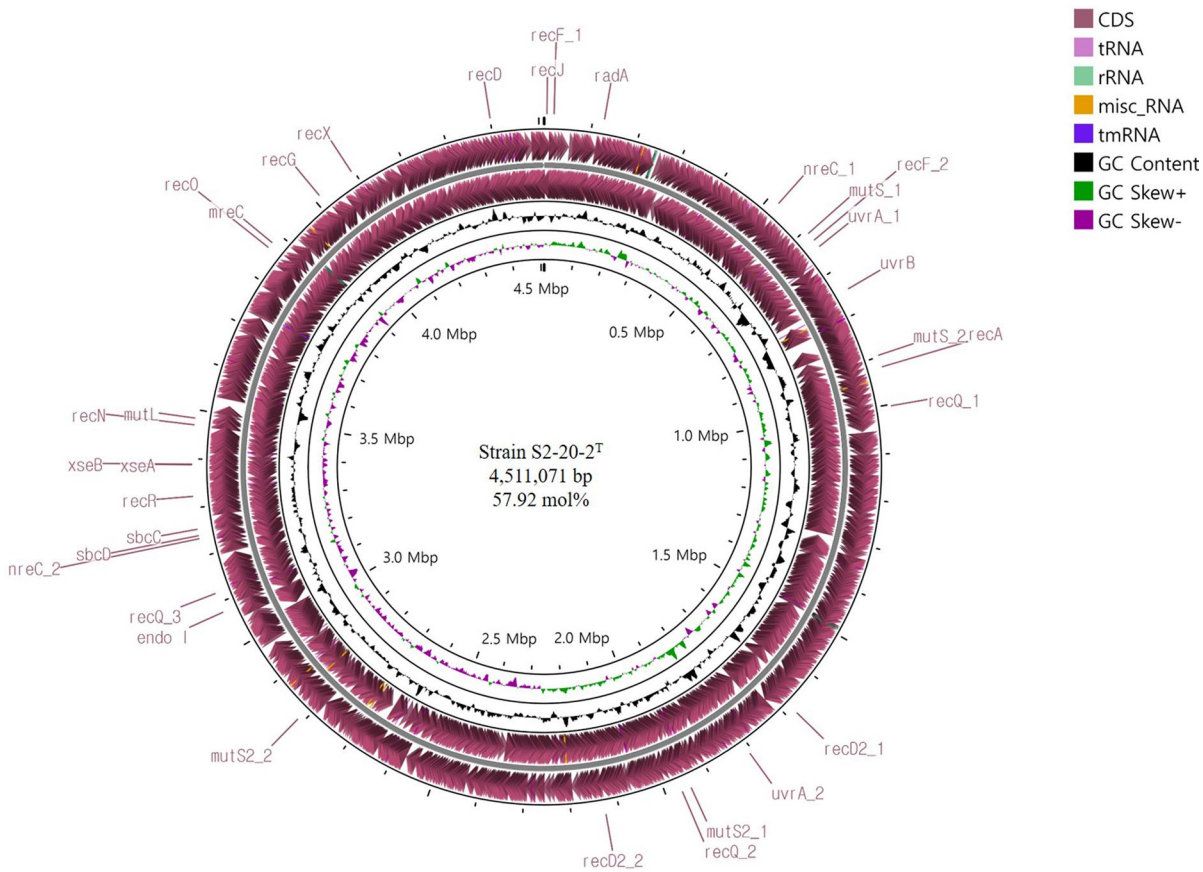

Fig. 4 Graphic representation of circular chromosome of S2-20-2^T. From inside to outside, the circles represent: the first and second circles represent protein-coding regions (CDS) and RNA-coding regions; the third and fourth circles represent sequence coverage; the fifth circle represents the genome's GC skew ([G C]/[G+C]) plot; and the seventh circle represents variation in G+C content

Cells are Gram-stain-negative, non-motile, rods grown for 48 h at 25 °C on R2A agar. Colonies are smooth, circular, convex, and pink-coloured on R2A agar. Growth occurs at 4–30 °C (optimum 25 °C), at pH 5.0–8.0 (optimum pH 7.0), and in 0–1% NaCl (0–2% for strain S2-21-1). Catalase-positive and oxidase-negative. Positive for gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, aesculin hydrolysis and β-galactosidase activities. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, summed feature 3 ($C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 7c/t$), and summed feature 4 ($C_{17:1}$ anteiso B and/or $C_{17:1}$ iso I). The major polar lipids are identified as phosphatidylethanolamine and an unidentified lipid. The DNA G+C content of the type strain is 57.9%.

The type strain S2-20-2^T (=CGMCC 1.18734^T=JCM 35801^T) was isolated from a sediment sample collected in Huaihe River, China. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains S2-20-2^T and S2-21-1 are MW073560 and MW073561, respectively. The GenBank accession number for the whole genome sequence of type strain S2-20-2^T is CP060202.

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Declarations

Conflict of interest The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Ethical approval No experiments with humans or animals were carried out.

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