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Flavobacterium circumlabens sp. nov. and *Flavobacterium cupreum* sp. nov., two psychrotrophic species isolated from Antarctic environmental samples☆

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ABSTRACT

A taxonomic study of 24 Gram-stain-negative rod-shaped bacteria originating from the Antarctic environment is described. Phylogenetic analysis using 16S rRNA gene sequencing differentiated isolated strains into two groups belonging to the genus *Flavobacterium*. Group I (n = 20) was closest to *Flavobacterium aquidurense* WB 1.1-56^T (98.3% 16S rRNA gene sequence similarity) while group II (n = 4) showed *Flavobacterium hydatis* DSM 2063^T as its nearest neighbour (98.5–98.9% 16S rRNA gene sequence similarity). Despite high 16S rRNA gene sequence similarity, these two groups represented two distinct novel species as shown by phenotypic traits and low genomic relatedness assessed by rep-PCR fingerprinting, DNA-DNA hybridization and whole-genome sequencing. Common to representative strains of both groups were the presence of major menaquinone MK-6 and *sym*-homospermidine as the major polyamine. Common major fatty acids were $C_{15:0}$ iso, $C_{15:1}$ iso G, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3—OH and Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*). Strain CCM 8828^T (group I) contained phosphatidylethanolamine, three unidentified lipids lacking a functional group, three unidentified aminolipids and single unidentified glycolipid in the polar lipid profile. Strain CCM 8825^T (group II) contained phosphatidylethanolamine, eight unidentified lipids lacking a functional group, three unidentified aminolipids and two unidentified glycolipids in the polar lipid profile. These characteristics corresponded to characteristics of the genus *Flavobacterium*. The obtained results showed that the analysed strains represent novel species of the genus *Flavobacterium*, for which the names *Flavobacterium circumlabens* sp. nov. (type strain CCM 8828^T = P5626^T = LMG 30617^T) and *Flavobacterium cupreum* sp. nov. (type strain CCM 8825^T = P2683^T = LMG 30614^T) are proposed.

Introduction

The genus *Flavobacterium*, the type genus of the family *Flavobacteriaceae* in the phylum *Bacteroidetes,* comprises Gram-stain-negative, rod-shaped and aerobic bacteria. This genus was proposed by Bergey *et al.* [5] and recently emended by Kuo et al. [29]. Members of this genus are characterised by producing yellowish pigments, containing MK-6 as the only or predominant respiratory quinone, homospermidine as major polyamine and $C_{15:0}$, $C_{15:0}$ iso, $C_{15:0}$ iso 3-OH, $C_{15:0}$ anteiso, $C_{15:1}$ *ω6c*, $C_{15:1}$ iso G, $C_{16:0}$ iso 3—OH, $C_{17:0}$ iso 3—OH and Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*) as the major fatty acids [6]. Sphingophospholipids were not detected among any members of this genus so far [6]. *Flavobacterium* species were found in many various sources, including soil, different types of water (i.e. freshwater, marine water, saline water), in warm and more often in polar areas; in food and dairy products; and importantly, some species are well recognized fish pathogens [6,7]. Majority of members of the genus *Flavobac-*

☆ The GenBank/EMBL/DDBJ accession number for the complete 16S rRNA gene sequence of *Flavobacterium circumlabens* (CCM 8828^T) is MH100898 and of *Flavobacterium cupreum* (CCM 8825^T) is MH100899. The Whole Genome Shotgun projects for *Flavobacterium circumlabens* (CCM 8828^T) and *Flavobacterium cupreum* (CCM 8825^T) have been deposited at DDBJ/ENA/GenBank under the accession QWDN00000000 and QWDM00000000, respectively. The versions described in this paper are versions QWDN01000000 and QWDM01000000.

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terium is well known for their eco-physiological adaptations to cold and/or polar climate, what makes them an attractive object of studies focused on cold-adaptive enzymes or cold-adaptive mechanisms [24,28,35].

Material and methods

Isolation, preservation and culture conditions

The present study describes a taxonomic investigation of 24 *Flavobacterium* strains isolated in the frame of a project focused on cultivable bacteria inhabiting Antarctic environment. This project has been performed at various terrestrial and aqueous habitats located close to the Johann Gregor Mendel Station, James Ross Island (east of the Antarctic Peninsula), Antarctica. The area of concern is the largest ice-free area in the whole Antarctic Peninsula region and is geologically made of partly calcareous Cretaceous marine sediments protruded by basic Neogene volcanic rocks [34]. Large ice-free area allowed the formation of different lake types [36] and proglacial streams [23], which represent hotspots of biological diversity [27]. Vegetation cover is very scarce with concentration spots mostly along streams, or around seal carcasses [38]. The mean annual air temperature at sea level in the vicinity of J. G. Mendel Station was −7.0 °C in the period 2006–2015, the mean daily air temperatures in summer can reach up to +5 °C, while it can drop below −30 °C during winter [21]. Strains analysed in this study were isolated from environmental materials sampled in above described area during the years 2008–2014 (Table S1, Supplementary Material). Sampling was carried out by dispersing 1 g of stone and soil fragments in 5 ml of sterile saline solution, subsequently 100 μl of this suspension was spread by L-loop on the surface of R2A (Oxoid) agar plates and cultivated at 15 °C for up to 5–7 days. Analogously, water samples were spread on R2A agar plates (150 μl) and cultivated at 15 °C for up to 5 days. Individual pigmented colonies were randomly selected, purified by subculturing and pure cultures were maintained on R2A slant agars until transported and analysed. After transportation, subcultures were transferred to −70 °C for long-term storage. The type strains of the closest phylogenetic relatives *Flavobacterium aquidurense* CCM 8768^T, *Flavobacterium hydatis* CCM 8769^T, *Flavobacterium collinsii* CCM 8772^T and *F. saccharophilum* CCM 8770^T were obtained from the Czech Collection of Microorganisms (www.sci.muni.cz/ccm) and tested parallelly with the analysed strains for comparison purposes.

Morphology

Colony morphology was assessed on R2A agar (Oxoid). The presence of flexirubin-type pigments was investigated using a 20% (w/v) KOH solution and the presence of capsule was evaluated by the Congo red adsorption test [8]. The motility was tested using the hanging-drop technique as described previously [8]. Cellular morphology was described by light microscopy following Gram-staining and by transmission electron microscopy using a Morgagni 268D Philips (FEI Company) electron microscope (Fig. S1, S2; Supplementary Material).

Temperature, NaCl and pH tolerance

Temperature range and NaCl tolerance were determined based on growth on R2A agar plates and pH tolerance was resolved in R2A broth adjusted accordingly. Inoculated plates and tubes were incubated for 48 h at 20 °C. Growth was tested at different temperatures ranging from 1 to 40 °C in 5 °C increments. Salt tolerance was assessed at 0.5, 1–4% of NaCl and pH tolerance at pH 5–10 at intervals of 1.0 pH unit at 20 °C by using the following buffer systems: pH 5.0–8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3. The pH value of the R2A agar was confirmed after autoclaving.

Biochemical and physiological characteristics

All 24 analysed strains and the type strains of the nearest neighbouring species *F. aquidurense* CCM 8768^T, *F. hydatis* CCM 8769^T, *F. collinsii* CCM 8772^T and *F. saccharophilum* CCM 8770^T were characterised by a set of relevant tests according to minimal standards protocols [8]. These test included: activities of catalase [3] (ID colour Catalase; bioMérieux) and oxidase (OXItest; Erba-Lachema) determined according to the manufacturers' instructions; oxidation-fermentation test (OF) [22]; urease production [12], arginine dihydrolase, ornithine and lysine decarboxylase production [9]; hydrolysis of aesculin, starch [4], gelatin, Tween 80 [41], casein, tyrosine [30], cellulose (R2A broth with strip of Whatman paper No.1) [1], and DNA (CM321; Oxoid); egg-yolk reaction [40]; ONPG [32]; nitrate and nitrite reduction; indol production; growth on Simmon's citrate agar [4]; utilization of acetamide [39] and sodium malonate [17]. Aerobic growth was tested on R2A, Nutrient Agar (Oxoid), Tryptone Soya Agar (Oxoid), Mac-Conkey agar (HiMedia), Endo agar (HiMedia) and anaerobic growth was tested on R2A agar using the Anaerocult A system (Merck). Microaerophilic growth was performed on R2A agar in 5% CO_2 atmosphere. The aforementioned biochemical and physiological tests were inoculated using cells grown on R2A agar at 20 °C and read daily for up to 7 days, except for tyrosine hydrolysis test (read daily for up to 10 days). Additional biotyping using identification test kits GEN III MicroPlate^{TN} (Biolog) and API ZYM (bioMérieux) according to the manufacturer's instructions enabled a comprehensive biochemical characterization of isolates.

Susceptibility tests

Sensitivity of strains to antibiotics was tested by using the disc diffusion method after spreading of cell suspensions (McFarland 0.5) on Mueller Hinton agar plates. The following antibiotic discs (Antimicrobial Susceptibility Discs, Oxoid) were tested: penicillin G (10 μg), ampicillin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg) and O/129 (150 μg). CLSI standards (CLSI, The Clinical & Laboratory Standards Institute) were strictly followed for cultivation and inhibition zone diameter reading [14] with exception of penicillin [37]. Sensitivity to vibriostatic compound O/129 (2,4-diamino-6,7-di-iso-propylpteridine phosphate) was evaluated according to Whitman [50].

Phylogenetic analysis

Genomic DNAs for the initial 16S rRNA gene sequence analysis were extracted and purified using High Pure PCR Template Preparation Kit (Roche Diagnostics) according to the manufacturer's recommendations. Fragments of the 16S rRNA gene corresponding to coordinates 8–1542 used for *Escherichia coli* were amplified by PCR with FastStart PCR Master (Roche Diagnostics) and conserved primers pA (AGAGTTTGATCCTGGCTCAG) and pH (AAGGAGGTGATCCAGCCGCA) described by Edwards et al.[16] and subsequently purified using High Pure PCR Product Purification Kit (Roche Diagnostics). Sequencing was performed using the same primers in the Eurofins MWG Operon sequencing facility. Obtained 16S rRNA gene sequences were initially identified using the EzTaxon database [26].

Genomic DNA was extracted using a High Pure PCR Template Preparation Kit as described above. The concentration of extracted DNA was estimated with a Spark^{TM} multimode microplate reader (Tecan) using the PicoGreen dsDNA quantitation assay. Whole-

Table 1
Genomic characteristics of *Flavobacterium circumlabens* sp. nov. and *Flavobacterium cupreum* sp. nov.

WGS Project no.	Strains	
	F. circumlabens CCM 8828^T = P5626	*F. cupreum* CCM 8825^T = P2683
	QWDN00000000	QWDM00000000
Assembly method	A5-miseq pipeline ver. 20160825 (assembler IDBA-UD)	SPAdes 3.12.0
Genome size (Mb)	5.86	5.89
Mean Coverage (×)	89.878	100.617
N50	316678	341169
N75	251333	155292
L50	6	7
L75	11	14
Largest contig (bp)	766548	652774
No. of contigs > 200 bp	531	276
No. of contigs > 1000 bp	56	49
GC content (%)	35.84	36.9
Coding sequences	6664	5475
No. of subsystems	286	133
No. of RNAs	53	61
No. of tRNAs	46	49
No. of prophages	ND	ND
No. of plasmids	ND	ND

genome shotgun (WGS) sequencing was performed using an Illumina Nextseq sequencing platform. The purified genomic DNA was used for preparing a 500-bp sequencing library with NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (NEB). The samples were loaded onto a Nextseq platform and sequenced using a MID output cartridge in a 150 bp paired-end mode (Illumina). The de novo assembly computation and error correction were performed using A5-miseq pipeline ver. 20160825 (assembler IDBA-UD) and SPAdes 3.12.0 [15]. Genome assembly evaluation was performed by QUAST 5.0.1 [20] with general genome characteristics referenced in Table 1. The comparisons and statistics analyses of individual assembly were performed using QUAST 5.0.0 [33]. The complete 16S rRNA gene sequence extracted from WGS data using the RNAmmer 1.2. server [31]. Phylogenetic comparison of the obtained sequences with 16S rRNA gene sequences of validly named *Flavobacterium* species retrieved from the GenBank database, was performed using MEGA 7.0 software [46]. Genetic distances were corrected using Kimura's two-parameter model and the evolutionary history was inferred using the neighbour-joining (NJ) and maximum-likelihood (ML) methods. Sequence similarities between individual strains were calculated using Bionumerics 7.6 software (Applied Maths). To estimate the DNA G + C content, the draft genome sequence was used.

Rep-PCR fingerprinting

Repetitive sequence-based PCR (rep-PCR) fingerprinting using the $(GTG)_5$ primer was performed to assess the genetic variability between individual strains. Isolation of DNA, rep-PCR fingerprinting method and numerical analysis of the resulting fingerprints using the BioNumerics 7.6 software was performed as described by Švec et al.[45].

DNA–DNA hybridization

Total high-molar-mass genomic DNA extraction for DNA–DNA hybridization (DDH) experiments was done as described by Cleenwerck et al. [13]. DDH was performed using the microplate technique described by Ezaki et al. [18] according to the protocols described previously [13,19]. DNA–DNA relatedness percentages were calculated as means based on at least three independent hybridizations. Reciprocal reactions were performed and considered as independent experiments. The standard deviation between reciprocal reactions was approximately 7%, as reported by Goris et al. [19].

Chemotaxonomic analyses

For cellular fatty acid analysis, all analysed Antarctic strains were grown on R2A agar (Oxoid) at 20 ± 2 °C for 48 h until bacterial communities reached the late-exponential stage of growth according to the four quadrants streak method [43]. Reference strains of closely related *Flavobacterium* spp. were cultured parallelly for comparison purposes. Extraction and subsequent analysis of fatty acid methyl esters was performed using an Agilent 7890 B gas chromatograph according to the standard protocol of the Sherlock MIDI Identification System (MIDI Sherlock version 6.2, MIDI database RTSBA 6.21).

Freeze-dried biomass for further chemotaxonomic analyses was prepared from bacterial cells grown in R2A broth cultivated at 20 °C for 72 h. Quinones, polar lipids and polyamines were extracted from freeze-dried biomass and analysed as described previously [2,10,44,47,48]. HPLC conditions applied for analyses of the polyamine patterns were as described by Busse et al. [11] and the HPLC apparatus was as described by Stolz et al. [44].

Results and discussion

Analysis of the partial 16S rRNA gene sequences differentiated Antarctic strains into two groups within the genus *Flavobacterium*. Group I consisted of 20 strains sharing 98.9–100% 16S rRNA gene sequence similarity and group II consisted of four isolates sharing 99.9–100% 16S rRNA gene sequence similarity within individual clusters. Strain CCM 8828^T representing group I showed the highest sequence similarity (98.3%) with that of *F. aquidurense* WB 1.1-56^T and strain CCM 8825^T representing the group II revealed the highest sequence similarity (98.9%) with that of *F. hydatis* DSM 2063^T. Remaining strains belonging to group II showed 98.5% 16S rRNA gene similarity with *F. hydatis* DSM 2063^T. Except for CCM 8825^T, all 16S rRNA gene similarities were below 98.65% similarity threshold suggested by Kim et al. [25]; however, the genus *Flavobacterium* represents one of those genera where high 16S rRNA gene sequence similarities may be accompanied by low DNA–DNA hybridization values demonstrating low genomic relatedness and novelty of some species [42,51]. The complete 16S rRNA gene sequences extracted from WGS data showed similarities with that obtained by Sanger sequencing and therefore were used for fur-

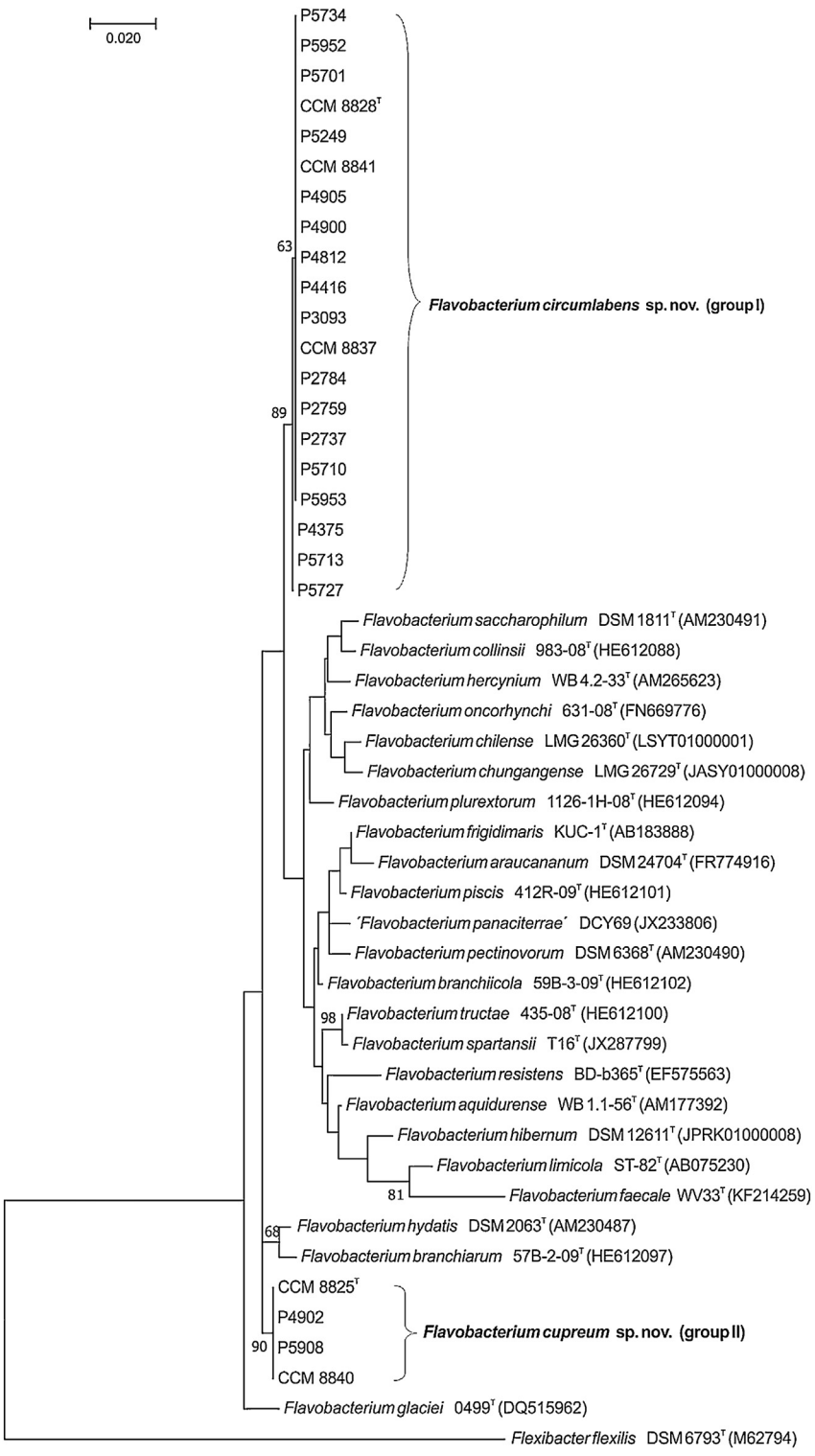

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences comparison showing the phylogenetic position of groups I and II among the closest relatives within the genus *Flavobacterium*. The evolutionary history was inferred by using the maximum likelihood method based on the Kimurás two-parameter model. All positions with less than 95% site coverage were eliminated. Bootstrap probability values (percentages of 1000 tree replications) greater than 50% are indicated at branch points. *Flexibacter flexilis* DSM 6793^T (M62794) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

ther phylogenetic comparison. In fact, ML (Fig. 1) and NJ clustering (Fig. S3, Supplementary Material) showed that both groups (I and II) formed separated clusters, that were differentiated from established *Flavobacterium* species suggesting they may represent novel species.

To precisely clarify taxonomic position of both groups, DDH experiments were undertaken in this study as well as whole-genome sequencing data comparison. The level of DDH obtained between strain CCM 8828^T (group I) and the closest phylogenetic neighbours, namely *F. aquidurense* CCM 8768^T and *F. hydatis*

Table 2
Phenotypic characteristics that differentiate *Flavobacterium circumlabens* sp. nov. and *Flavobacterium cupreum* sp. nov. from closely related *Flavobacterium* spp. Strains: (1) *Flavobacterium circumlabens* sp. nov. CCM 8828^T; (2) *Flavobacterium cupreum* sp. nov. CCM 8825^T; (3) *F. aquidurense* CCM 8768^T; (4) *F. hydatis* CCM 8769^T; (5) *F. collinsii* CCM 8772^T; (6) *F. saccharophilum* CCM 8770^T. All data were taken from this study.

Test	1[a]	2[a]	3	4	5	6
Growth in 1% NaCl	+	–	+	+	+	–
Growth in 2% NaCl	–	–	w	+	+	–
Hydrolysis of tyrosine	+	–	–	+	+	+
Hydrolysis of DNA	+	+	+	+	+	–
Nitrate reduction	–	–	–	+	–	–
Arginine dihydrolase	–	–	–	–	w	–
Urease	–	–	+	–	–	–
Simmons citrate	–	–	–	–	+	–
Ampicillin (10 μg)	R	S	S	R	R	R
Spreading motility	+	–	–	+	w	–

+, positive; w, weak positive; –, negative; S, sensitive; R, resistant.
[a] data are uniform for all isolates of described species.

CCM 8769^T was 23% and 12%, respectively. The hybridization values between strains CCM 8825^T (group II), *F. aquidurense* CCM 8768^T and *F. hydatis* CCM 8769^T were 23% and 13%, respectively. DNA–DNA similarity between strains CCM 8828^T (group I) and CCM 8825^T (group II) was 53%. All DDH values obtained were well below the 70% threshold [49], which confirmed that each of the analysed strains was a representative of novel species. Moreover, the calculated ANI values for pairwise genomic comparison between proposed novel species and type strains of their closest relatives were well below 95–96% threshold suggested by Kim *et al.* [25]. The calculated ANI values between *Flavobacterium circumlabens* sp.nov. (CCM 8828^T) and *F. aquidurense* CCM 8768^T, and between *Flavobacterium cupreum* sp.nov. (CCM 8825^T) and *F. hydatis* CCM 8769^T were 82.69% and 89.28%, respectively. Both novel species were also distinct from each other, confirmed by 80.50% ANI value. The genomic G + C %mol is 35.84 and 36.9 for CCM 8828^T and CCM 8825^T, respectively.

Rep-PCR fingerprinting also differentiated groups I and II from the closest relatives (Fig. S4, Supplementary Material). Numerical analysis revealed genetic heterogeneity among strains assigned to group I, where at least four separated subclusters and two detached branches were formed. Strains belonging to group II formed separated cluster except for strain P4902 revealing a distinct pattern.

Both groups consist of aerobic, Gram-stain-negative rods with rounded ends, occurring either singly, in pairs or irregular clusters. Members of group I also form filamentous cells (Fig. S1, S2, Supplementary Material). Members of both groups formed yellowish pigmented 1–2 mm colonies with opalescence on R2A agar. All strains were catalase positive while oxidase test yielded variable results. All members were able to grow under aerobic and microaerophilic conditions. Strains of group I exhibited strong gliding motility on R2A agar plates, however no motility was observed by the hanging-drop technique. Spectrum of biochemical tests that can be used for distinguishing members of both groups from closely related species and between each other is listed in Table 2. Due to high physiological heterogeneity among strains of both groups these tests are limited to ability to growth in 1% NaCl, tyrosine hydrolysis, susceptibility to ampicillin (10 μg) and exhibition of gliding motility on R2A agar. All phenotypical characteristics revealed by group I and II strains are described in the appropriate protologues given below.

The predominant fatty acids for both groups were $C_{15:0}$ iso, $C_{15:1}$ iso G, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3—OH and Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*), which corresponded to the fatty acids typically found in other *Flavobacterium* species [6] but they can be distinguished from the closest relatives by quantitative differences in fatty acids profiles, specifically in amounts of $C_{15:0}$ iso and $C_{15:1}$ iso G. Moreover, group I strains revealed higher presence of minor fatty acids, namely $C_{16:1}$ iso H, $C_{15:1}$ anteiso A and $C_{15:0}$ 2—OH (1.0–1.2%) compared to trace amounts of these fatty acids among members of group II. The complete cellular fatty acid composition of type and representative strains of both groups and type strains of the closest relatives is shown in Table 3. The complete cellular fatty acid composition of all strains belonging to groups I and II is shown in Table S2 (Supplementary Material).

Further chemotaxonomic analyses revealed the presence of the major menaquinone MK-6 in representatives of both groups and additionally, also minor amounts of MK-7 in strain CCM 8825^T (group II). In the polar lipid profile, common to both strains were the major polar lipids phosphatidylethanolamine, the unidentified lipids L3 and L7, unidentified aminolipid AL1 and moderate to minor amounts of unidentified lipid L1, two unidentified aminolipids AL2, AL3 and unidentified glycolipid GL1 (Fig. S5 and S6, Supplementary Material). Strain CCM 8825^T (group II) was distinguishable from CCM 8828^T (group I) by presence of unidentified lipids L4, L6 and L8–L10 and by presence of unidentified glycolipid GL2. Generally, strain CCM 8825^T (group II) showed more complex polar lipid profile than strain CCM 8828^T (group I). *sym*-Homospermidine was the major polyamine for both strains, which is in accordance with the family description [7]. Moreover, both strains contained also minor amounts of spermine, spermidine and traces of cadaverine and putrescine. The aforementioned chemotaxonomic characteristics of strains CCM 8828^T (group I) and CCM 8825^T (group II) corresponded with their assignment to the genus *Flavobacterium* [6].

Description of *F. circumlabens* sp. nov.

F. circumlabens (cir.cum.la'bens. L. pref. *circum*, around; L. pres. part. *labens*, tumbling; N.L. part. adj. *circumlabens*, tumbling around, referring to its gliding motility).

Cells are Gram-stain-negative, rounded-end rods, 2.0–4.0 × 0.3–0.4 μm, forming filamentous structures up to 10 μm. Cells are occurring predominantly singly, in pairs or in irregular clusters. Endospores are not formed. They reveal gliding motility on R2A agar plates, but no motility was observed by hanging-drop method. Presence of capsule negative. Produce flexirubin type of yellowish pigment. Growth occurs on R2A, TSA and Nutrient agar at 15 °C. No growth is observed on Marine agar and MacConkey agar, growth on Endo agar is strain-dependent. There is good growth on R2A agar in 5% CO_2, but no growth under anaerobic conditions. Growth temperatures ranges between 1 °C and 25 °C. Cells grow in pH range of 6.0–9.0. Good growth on R2A medium in presence of 0.5% NaCl (w/v) and weak in presence of 1% NaCl; the presence of 2% NaCl inhibits growth. Catalase-positive and oxidase-variable. Glucose is not fermented to acid in OF test medium. Alkaline phosphatase, acid phosphatase, leucine arylamidase, naphthol-AS-Bi-phosphohydrolase (weak) and α-glucosidase positive by API ZYM. Acid production from glucose, xylose and maltose positive. Gelatine, casein, esculin, ONPG, DNA, tyrosine and starch hydrolysis positive. Lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl- β-glucosaminidase, α-mannosidase and α-fucosidase negative by API ZYM. Nitrate and nitrite reduction, indole production, fluorescein (King B medium), urease, lysine and ornithine decarboxylase, arginine dihydrolase, Simmons citrate, acetamide and malonate utilization negative. Hydrolysis of Tween 80 and lecithin negative. Cellulose degradation negative. Variable phenotypic reactions of *F. circumlabens* strains are listed in Table S3 (Supplementary Material). Resistant to ampicillin, but sensitive to chloramphenicol and tetracycline. Sensitivity to O/129

Table 3
Cellular fatty acid composition (as a percentage of the total) of strain CCM 8828^T, CCM 8841, CCM 8837; CCM 8825^T, CCM 8840 and the closest relative species *F. aquidurense* CCM 8768^T, *F. hydatis* CCM 8769^T and *F. collinsii* CCM 8772^T and *F. saccharophilum* CCM 8770^T. All data were obtained in this study. Values of less than 1% are not shown.

Fatty acid	Group I			Group II		CCM 8768^T	CCM 8769^T	CCM 8772^T	CCM 8770^T
	CCM 8828^T	CCM 8841	CCM 8837	CCM 8825^T	CCM 8840				
$C_{15:0}$iso	17.6	18.6	18.5	22.0	22.0	13.5	16.6	24.9	11.8
Summed feature 3[a]	14.5	12.5	13.4	14.6	14.5	12.5	15.4	15.4	21.6
$C_{15:1}$ iso G	10.0	10.1	9.7	8.5	8.5	7.3	8.3	8.1	5.6
$C_{15:0}$ iso 3—OH	9.2	9.8	8.8	8.8	8.9	7.7	7.8	8.7	5.6
$C_{17:0}$ iso 3—OH	8.2	9.0	8.1	8.3	7.8	9.4	7.4	6.8	7.7
$C_{16:0}$ iso 3—OH	5.7	6.2	5.9	5.0	5.7	5.6	6.9	4.5	6.1
$C_{15:1}$ *ω6c*	4.7	5.0	6.1	5.2	5.9	10.1	7.9	5.1	5.5
$C_{15:0}$ anteiso	4.5	4.1	4.2	3.8	3.8	3.4	2.7	1.9	5.4
Summed feature 9[b]	4.2	4.4	3.9	3.1	3.1	4.8	4.3	3.4	5.4
$C_{17:1}$ *ω6c*	3.2	3.3	3.5	3.3	4.2	9.0	5.8	4.9	4.6
$C_{15:0}$ 3—OH	2.5	2.7	2.6	1.9	2.3	2.9	2.3	2.5	ND
$C_{16:0}$ 3—OH	2.4	2.1	2.3	3.4	3.6	2.2	2.6	4.3	2.6
$C_{16:0}$ iso	2.1	2.1	2.4	2.1	2.1	1.3	2.4	1.5	4.0
$C_{16:0}$	1.8	1.3	1.5	2.0	1.5	1.6	2.0	1.9	3.8
$C_{16:1}$ iso H	1.6	1.6	1.7	0.8	TR	1.0	1.6	TR	2.0
$C_{17:0}$ 2—OH	1.5	1.4	1.4	1.2	1.0	1.0	TR	TR	1.6
$C_{15:1}$ anteiso A	1.5	1.3	1.3	0.8	TR	TR	TR	TR	TR
$C_{15:0}$ 2—OH	1.1	1.1	1.0	0.8	TR	1.4	TR	TR	1.4
$C_{17:1}$ *ω8c*	TR	TR	TR	TR	TR	1.2	TR	TR	TR
$C_{14:0}$ iso	TR	TR	TR	TR	TR	TR	1.1	TR	1.0

[a] $C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*.
[b] $C_{16:0}$ 10-methyl/$C_{17:1}$ iso *ω9c*; ND, not detected; TR, trace amounts.

vibrostatic agent and penicillin G is strain-dependent (Table S3, Supplementary Material).

Carbon source utilization ability via respiration, determined in Biolog GEN III MicroPlate test panels, is positive for dextrin, *D*-maltose, *D*-trehalose, *D*-cellobiose, gentiobiose, *N*-acetyl-*D*-glucosamine, *N*-acetyl-*D*-galactosamine, α-*D*-glucose, *D*-mannose, *D*-galactose, gelatine, glycyl-*L*-proline, *L*-glutamic acid, *D*-galacturonic acid and *D*-galactonic acid lactone and negative for *D*-turanose, stachyose, *N*-acetyl- β-*D*-mannosamine, *N*-acetyl neuraminic acid, 3-methyl glucose, *D*-fucose, *L*-fucose, *L*-rhamnose, inosine, *D*-sorbitol, *D*-mannitol, *D*-arabitol, myo-inositol, glycerol, *D*-aspartic acid, *D*-serine, *L*-alanine, *L*-arginine, *L*-histidine, *L*-pyroglutamic acid, *L*-serine, *D*-gluconic acid, *D*-glucuronic acid, glucuronamide, mucic acid, quinic acid, *D*-saccharic acid, *p*-hydroxy phenylacetic acid, methyl pyruvate, *D*-lactic acid methyl ester, *L*-lactic acid, citric acid, α-keto glutaric acid, *D*-malic acid, *L*-malic acid, bromo-succinic acid, γ-amino-butyric acid, α-hydroxy-butyric acid, β-hydroxy-D, *L*-butyric acid, α-keto butyric acid, acetoacetic acid, propionic acid and formic acid. Variable results of *F. circumlabens* strains obtained on the Biolog GEN III Micro Plate are shown in Table S4 (Supplementary Material). The major cellular fatty acids are $C_{15:0}$ iso (18.9%), $C_{15:1}$ iso G (10.1%), $C_{15:0}$ iso 3—OH (9.1%), $C_{17:0}$ iso 3—OH (8.3%) and Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*) (13.2%). The polar lipid profile contains phosphatidylethanolamine, two unidentified lipids (L3, L7) lacking a functional group and unidentified aminolipid AL1 as the major polar lipids, moderate amounts of lipid L1, aminolipid AL2 and minor amounts of unidentified aminolipid AL3 and unidentified glycolipid GL1. The major polyamine is *sym*-homospermidine and spermine, spermidine and traces of cadaverine and putrescine were also present. The major respiratory quinone is MK-6. The DNA G + C content of the type strain is 35.84 mol%.

Type strain is CCM 8828^T (=P5626^T = LMG 30617^T) isolated from sediment recovered from upper reaches of temporary stream, Panorama Pass (GPS: 57°51′05″ W; 63°48′50″ S). All characteristics of the type strain CCM 8828^T are in agreement with the species description provided in this study. The strain-dependent test results are as follows: positive for oxidase (weak), growth at 30 °C (weak), and acid from fructose, esterase (C 4), esterase lipase (C 8), valine arylamidase, and β-glucosidase activity. Negative for acid production from mannitol. Resistant to ampicillin, but sensitive to O/129 vibrostatic agent. On the Biolog GEN III Micro Plate, the type strain CCM 8828^T is able to utilise *D*-salicine, *D*-fructose, Tween 40 and acetic acid, but unable to utilise sucrose, *D*-raffinose, α-*D*-lactose, *D*-melibiose, *D*-glucose-6-PO_4, *L*-aspartic acid and, pectin. The utilization of β-methyl-*D*-glucoside and *D*-fructose-6-PO_4 was borderline. The strains of *F. circumlabens* species were isolated from inorganic materials (stone fragments, soil, freshwater) in Antarctica. The formal proposal of the new species "*Flavobacterium circumlabens* sp. nov." is given in Table 4 with the Taxonumber TA00465.

Description of *F. cupreum* sp. nov.

F. cupreum (cu'pre.um. L. neut. adj. *cupreum*, copperish opalescent).

Cells are Gram-stain-negative, rounded-end rods, 2.0–6.0 × 0.4–0.6 μm occurring predominantly singly, in pairs or in irregular clusters. Endospores are not formed. Non-motile rods. Presence of capsule negative. Produce flexirubin type of yellowish pigment. Growth occurs on R2A, TSA and Nutrient agar at 15 °C. No growth is observed on Marine agar, MacConkey agar and Endo agar. There is good growth on R2A agar in 5% CO_2 but no growth on R2A agar under anaerobic conditions. Growth temperature ranges between 1 °C (weak) and 25 °C, but no growth at 35 °C. Cells only grow in pH range 6.0–9.0. Good growth on R2A medium in presence of 0.5% NaCl (w/v); the presence of 1% NaCl inhibits growth. Catalase and oxidase positive. Glucose is not fermented to acid in OF test medium. Alkaline phosphatase, acid phosphatase, esterase lipase (C 8) (weak), leucine arylamidase, valine arylamidase, α-glucosidase (weak) and β-glucosidase positive by API ZYM. Acid production from glucose, fructose, xylose and maltose positive. Gelatine, casein, esculin, ONPG, DNA and starch hydrolysis positive. Esterase (C 4), lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase negative by API ZYM. Nitrate and nitrite reduction, indole production, fluorescein (King B medium), urease, lysine and ornithine decarboxylase, arginine dihydrolase, Simmons citrate, acetamide and malonate utilization negative.

Table 4
Description of *Flavobacterium circumlabens* sp. nov. according to Digital Protologue TA00465 assigned by the www.imedea.uib.es/dprotologue website.

Taxonumber	TA00465
Species name	*Flavobacterium circumlabens*
Genus name	*Flavobacterium*
Specific epithet	*circumlabens*
Species status	sp. nov.
Species etymology	cir.cum.la'bens. L. pref. circum, around; L. pres. part. labens, tumbling; N.L. part. adj. *circumlabens*, tumbling around, referring to its swarming motility
Authors	Králová Stanislava, Busse Hans-Jürgen, Švec Pavel, Mašlaňová Ivana, Staňková Eva, Barták Miloš, Sedláček Ivo
Title	*Flavobacterium circumlabens* sp. nov. and *Flavobacterium cupreum* sp. nov., two psychrotrophic species isolated from Antarctic environmental samples
Corresponding author	Stanislava Králová
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Submitter	Stanislava Králová
E-mail of the submitter	stanis.kralova@gmail.com
Designation of the type strain	P5626
Strain collection numbers	CCM 8828 = LMG 30617
16S rRNA gene accession number	MH100898
Genome accession number [RefSeq]	QWDN00000000
Genome status	Draft
GC mol%	35.84
Country of origin	Antarctica
Region of origin	James Ross Island
Date of isolation	1/1/2014
Source of isolation	Sediment from upper reaches of temporary stream, Panorama Pass
Sampling date	1/1/2014
Geographic location	Panorama Pass
Latitude	63°48'50"N/S
Longitude	57°51'05"E/W
Number of strains in study	20
Source of isolation of non-type strains	Regolith, stone fragments, water, algae matter
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	R2A, 20 °C, pH = 7.2 (two days), aerobically
Is a defined medium available	R2A
Alternative medium 1	TSA
Alternative medium 2	NA
Conditions of preservation	Cryovials containing glass beads and R2A broth with 15% glycerol at −70 °C
Gram stain	Negative
Cell shape	Rod
Cell size (length or diameter)	2.0–4.0 × 0.3–0.4 μm; filamentous structures up to 10 μm
Motility	Motile
If motile	Unknown mechanism
Sporulation (resting cells)	None
Colony morphology	1–2 mm, circular, regular shape, strong swarming motility on solid media, yellowish pigment of flexirubin type, often opalescence — copperish, greenish
Temperature range	1–25 °C
Lowest temperature for growth	1 °C
Highest temperature for growth	25 °C
Temperature optimum	15–20 °C
Lowest pH for growth	6.0
Highest pH for growth	9.0
pH optimum	7.0
pH category	Neutrophile
Lowest NaCl concentration for growth	0.0%, R2A, 20 °C
Highest NaCl concentration for growth	1.0%, R2A, 20 °C
Salinity optimum	0–0.5%
Salinity category	Nonhalophile (NaCl inhibitory at <1% NaCl)
Relationship to O_2	Aerobe
O_2 conditions for strain testing	Aerobiosis
Carbon source used [class of compounds]	Sugars, amino acids
Carbon source used [specific compounds]	Glucose, maltose, xylose
Carbon source not used [specific compounds]	Acetamid, malonate, Simmons citrate
Carbon source variable [specific compounds]	Fructose, mannitol
Positive tests with BIOLOG	Dextrin, *D*-maltose, *D*-trehalose, *D*-cellobiose, gentiobiose, *N*-acetyl-*D*-glucosamine, *N*-acetyl-*D*-galactosamine, α-*D*-glucose, *D*-mannose, *D*-galactose, gelatine, glycyl-*L*-proline, *L*-glutamic acid, *D*-galacturonic acid, *D*-galactonic acid lactone
Negative tests with BIOLOG	*D*-turanose, stachyose, *N*-acetyl- β-*D*-mannosamine, *N*-acetyl neuraminic acid, 3-methyl glucose, *D*-fucose, *L*-fucose, *L*-rhamnose, inosine, *D*-sorbitol, *D*-mannitol, *D*-arabitol, myo-inositol, glycerol, *D*-aspartic acid, *D*-serine, *L*-alanine, *L*-arginine, *L*-histidine, *L*-pyroglutamic acid, *L*-serine, *D*-gluconic acid, *D*-glucuronic acid, glucuronamide, mucic acid, quinic acid, *D*-saccharic acid, *p*-hydroxyphenylacetic acid, methyl pyruvate, *D*-lactic acid methyl ester, *L*-lactic acid, citric acid, α-keto glutaric acid, *D*-malic acid, *L*-malic acid, bromo-succinic acid, γ-amino-butyric acid, α-hydroxy-butyric acid, β-hydroxy-D, *L*-butyric acid, α-keto butyric acid, acetoacetic acid, propionic acid, formic acid
Variable tests with BIOLOG	Sucrose, *D*-raffinose, α-*D*-lactose, *D*-melibiose, β-methyl-*D*-glucoside, *D*-salicin, *D*-fructose, *D*-glucose-6-PO4, *D*-fructose-6-PO4, *L*-aspartic acid, pectin, Tween 40, Acetic acid

Table 4 (*Continued*)

Positive tests with API	Alkaline phosphatase, acid phosphatase, leucine arylamidase, naphthol-AS-Biphosphohydrolase (weak), α-glucosidase
Negative tests with API	Lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl- β-glucosaminidase, α-mannosidase, α-fucosidase
Acid formation from carbohydrates (all positive)	Glucose, maltose, xylose
Acid formation from carbohydrates (variable)	Fructose, mannitol
Commercial kits used?	API ZYM, Biolog
Terminal electron acceptor	Oxygen
Energy metabolism	Chemoorganotroph
Oxidase	Variable
Catalase	Positive
Positive tests	Casein, DNA, esculin, gelatine, ONPG, starch, tyrosine
Negative tests	Nitrate reduction, nitrite reduction, indole production, fluorescein (King B medium), urease, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, Simmons citrate, acetamide, malonate utilization, Tween 80 hydrolysis, lecithin hydrolysis, cellulase degradation
Variable tests	Sensitivity to 0/129
Quinone type	MK-6
Major fatty acids	$C_{15:0}$ iso; $C_{15:1}$ iso G, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3-OH; Summed Feature 3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$)
Phospholipide pattern or diagnostic phospholipid	Phosphatidylethanolamine is the major polar lipid. Three unidentified lipids, three unidentified aminolipids.
Glycolipids	Single unidentified glycolipid
Polyamine pattern	The major polyamine is sym-homospermidin. Spermin, spermidin and traces of cadaverine, putrescine were also present.
Habitat	Terrestrial, aquatic
Biotic relationship	Free-living

Table 5
Description of *Flavobacterium cupreum* sp. nov. according to Digital Protologue TA00466 assigned by the www.imedea.uib.es/dprotologue website.

Taxonumber	TA00466
Species name	*Flavobacterium cupreum*
Genus name	*Flavobacterium*
Specific epithet	*cupreum*
Species status	sp. nov.
Species etymology	cu'pre.um. L. neut. adj. *cupreum*, copperish opalescent
Authors	Králová Stanislava, Busse Hans-Jürgen, Švec Pavel, Mašlaňová Ivana, Staňková Eva, Barták Miloš, Sedláček Ivo
Title	*Flavobacterium circumlabens* sp. nov. and *Flavobacterium cupreum* sp. nov., two psychrotrophic species isolated from Antarctic environmental samples
Corresponding author	Stanislava Králová
E-mail of the corresponding author	stanci@mail.muni.cz
Submitter	Stanislava Králová
E-mail of the submitter	stanis.kralova@gmail.com
Designation of the type strain	P2683
Strain collection numbers	CCM 8825 = LMG 30614
16S rRNA gene accession number	MH100899
Genome accession number [RefSeq]	QWDM00000000
Genome status	draft
GC mol%	36.9
Country of origin	Antarctica
Region of origin	James Ross Island
Date of isolation	1/1/2008
Source of isolation	Regolith (nearby skua nest), hillside of Bibby Point
Sampling date	1/1/2008
Geographic location	Hillside of Bibby Point
Latitude	63°48'25"N/S
Longitude	57°55'30"E/W
Number of strains in study	4
Source of isolation of non-type strains	Freshwater
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	R2A, 20 °C, pH = 7.2 (two days), aerobically
Is a defined medium available	R2A
Alternative medium 1	TSA
Alternative medium 2	NA
Conditions of preservation	Cryovials containing glass beads and R2A broth with 15% glycerol at −70 °C
Gram stain	Negative
Cell shape	Rod
Cell size (length or diameter)	2.0–6.0 × 0.4–0.6 μm
Motility	Nonmotile
Sporulation (resting cells)	None
Colony morphology	1–2 mm colonies, circular, regular, flexirubine yellowish pigmentation with copperish opalescence
Temperature range	1–25 °C
Lowest temperature for growth	1 °C
Highest temperature for growth	25 °C
Temperature optimum	15–20 °C
Lowest pH for growth	6.0

Table 5 (*Continued*)

Highest pH for growth	9.0
pH optimum	7.0
pH category	Neutrophile
Lowest NaCl concentration for growth	0.0%, R2A, 20° C
Highest NaCl concentration for growth	0.5%, R2A, 20 °C
Salinity optimum	0–0.5%
Salinity category	Nonhalophile (NaCl inhibitory at <1% NaCl)
Relationship to O_2	Aerobe
O_2 conditions for strain testing	Aerobiosis
Carbon source used [class of compounds]	Sugars, amino acids
Carbon source used [specific compounds]	Fructose, glucose, maltose
Carbon source not used [specific compounds]	Acetamid, malonate, Simmons citrate
Carbon source variable [specific compounds]	Fructose, mannitol
Positive tests with BIOLOG	Dextrin, *D*-maltose, *D*-trehalose, gentiobiose, sucrose (weak), *D*-raffinose, α-*D*-glucose, *D*-mannose, *D*-fructose, *D*-galactose, *D*-galacturonic acid
Negative tests with BIOLOG	*D*-turanose, stachyose, α-*D*-lactose, *D*-melibiose, *N*-acetyl-β-*D*-mannosamine, *N*-acetyl neuraminic acid, 3-methyl glucose, *D*-fucose, *L*-fucose, *L*-rhamnose, inosine, *D*-sorbitol, *D*-mannitol, *D*-arabitol, myo-inositol, glycerol, *D*-glucose-6-PO4, *D*-aspartic acid, *D*-serine, *L*-alanine, *L*-arginine, *L*-histidine, *L*-pyroglutamic acid, *D*-gluconic acid, *D*-glucuronic acid, glucuronamide, mucic acid, quinic acid, *D*-saccharic acid, *p*-hydroxy phenylacetic acid, methyl pyruvate, *D*-lactic acid methyl ester, *L*-lactic acid, citric acid, α-keto glutaric acid, *D*-malic acid, *L*-malic acid, bromo-succinic acid, γ-amino-butyric acid, α-hydroxy-butyric acid, β-hydroxy-D, *L*-butyric acid, α-keto butyric acid, acetoacetic acid, propionic acid, formic acid
Variable tests with BIOLOG	*D*-cellobiose, β-methyl-*D*-glucoside, *D*-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-*D*-galactosamine, D-fructose-6-PO4, Gelatine, Glycyl-*L*-proline, *L*-aspartic acid, *L*-glutamic acid, *L*-serine, Pectin *D*-galactonic acid lactone, Tween 40, Acetic acid
Positive tests with API	Alkaline phosphatase, acid phosphatase, esterase lipase (C 8) (weak), leucine arylamidase, valine arylamidase, α-glucosidase (weak), β-glucosidase
Negative tests with API	Esterase (C 4), lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase
Acid formation from carbohydrates (all positive)	Glucose, fructose, xylose, maltose
Acid formation from carbohydrates (variable)	Mannitol
Commercial kits used?	API ZYM, Biolog
Terminal electron acceptor	Oxygen
Energy metabolism	Chemoorganotroph
Oxidase	Positive
Catalase	Positive
Positive tests	Casein, DNA, esculin, gelatine, ONPG, starch
Negative tests	Nitrate reduction, nitrite reduction, indole production, fluorescein (King B medium), urease, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, Simmons citrate, acetamide, malonate utilization, tyrosine hydrolysis, Tween 80 hydrolysis, lecithin hydrolysis, cellulose degradation
Variable tests	Sensitivity to 0/129
Quinone type	MK-6
Major fatty acids	$C_{15:0}$ iso; $C_{15:1}$ iso G, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3-OH; Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*)
Phospholipide pattern or diagnostic phospholipid	Phosphatidylethanolamine is the major polar lipid. Eight unidentified lipids, three unidentified aminolipids.
Glycolipids	Two unidentified glycolipid
Polyamine pattern	The major polyamine is sym-homospermidin. Spermin, spermidin and traces of cadaverine, putrescine were also present.
Habitat	Terrestrial, aquatic
Biotic relationship	Free-living

Hydrolysis of tyrosine, Tween 80 and lecithin negative. Cellulose degradation negative. Variable phenotypic reactions of *F. cupreum* strains are listed in Table S5. Sensitive to ampicillin, penicillin, chloramphenicol and tetracycline. Sensitivity or resistance to O/129 was strain-dependent (Table S5).

Carbon source utilization ability via respiration, determined in Biolog GEN III MicroPlate test panels is positive for dextrin, *D*-maltose, *D*-trehalose, gentiobiose, sucrose (weak), *D*-raffinose, α-*D*-glucose, *D*-mannose, *D*-fructose, *D*-galactose and *D*-galacturonic acid as carbon sources and negative for utilization of *D*-turanose, stachyose, α-*D*-lactose, *D*-melibiose, *N*-acetyl-β-*D*-mannosamine, *N*-acetyl neuraminic acid, 3-methyl glucose, *D*-fucose, *L*-fucose, *L*-rhamnose, inosine, *D*-sorbitol, *D*-mannitol, *D*-arabitol, myo-inositol, glycerol, *D*-glucose-6-PO_4, *D*-aspartic acid, *D*-serine, *L*-alanine, *L*-arginine, *L*-histidine, *L*-pyroglutamic acid, *D*-gluconic acid, *D*-glucuronic acid, glucuronamide, mucic acid, quinic acid, *D*-saccharic acid, *p*-hydroxy phenylacetic acid, methyl pyruvate, *D*-lactic acid methyl ester, *L*-lactic acid, citric acid, α-keto glutaric acid, *D*-malic acid, *L*-malic acid, bromo-succinic acid, γ-amino-butyric acid, α-hydroxy-butyric acid, β-hydroxy-*D*, *L*-butyric acid, α-keto butyric acid, acetoacetic acid, propionic acid and formic acid. Variable results of *F. cupreum* strains obtained on the Biolog GEN III Micro Plate are shown in Table S6 (Supplementary Material). The major cellular fatty acids are $C_{15:0}$ iso (22.1%), $C_{15:1}$ iso G (9.1%), $C_{15:0}$ iso 3—OH (9.1%), $C_{17:0}$ iso 3—OH (7.9%) and Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*) (14.4%). The polar lipid profile contains major amounts of phosphatidylethanolamine, two unidentified lipids (L3, L7) lacking a functional group and unidentified aminolipid AL1 as the major polar lipids, moderate amounts of lipid L1, aminolipid AL2 and minor amounts of unidentified aminolipid AL3 and unidentified glycolipid GL1 glycolipids (GL1–GL2). The major polyamine is *sym*-homospermidine and spermine, spermidine and traces of cadaverine and putrescine were also present. The major respiratory quinone is MK-6. The DNA G + C content of the type strain is 36.9 mol%.

Type strain is CCM 8825^T (=P2683^T = LMG 30614^T) isolated from regolith (nearby skua nest), hillside of Bibby Point (GPS: 57°55′30″ W 63°48′25″ S). All characteristics of the type strain CCM 8825^T are in agreement with the species description provided in this study. The strain-dependent test results are as follows: positive for growth at 30 °C and acid production from mannitol. Negative for naphthol-AS-Bi-phosphohydrolase. Resistant to

O/129 discs. On the Biolog GEN III Micro Plate, the type strain CCM 8825^T is able to utilise *D*-cellobiose, *D*-raffinose (weak), β-methyl-*D*-glucoside (weak), *D*-salicine, *N*-acetyl-*D*-glucosamine, *N*-acetyl-*D*-galactosamine, gelatine, glycyl-*L*-proline, *L*-aspartic acid, *L*-glutamic acid, *L*-serine, pectin, *D*-galactonic acid lactone (weak), Tween 40 and acetic acid, but unable to utilise D-fructose-6-PO_4. The strains of *F. cupreum* species were isolated from stone fragment and waters in Antarctica. The formal proposal of the new species "*Flavobacterium cupreum* sp. nov." is given in Table 5 with the Taxonumber TA00466.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2018.12.005.

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