INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Kämpfer *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:211–219

DOI 10.1099/ijsem.0.003739

MICROBIOLOGY SOCIETY

Psychrobacter pygoscelis sp. nov. isolated from the penguin *Pygoscelis papua*

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Abstract

One slightly beige-white pigmented, Gram-stain-negative, rod-shaped bacterium, strain I-STPP5b^T, was isolated from the trachea of a Gentoo penguin chick individual (*Pygoscelin papua*) investigated in Fildes Bay, Chilean Antarctic (62° 12′ S, 58° 57′ W). I-STPP5b^T consists of a 3.4 Mb chromosome with a DNA G+C content of 44.4 mol%. Of the 3056 predicted genes, 1206 were annotated as hypothetical proteins and 51 were tRNAs. Phylogenetic analysis based on nearly full-length 16S rRNA gene sequences showed that the isolate shared a 16S rRNA gene sequence identity to the type strains of *Psychrobacter phenylpyruvicus* (98.8%), *Psychrobacter arenosus* and *Psychrobacter pasteurii* (both 98.3%), *Psychrobacter piechaudii* (98.2%) and *Psychrobacter sanguinis* (98.1%), but 16S rRNA gene sequence similarities to all other *Psychrobacter* species were ≤98.0%. Partial *gyrB* nucleotide and amino acid sequence similarities among strain STPP5b^T and the next related type strains were all below 81.8 and 92.9%, respectively. DNA–DNA hybridisation (DDH) with *P. phenylpyruvicus* LMG 5372^T, *P. arenosus* DSM 15389^T and *P. sanguinis* DSM 23635^T also showed low values (all below 30%). The main cellular fatty acids of the strain were $C_{18:1}\omega9c$ and $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$. Based on phylogenetic, chemotaxonomic, genomic and phenotypic analyses we propose a new species of the genus *Psychrobacter,* with the name *Psychrobacter pygoscelis* sp. nov. and strain I-STPP5b^T (=CIP 111410^T= CCM 8799^T=LMG 30301^T) as type strain.

The genus *Psychrobacter* is classified into the family *Moraxellaceae* along with the genera *Moraxella* and *Acinetobacter*. Originally proposed by Juni and Heym [1] the genus accommodates obligate or facultative psychrophilic bacteria possessing a strictly oxidative metabolism. At the time of writing, 40 species have been described (January 2019) [2]. Most of the species of the genus *Psychrobacter* have been frequently found to be associated with the Antarctic and marine environments [3] as well as with poikilothermic animals, but some of them have also been isolated from human samples [4] and some have been found to be responsible for infections in humans [5, 6] including ocular infection [7], bacteremia, endocarditis [8], and also meningitis [9–11].

Here we describe a slightly beige-white pigmented bacterial strain (I-STPP5b^T) isolated from the trachea of the Gentoo penguin (*Pygoscelin papua*) studied in the Chilean Antarctic during the 51st expedition in January 2015. This procedure was done by employing a sterilized cotton swab and all procedures was done avoiding any animal injury and the most quickly possible. All sampling was previously authorized by INACH-Chile in according to the Antarctic Treaty.

Each penguin was carefully caught using a short hand net of 60 cm diameter and without disturbing the bird colony. The sampling was done by at least two persons. The bird specimen was firmly immobilized by both legs using the right hand and the whole body was fixed firmly under the left armpit, protecting the flippers from injuries. The second sampler fixed the bird's head with the hand and opened the bird's mouth with the fingers; the glottis sampling was done using a sterile cotton swab stick with the twist movement over the bird's glottis. The swab stick was immediately introduced to the closed sterile tube and kept refrigerated and transported

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Keywords: *Psychrobacter*; *Psychrobacter pygoscelis*; *penguin*.
The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene and *gyrB* sequence of strain I-STPP5b^T are MH065724 and MH165857, respectively. The whole genome sequence (WGS) project of strain STPP5b^T has been deposited at GenBank/EMBL/DDBJ under accession number RQRU00000000.

to the laboratory at Antarctic Research Station *Professor Julio Escudero Base* for bacteriological examination (62° 12′ S 58° 57′ W). Samples were directly streaked onto tryptone soya agar (Oxoid) supplemented with 1% (w/v) NaCl (TSA-1) and incubated for one week at 18 °C under aerobic conditions. Isolation of pure cultures was obtained from plates and a representative colony was selected, which was streaked onto a new TSA-1 plate to obtain pure cultures, and then stored at −80 °C in Criobille tubes (AES Laboratories, France) as well as in tubes containing tryptone soya broth supplemented with 1% (w/v) NaCl (TSB-1) and 20% (v/v) glycerol.

The original colonies had a diameter of approximately 2 mm, with smooth edges and a beige pigmentation. The strain was sub-cultivated on TSA-1 at 18 °C and can be maintained for several weeks at 6–8 °C.

The nearly full-length 16S rRNA gene of strain I-STPP5b^T was PCR amplified with universal 16S rRNA gene targeting primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) [12] and sequenced by the Sanger method with primer 27F (5′-GAGTTTGATCMTGGCTCAG-3′) and E786F (5′-GATTAGATACCCTGGTAG-3′) at LGC Genomics (Berlin, Germany). The manually corrected 16S rRNA gene sequence had a length of 1448 nucleotides spanning gene termini 46 to 1500 (numbered according to the *Escherichia coli rrnB* reference sequence published by Brosius *et al.* [13]). *P. phenylpyruvicus* was obtained as the closest related species with 98.8% 16S rRNA gene sequence similarity by a first BLAST search in the EzTaxon 16S rRNA gene sequence type strain database [14]. The phylogenetic placement of strain I-STPP5b^T was investigated in detail by the construction of phylogenetic trees using the 'All species living tree project' database (LTPs [15]) release LTPs128 (February 2017) and the ARB software package release 5.2 [16]. Sequences not included in the LTP database were added after alignment with the SILVA Incremental Aligner (SINA) v1.2.11 [17]. After manual correction of the alignment, considering the secondary structure of the 16S rRNA, phylogenetic trees were calculated with the maximum-likelihood (ML) method using RAxML version 7.04 [18] with the General-Time-Reversible (GTR)-GAMMA and rapid bootstrap analysis, the maximum-parsimony (MP) method using DNAPARS v 3.6 [19], and the neighbour-joining (NJ) methods using ARB Neighbour-joining and the Jukes-Cantor correction [20]. The NJ tree based on 1000, the ML and MP trees on 100 re-samplings (bootstrap analysis; [21]). Sequence positions between gene termini 74 and 1440 (according to Brosius *et al.* [13]) were considered in the phylogenetic analysis. The topology of the three calculated phylogenetic trees were compared and conserved nodes marked in the depicted tree (Fig. 1). All *Psychrobacter* type strains and further type strains of next closest related genera of the *Moraxellaceae* (*Moraxella*, *Enhydrobacter*, and *Faucicola*) were included in the analysis to show the monophyletic character of the genus *Psychrobacter*; type strains of the genus *Acinetobacter* (also *Moraxellaceae*) were used as outgroup. Pairwise sequence similarities of the *Psychrobacter* type strain 16S rRNA gene sequences were calculated within ARB using the ARB Neighbour-joining tool, without considering evolutionary models.

The pairwise 16S rRNA gene sequence similarity of strain I-STPP5b^T was highest to the type strains of *P. phenylpyruvicus* (98.8%), *P. arenosus* and *P. pasteurii* (both 98.3%), and *P. sanguinis* (98.1%). Sequence similarities to all other *Psychrobacter* species were ≤98.0%. Phylogenetic trees confirmed the placement of strain I-STPP5b^T into the monophyletic cluster of the genus *Psychrobacter* (Fig. 1). Independent of the applied treeing methods strain I-STPP5b^T clustered together with type strains of the species *P. arenosus, P. pasteurii, P. sanguinis, P. phenylpyruvicus, P. piechaudii*, and *Psychrobacter lutiphocae*.

The phylogenetic relationship to *P. phenylpyruvicus* is problematic due to different 16S rRNA gene sequences available for the respective type strain in the public databases. Considering the genome sequence derived 16S rRNA gene sequences (two genome sequences are available: BCUH01000000 and JMKP01000000) the phylogenetic relationship was as described here. But, if the first published 16S rRNA gene sequence for *P. phenylpyruvicus* ATCC 23333^T (U46144 [22]) is considered, which is also given in the LPSN and LTP databases (http://www.bacterio.net/index.html and LTPs132 database), strain I-STPP5b^T shared only 95.9% 16S rRNA gene sequence similarity with the type strain of *P. phenylpyruvicus.* Both *P. phenylpyruvicus* type strain 16S rRNA gene sequences were considered in the phylogenetic analyses and represented in the phylogenetic tree (Fig. 1), the two sequences among each other also only share 96.1% sequence similarity. Re-sequencing of the 16S rRNA gene of the type strain *P. phenylpyruvicus* LMG 5372^T obtained from the respective strain collection showed that this sequence was identical to the genome derived 16S rRNA gene sequences, which indicates that the original published 16S rRNA gene sequence (published under Acc. number U46144) did not represent the type strain of the species *P. phenylpyruvicus.*

To obtain a higher phylogenetic resolution further phylogenetic analysis was performed based on partial nucleotide and amino acid sequences of the gyrase, beta subunit (*gryB*). This gene is a general applied phylogenetic marker for the genus *Psychrobacter* (e.g. [23]). The *gyrB* gene was sequenced for strain I-STPP5b^T and if not available for next related type strains using primers gyrB_UP-1 and gyrB_UP-2r for PCR amplification and primers gyrB_UP-1S and gyrB_UP-2Sr for Sanger sequencing, respectively. Primer sequences were derived from Yamamoto and Harayama [24]. PCRs were performed in a total volume of 50 µl including one-fold PCR buffer, 0.2 mM of each dNTP, 0.4 µM of each primer, 0.04 mg ml^{-1} BSA, 0.02 U µl^{-1} Dream *Taq* DNA polymerase, and 2 µl cell lysate as DNA template. All chemicals except primers were purchased from Thermo Scientific (formerly Fermentas, St. Leon Roth, Germany). Cycle conditions were as followed, 95 °C for 3 min, 34 cycles of 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 1.5 min, and finally 72 °C for 5 min. PCR products were purified with the Qiagen PCR purification kit and sequenced at LGC Genomics. Phylogenic analyses were performed in MEGA 7 [25]. Nucleotide sequences based

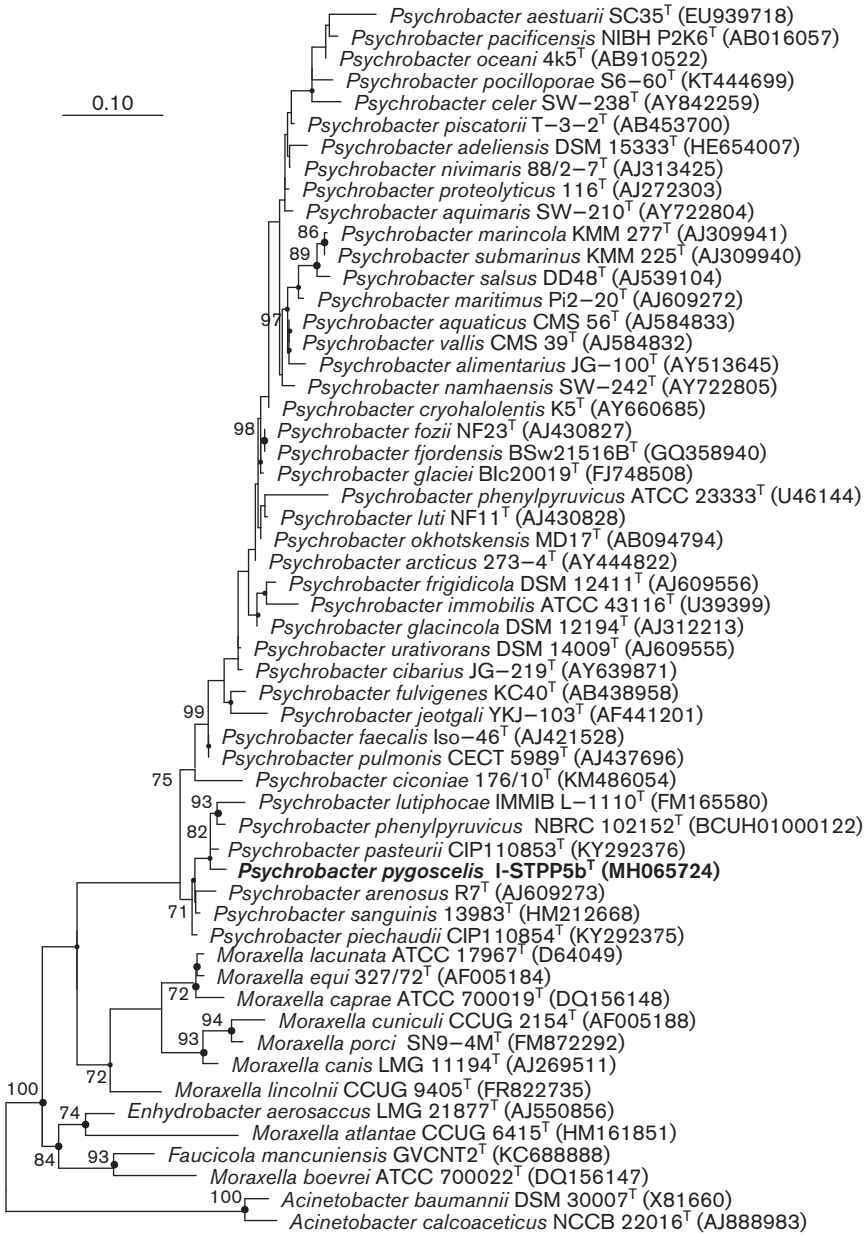


Fig. 1. Phylogenetic tree based on nearly full-length 16S rRNA gene sequences showing the placement of strain I-STPP5b^T within the genus *Psychrobacter* (*Moraxellaceae*). The phylogenetic tree was generated in ARB with the maximum-likelihood method and based on sequences among gene termini 74 and 1440 (numbering according to Brosius *et al.* [12]) and 100 replications (bootstrap support). Bootstrap values >70% are depicted at nodes. Circles mark nodes, which were also present in the maximum-parsimony and neighbour-joining tree. Larger circles represent nodes which had a bootstrap value >70% in at least one of the two trees. *Moraxella* and *Acinetobacter* spp. type strains were used as outgroup. Bar: 0.1 substitutions per nucleotide positions.

on the performed amino acid sequences alignmentusing ClustalW [26] after nucleotide sequence transcription. Phylogenetic trees were calculated with the maximum-likelihood method using the GTR-GAMMA model by using a discrete GAMMA-distribution (+G) with five rate categories and by assuming that a curtain fraction of sides are evolutionary invariable (+I) (for nucleotide sequences) and the Jones-Thornton-Taylor model (JTT [27])+G+I (for amino acid sequences). Pairwise nucleotide and amino acid sequence similarities were determined based on the calculation of *p*-distances without considering evolutionary models. All performed phylogenetic analysis based on 984 nucleotide and 325 amino acid sequence positions, respectively.

Strain I-STPP5b^T formed at the level of *gyrB* nucleotide sequences a cluster with the same six *Psychrobacter* species as

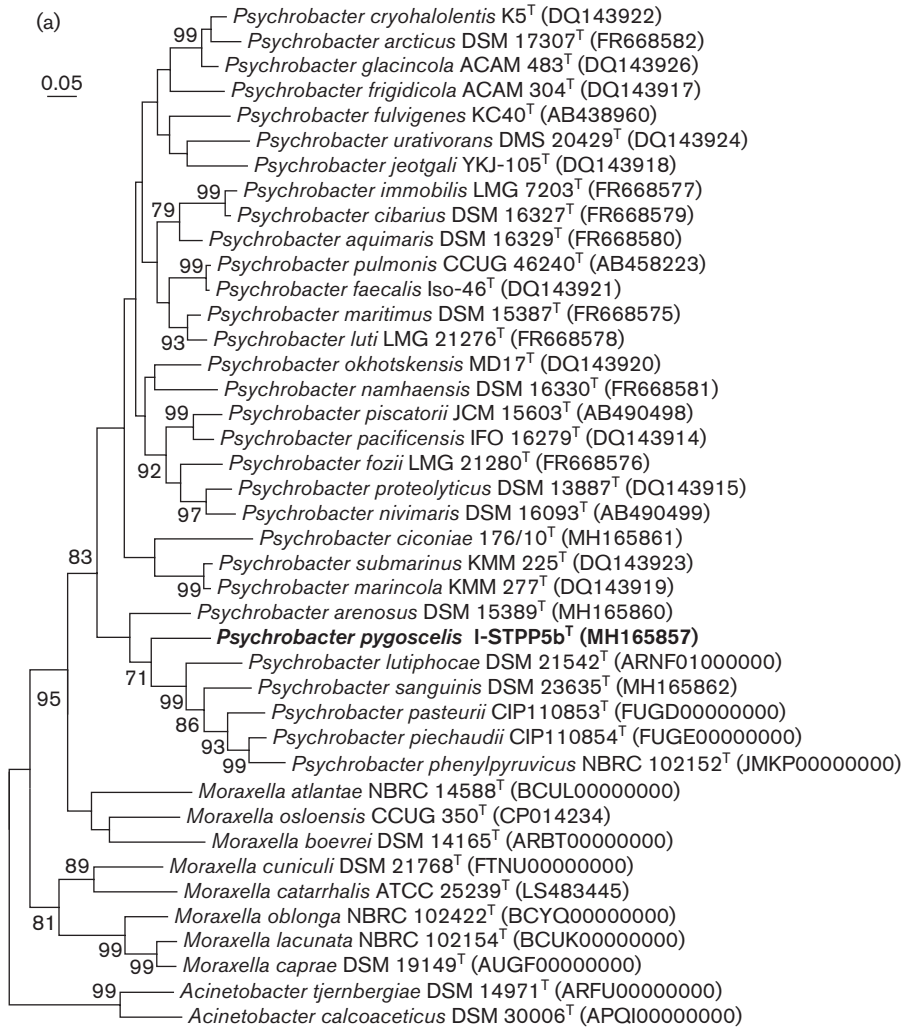
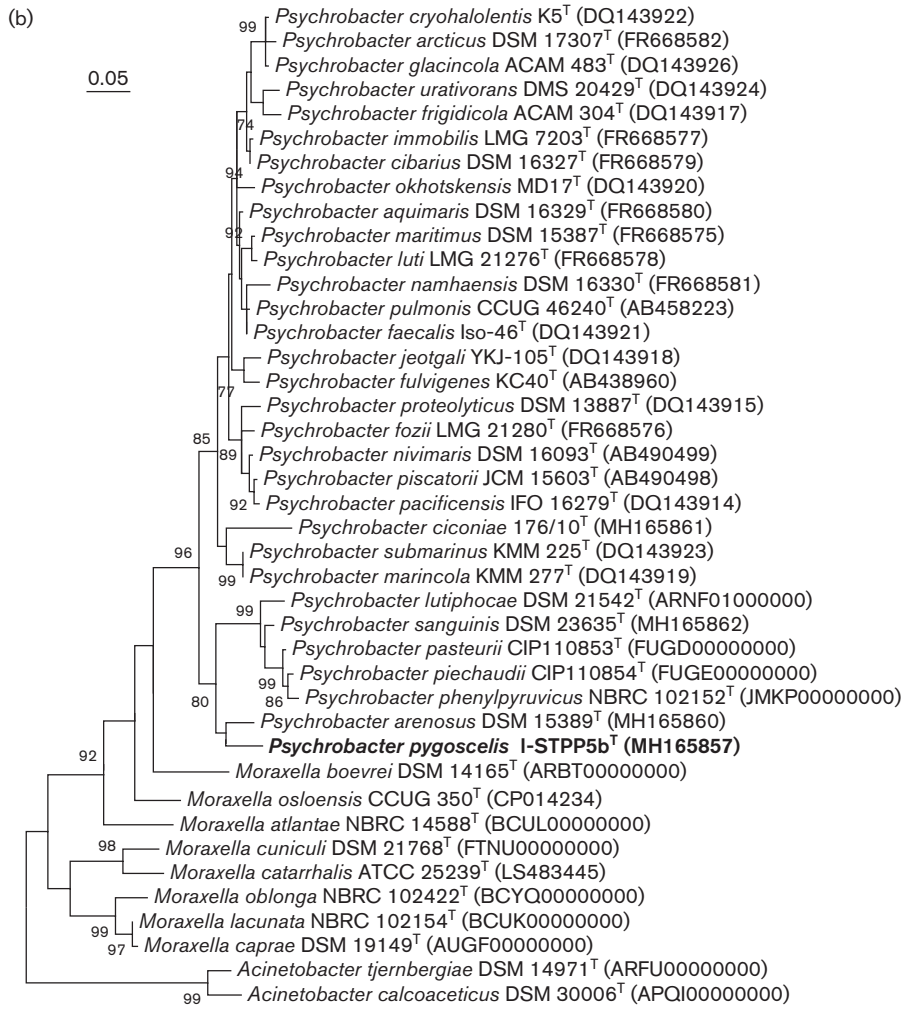


Fig. 2. Phylogenetic tree based on partial *gyrB* nucleotide (a) and amino acid sequences (b) showing the placement of strain I-STPP5b^T within the genus *Psychrobacter* (*Moraxellaceae*). The maximum-likelihood trees were calculated in MEGA7 using the GTR-GAMMA model +G+I (for nucleotide sequences) and the JTT-model (for amino acid sequences). The trees based on 984 nucleotide (a) and 325 amino acid sequence positions and was calculated based on 100 replications. Gene sequence Accession numbers are given in brackets. Genome sequence Accession numbers are given respectively, if gene sequences were downloaded from respective genomes (determined by BLAST search in genome sequences). Bootstrap values >70% are depicted at nodes. *Moraxella* and *Acinetobacter* spp. type strains were used as outgroup. Bar: 0.1 substitutions per nucleotide and amino acid positions, respectively.

Fig. 2. (Cont.)



obtained by the 16S rRNA gene sequence phylogeny; however, phylogenetic relationships within the cluster were different (Fig. 2a). The next related species in the *gyrB* nucleotide sequence based phylogenetic analysis was *P. arenosus*, which was even more pronounced at the level of *gyrB* amino acid sequences (Fig. 2b). Pairwise *gyrB* nucleotide and amino acid sequence similarities were highest for I-STPP5b^T and the type strain of *P. arenosus,* with 81.8 and 92.9%, respectively. Sequence similarities of strain I-STPP5b^T to all other type strains considered in the *gyrB* based phylogenetic analysis (see Fig. 2) were lower. Nucleotide sequence similarities were in the range of 80.7 to 77.3 % and amino acid sequence similarities in the range of 91.4 to 86.2%, respectively.

To evaluate the genomic properties of I-STPP5b^T, Fraunhofer Chile Research Foundation performed whole-genome sequencing. Libraries were constructed using the NEXTERA XT library preparation kit, and sequenced on a MiSeq instrument using the 2×300 cycles, paired-end V3 chemistry (Illumina, Inc., San Diego, CA, USA), leading to 1094323 reads pairs (35–301 bp length), which were assembled using SPAdes v3.11.1 software [28]. The draft genome obtained has a total size of 3407403 bps, conformed by 15 scaffolds (>500 bp), an N50 value of 622449 bps, and G+C content of 44.4%. This group of scaffold sequences were processed with Tychus pipeline [29] in order to get the prediction and annotation of the genes. Of the 3056 predicted genes, 1206 were annotated as hypothetical proteins, and 51 were tRNAs.

To test if strain I-STPP5b^T was closely related to other species previously described, Average Nucleotide Identity (ANI) analyses were performed. The genomic sequence information from the twelve *Psychrobacter* strains available in the RSAT-prokaryotes data base [30] was downloaded (data not shown). With these phylogenetically close genomes, we performed a pairwise ANI calculation using the JSpecies Web server [31], and the following parameters to establish a significantly different species: <94% identity for BLAST analysis (ANIb),

Table 1. Differential characteristics of strain I-STPP5b^T and the closest phylogenetic neighbours

Strains: 1, I-STPP5b^T; 2, *P. arenosus* DSM 15389^T; 3, *P. pasteurii* A1019^T; 4, *P. sanguinis* CIP 110993^T; 5, *P. piechaudii* 1232^T; 6, *P. phenylpyruvicus* CIP 82.27^T; 7, *P. lutiphocae* CIP 110018^T; 8, *P. immobilis* CIP 102557^T. Data for strains 1–3 from this study; data from Wirth *et al.* [4] for *P. sanguinis* CIP 110993^T, from Bowman *et al.* [22] for *P. phenylpyruvicus* CIP 82.27^T, from Yassin and Busse [38] for *P. lutiphocae* CIP 110018^T, and from Juni and Heym [1] for *P. immobilis* CIP 102557^T. +, Positive; -, negative; (+), weak positive reaction/growth; (-), weak negative reaction/growth; NA, data not available. All strains were positive for oxidase and negative for acid production from glucose, arabinose, mannose, and citrate.

Characteristic	1	2	3	4	5	6	7	8
Urease	+	(+)	+	+	+	+	+	–
Carbon source								
Tween 80	–	–	+	–	+	+	+	–
Dextrin	+	–	+	–	–	–	–	–
D-Glucosamine	–	–	+	+	+	–	+	+
5-keto-D-Gluconic acid	–	-+	+	+	+	–	+	+
Enzyme activities (API ZYM)								
Alkaline phosphatase	–	+	+	+	–	+	+	–
Esterase	(+)	+	+	+	+	+	+	+
Lipase	+	(+)	+	–	–	+	–	+
Cystine arylamidase	–	–	–	–	–	–	+	–
Growth at (°C)								
4	+	+	+	(+)	+	NA	(+)	NA
37	(+)	+	+	(+)	+	(+)	(+)	(-)
Tolerates (% NaCl)								
0	(+)	+	+	(+)	+	(+)	(+)	NA
5	+	+	+	(-)	+	(+)	(+)	NA
10	+	+	+	(-)	+	(-)	(-)	NA

<96% MUMer analysis (ANIm), and <0.99 for tetranucleotides frequency analysis (TETRA [32]). All the three analyses performed, results shows low values with averages near to 73 and 78% for ANIb and ANIm analysis, respectively, and 0.92 for TETRA analysis, which are below from accepted species cut-off value for those analyses (data not shown). In particular the best value for ANIb was 73.7% with *Psychrobacter cryohalolentis*, for ANIm was 80.99% with *Psychrobacter* sp strain AntiMin-1 and 0.937 for TETRA analysis with a *Psychrobacter* sp P2G3 strain. DNA–DNA hybridisation (DDH) experiments were performed with strain I-STPP5b^T and the type strains of *P. phenylpyruvicus* LMG 5372^T, *P. arenosus* DSM 15389^T, and *P. sanguinis* DSM 23635^T according to the method of Ziemke *et al.* [33] (except that for nick translation 2 µg of DNA were labelled during 3 h of incubation at 15 °C) using DNA extracted by the method of Pitcher *et al.* [34]. Strain I-STPP5b^T showed DNA–DNA similarities below 30 % to all of these type strains.

For more detailed phenotypic characterization the strain was cultured on TSA-1 at 18 °C for 3 days. Gram-staining was performed by using the modified Hucker method according to Gerhardt *et al.* [35]. Cell morphology was analysed by light microscopy at 1000× magnification with a Leica DFC 3000G microscope (Leica, Germany) using glass slides covered with three times washed (each time 20 min in pure water) and autoclaved 2% (w/v) agar (Becton Dickinson). Cell motility was determined by observing a 24 h cell culture incubated in TSB-1 at 18 °C. Cytochrome oxidase activity was tested by using Microbiology Bactident oxidase test strips (Merck) and catalase activity by testing gas bubble formation after dropping 3% H_2O_2 (v/v) onto a fresh culture grown on TSA-1.

Growth was tested on R2A (Oxoid), nutrient agar (NA, Oxoid), K7 [0.1% (w/v) of yeast extract, peptone, and glucose, 15 g l^{-1} agar, pH 6.8], medium 65 according to DSMZ [4.0 g of yeast extract, 10.0 g of malt extract, 4.0 g of D-glucose, 2.0 g of $CaCO_3$, 12 g l^{-1} agar, pH 6.8], and PYE [0.3% (w/v) yeast extract and 0.3% (w/v) casein peptone, respectively, 15 g agar l^{-1}, pH 7.2], Luria Bertani (LB, Sigma-Aldrich), tryptic soy agar with and without the addition of 1% w/v NaCl (TSA-1 and TSA respectively, Becton Dickinson), malt agar (Merck), DEV agar (DEV, 'Deutsche Einheitsverfahren' Merck), marine agar 2216 (MA, Becton Dickinson), Columbia agar with sheep blood (BA, Liofilchem), glycine arginine agar, MacConkey agar (McC, Oxoid), thiosulfate-citrate-bile

Table 2. Cellular fatty acid compositions of strain I-STPP5b^T and the closest phylogenetic neighbours

Data for strains 1–3 from this study. 1, I-STPP5b^T; 2, *P. arenosus* DSM 15389^T (data in brackets from Romanenko *et al.* [39]); 3, *P. pasteurii* A1019^T (data in brackets from Hurtado-Ortiz *et al.* [6]); 4, *P. piechaudii* 1232^T (data from Hurtado-Ortiz *et al.* [6]); 5, *P. sanguinis* CIP 110993^T data obtained after growth on Blood agar (Oxoid) (data in brackets from Hurtado-Ortiz *et al.* [6]); 6, *P. phenylpyruvicus* CIP 82.27^T data obtained after growth on Blood agar (data in brackets from Bowman *et al.* [22]); 7, *P. lutiphocae* CIP 110018^T data obtained after growth on Blood agar (data in brackets from Yassin and Busse) [38]. The most prevalent fatty acids for each column are in bold type; ND, fatty acid not detected in the strain.

Fatty acid %	1	2	3	4	5	6	7
Saturated							
$C_{9:0}$	ND	ND (ND)	1.8	ND	ND (ND)	ND (ND)	ND (ND)
$C_{10:0}$	**1.9**	**2.8 (2.9)**	**7.8**	3.8	ND (3.2)	1.6 (ND)	ND (ND)
$C_{11:0}$	ND	ND (ND)	6.9	0.9	ND (0.6)	6.1 (ND)	ND (ND)
iso-$C_{11:0}$	ND	ND (ND)	1.4	5.0	ND (1.0)	2.9 (ND)	ND (ND)
$C_{12:0}$	ND	**2.5 (2.6)**	3.2	1.9	ND (1.0)	ND (ND)	2.8 (ND)
$C_{14:0}$	ND	nd (ND)	0.7	0.7	ND (1.5)	ND (ND)	ND (ND)
$C_{15:0}$	ND	ND (ND)	0.5	0.3	ND (0.3)	ND (ND)	ND (ND)
$C_{16:0}$	**2.0**	**4.0 (0.7)**	**8.5**	**12.3**	**8.0 (8.8)**	**7.0 (6.0)**	1.5 (1.7)
$C_{17:0}$	ND	ND (ND)	0.5	0.7	ND (0.4)	ND (ND)	ND (1.9)
iso-$C_{17:0}$	**7.6**	**1.8 (1.9)**	0.3	0.3	ND (0.3)	ND (ND)	ND (ND)
$C_{18:0}$	**2.0**	**3.7 (2.0)**	**7.3**	**11.2**	**6.6 (6.0)**	5.1 (3.0)	**2.0 (3.7)**
Unsaturated							
$C_{14:1}$ ω5c	ND	ND (ND)	ND	ND	ND (0.9)	ND (ND)	ND (ND)
$C_{15:1}$ ω6c	ND	ND (ND)	ND	ND	ND (0.3)	ND (ND)	ND (ND)
$C_{16:1}$ ω7c	ND	ND (ND)	ND	ND	ND (ND)	ND **(33.0)**	ND **(8.3)**
$C_{16:1}$ ω9c	ND	ND (ND)	ND	ND	ND (ND)	ND (ND)	ND (ND)
$C_{17:1}$ ω8c	**3.9**	**6.2 (13.9)**	0.8	0.8	ND (1.5)	ND **(6.0)**	2.4 (2.6)
$C_{18:1}$ ω9c	**66.2**	**70.3 (61.3)**	**28.3**	**37.5**	**45.9 (45.2)**	**35.1 (43.0)**	**55.9 (69.3)**
$C_{18:1}$ *9c/11*c	ND	ND (ND)	ND	ND	ND (ND)	ND (ND)	ND **(4.7)**
$C_{18:2}$	ND	ND (ND)	ND	ND	ND (ND)	ND **(8.0)**	ND (ND)
$C_{18:3}$ *ω6,9,12c*	**1.8**	**3.1 (ND)**	ND	ND	ND 5.0 (ND)	ND 2.7 (ND)	ND (ND)
$C_{20:4}$ *ω6,9,12,15c*	ND	ND (ND)	0.9	1.3	ND (1.6)	ND (ND)	ND (ND)
Hydroxy							
$C_{10:0}$ 3-OH	ND	ND (ND)	0.3	0.3	ND (0.3)	ND (ND)	ND (ND)
$C_{11:0}$ 3-OH	ND	ND (ND)	2.7	0.5	ND (0.7)	2.1 (ND)	ND (ND)
$C_{12:0}$ 2-OH	ND	ND (ND)	0.5	0.3	ND (ND)	ND (ND)	ND (ND)
$C_{12:0}$ 3-OH	**1.8**	**3.8 (3.4)**	**14.4**	**8.2**	4.0 (4.1)	6.3 (2.0)	**2.8 (5.5)**
Iso-$C_{13:0}$ 3-OH		ND (ND)	ND	0.4	ND (ND)	ND (ND)	ND (ND)
Summed Features*							
3	**13.3**	**5.6 (6.6)**	4.6	4.6	**18.6 (14.2)**	10.3 (ND)	4.3 (ND)
5	**3.5**	ND (ND)	6.0	**7.4**	**11.6 (7.8)**	12.1 (ND)	26.7 (ND)
8	ND	ND (ND)	0.8	1.1	ND (ND)	ND (ND)	1.5 (ND)

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of $C_{16:1}\omega7c$ and /or $C_{16:1}\omega6c$. Summed feature 5 consisted of $C_{18:3}\omega6,9c$ and/or anteiso-$C_{18:0}$. Summed feature 8 consisted of $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

salts agar (TCBS, Oxoid), and Müller-Hinton agar with and without the addition of 1% w/v NaCl (MH and MH-1, respectively, Oxoid). Temperature-dependent growth was tested on TSA-1 at 0.5, 4, 10, 15, 20, 25, 28, 30, 33, 37, 45, 50, and 55 °C, salinity-dependent growth at 18 °C in TSB broth supplemented with 0 to 10.0% (w/v) NaCl, and pH-dependent growth was tested using TSB broth adjusted to pH values of pH 4 to 10 (in 0.5 pH units intervals). The pH values were adjusted using 1 M HCl and 1 M KOH and stabilized by the addition of 5 mM phosphate buffer adjusted to the same pH values. Further physiological tests were performed with API 20NE and API ZYM test strips (bioMérieux) as described by the manufacturer's instruction, except for the incubation temperature that was set at 18 °C. For the inoculation of the API 20NE and API ZYM bacterial biomass was suspended in 0.2% (w/v) autoclaved NaCl to a McFarland standard value of 0.5 and 6.0, respectively. All panels were analysed after three days of incubation at 18 °C, except nitrate reduction and indol production (API 20NE) were analysed after 48 h. Further comparative phenotypic characterization of the strain was carried out using the 96 well physiological test panel according to Kämpfer *et al.* [36]. For inoculation of the panel, stains were suspended in 0.2% NaCl to a McFarland value of 0.5. Analysis of acid production, carbon substrate assimilation, and enzyme activity was performed after 3 days of growth at 25 °C.

The strain showed a coccoid-shaped morphology, single or in pairs, with a cell size of 1.5×3.0 µm. The strain grew well at 0.5 to 30 °C on BA, LB, MA, MH, MH-1, NA, TSA and TSA-1, K7, medium 65, PYE, but no growth on R2A, McC, TCBS, and DEV.

Good growth was obtained after two days of incubation between 4 and 37 °C (no growth at 50 °C or above), in the presence of 0–10.0% (w/v) NaCl but the best growth occurred with 1–5% NaCl. Growth was observed between pH 5.5 and 10.0.

Results of the test panel according to Kämpfer *et al.* [36] showed growth with the different carbon sources but acid production from several sugars and sugar-related compounds was not observed (Table 1). Physiological tests performed with the API test systems resulted in differences in comparison to the *Psychrobacter* type strains. The differentiating physiological characteristic of the strain compared to other *Psychrobacter* type strains are listed in Table 1. All physiological properties of the new strains are provided in the species description.

Biomass for fatty acid analysis was harvested after growth on TSA at 28 °C for 48 h. The analysis was performed as described by Kämpfer and Kroppenstedt [37]. Fatty acids were separated with a 5898A gas chromatograph (Hewlett Packard), respective peaks were automatically integrated and fatty acid names and percentages were determined with the Sherlock MIDI software v. 2.1 (TSBA v. 4.1). The fatty acid profile of the strain was consistent with the profiles described for species of genus *Psychrobacter* as shown in Table 2, with the predominant unsaturated fatty acid $C_{18:1}\omega9c$ and $C_{16:1}\omega7c$ shown as summed feature 3 (Table 2).

Based on genotypic, chemotaxonomic, and physiological data we propose a new species of the genus *Psychrobacter*, *Psychrobacter pygoscelis* with I-STPP5b^T (=CIP 111410^T=CCM 8799^T=LMG 30301^T) as type strain.

DESCRIPTION OF *PSYCHROBACTER PYGOSCELIS* SP. NOV.

Psychrobacter pygoscelis (py.go.sce'lis. N.L. gen. n. *pygoscelis* of the penguin genus *Pygoscelis*).

Gram-stain-negative, coccobacillus-shaped cell morphology with a cell size of approximately 1.5×3.0 µm often associated in chains of 2–6 cells. Colonies grown after 3 days on TSA-1 at 18 °C are circular, shiny and slightly white-beige pigmented with a smooth border. Non-motile. Aerobic. Catalase and cytrochrome oxidase positive. Good growth at 18 °C after three days of incubation on BA, LB, MA, MH, MH-1, NA, TSA and TSA-1, no growth on Malt, CASO, glycine arginine, DEV, McC, R2A, TCBS agar. Optimal growth between 4 to 30 °C, weak growth at 37 °C, no growth at 50 °C and above. Good growth in the presence of 1–10% (w/v) NaCl, at pH 5.5 to 6.5, slightly less growth can be observed at pH 7.0 and 7.5, but not at pH 4.0 or below or above pH 10.0.

Negative for indole production, arginine dihydrolase, β-galactosidase, acid production from L-arabinose and potassium gluconate. Positive for assimilation of dextrin. Negative for the assimilation of *N*-acetyl-glucosamine (API 20NE), capric acid, adipic acid, trisodium citrate, and phenylacetic acid, but positive for the assimilation of malic acid, urease, and reduction of nitrate to nitrite. Results for Simmons' citrate and glucose fermentation/oxidation were negative.

For the API ZYM galleries, enzymatic activity was observed for esterase (C4), lipase esterase (C8), leucine arylamidase, acid phosphatase, and naphtol-AS-BI-phosphydrolase activity, but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-D-glucosidase, α-mannosidase, and α-fucosidase (API ZYM). The fatty acid profile contains $C_{18:1}\omega9c$ and $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ as predominant fatty acids.

The draft genome obtained has a total size of 3.40 Mb with a DNA G+C content of 44.4%. The type strain I-STPP5b^T (=CIP 111410^T=CCM 8799^T=LMG 30301^T) was isolated from a Gentoo penguin chick in the Chilean Antarctic in January 2015. The 16S rRNA gene sequence and the whole genome sequence (WGS) project of strain STPP5b^T has been deposited at GenBank/EMBL/DDBJ under accession numbers MH065724 and RQRU00000000.

Funding information
RA-H acknowledges support received by the Grants FONDAP N° 15110027 and FONDECYT N° 1150695, awarded by the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT, Chile) and Grant RT_08_13 entitled 'Study of viral and bacterial diversity in seawater and Antarctic fish species: Finding of natural reservoir of salmonid pathogens' by the Instituto Antártico Chileno (INACH).

Acknowledgements
We thank Katja Grebing, Maria Sowinsky, and Gundula Will for excellent technical assistance and Professor Aharon Oren for his help with the etymology of the species name.

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

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

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