Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*

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Several orange- and yellow-pigmented, halophilic, strictly aerobic, chemoheterotrophic, Gram-negative strains were isolated during investigations of maritime Antarctic habitats, including coastal fast sea-ice brine and algae, crustaceans and quartz stone sublithic cyanobacterial biofilms. Isolates investigated in this study belonged to the marine clade of the family *Flavobacteriaceae* and represented lineages that were either distinct from species with validly published names or appeared to be distinct species within existing genera. A polyphasic taxonomic analysis demonstrated the novelty of these strains, and several new taxa are proposed. Strains from quartz stone sublithic communities were grouped into two new genera designated *Subsaximicrobium* gen. nov. and *Subsaxibacter* gen. nov. The genus *Subsaximicrobium* included the species *Subsaximicrobium wynnwilliamsii* sp. nov. (type species; type strain G#7^T = ACAM 1070^T = CIP 108525^T) and *Subsaximicrobium saxinquilinus* sp. nov. (type strain Y4-5^T = ACAM 1063^T = CIP 108526^T). The genus *Subsaxibacter* contained a single species designated *Subsaxibacter broadyi* sp. nov. (type strain P7^T = ACAM 1064^T = CIP 108527^T). A novel bacterial strain isolated from the lake-dwelling, calanoid copepod *Paralabidocera antarctica* was given the name *Lacinutrix copepodicola* gen. nov., sp. nov. (type strain DJ3^T = ACAM 1055^T = CIP 108538^T). Four novel species of the genus *Bizionia* were discovered, *Bizionia aloritergicola* sp. nov. (type strain APA-1^T = ACAM 1056^T = CIP 108533^T) and *Bizionia myxarmorum* sp. nov. (type strain ADA-4^T = ACAM 1058^T = CIP 108535^T), which were isolated from the carapace surfaces of sea-ice algae-feeding amphipods, and *Bizionia gelidisalsuginis* sp. nov. (type strain IC164^T = ACAM 1057^T = CIP 108536^T) and *Bizionia saleffrena* sp. nov. (type strain HFD^T = ACAM 1059^T = CIP 108534^T), which were isolated from sea-ice brines. Several other novel species were also isolated from sea-ice samples, including two novel species of the genus *Gelidibacter*, *Gelidibacter gilvus* sp. nov. (type strain IC158^T = ACAM 1054^T = CIP 108531^T) and *Gelidibacter salicanalis* sp. nov. (type strain IC162^T = ACAM 1053^T = CIP 108532^T), as well as three novel species of the genus *Gillisia*, *Gillisia illustrilutea* sp. nov. (type strain IC157^T = ACAM 1062^T = CIP 108530^T), *Gillisia sandarakina* sp. nov. (type strain IC148^T = ACAM 1060^T = CIP 108529^T) and *Gillisia hiemivivida* sp. nov. (type strain IC154^T = ACAM 1061^T = CIP 108528^T).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains G#7^T, Y4-5^T, P7^T, 06-2, DJ3^T, ADA-4^T, APA-1^T, IC164^T, HFD^T, IC154^T, IC148^T, IC157^T and IC162^T are AY693997–AY694009, respectively.

A figure showing the cellular morphologies of *Subsaximicrobium wynnwilliamsii*, *Subsaxibacter broadyi* and *Lacinutrix copepodicola* is available as supplementary material in IJSEM Online.

INTRODUCTION

The family *Flavobacteriaceae* (Bernardet *et al.*, 1996, 2002) is one of the major branches of the *Cytophaga-Flavobacterium-Bacteroides* phylum, which was renamed (so far unofficially) '*Bacteroidetes*' in volume 1 of the second edition of *Bergey's Manual of Systematic Bacteriology* (Garrrity & Holt, 2001). The family *Flavobacteriaceae* includes many marine species that cluster together in 16S rRNA gene sequence phylogenetic trees as a well-defined 'marine clade' (Bowman, 2004). *Flavobacterium*, the type genus of the family, is positioned at the periphery of this marine clade. This genus includes species that are more adapted to freshwater and brackish ecosystems. Genera belonging to the family *Flavobacteriaceae* positioned beyond the genus *Flavobacterium* are generally adapted to non-marine ecosystems, including soil, water and animal habitats. Functionally, the members of this family are chemo-organotrophs and seem to play important roles in natural carbon cycles (Kirchman, 2002).

The marine clade of the family *Flavobacteriaceae* is particularly important in marine and marine-derived surface waters, where its members contribute strongly to the mineralization of primary-produced organic matter. They are major inhabitants of marine aggregates, conglomerations of organic detritus, which sink in the pelagic water column and which represent active biological zones in the oceans (Kirchman, 2002). Members of the marine clade also concentrate around or inhabit the surfaces of marine biota, where they seem to act primarily as commensals. Some are opportunistic pathogens, such as some *Tenacibaculum* species (Suzuki *et al.*, 2001), or can potentially exacerbate existing disease (Bowman & Nowak, 2004). From molecular surveys, members of this family also make up a large community component in surface benthic sediments (Bowman & McCuaig, 2003), and are very abundant in sea ice (Brinkmeyer *et al.*, 2003). In the cold, comparatively more nutrient-replete, surface waters of the Southern Ocean south of the Polar Front (located at about latitudes 55–60° S), many so far uncultured members of the family *Flavobacteriaceae* proliferate. Molecular analyses suggest that the abundance and diversity of this group markedly increases in Antarctic Zone waters, compared with the warmer, highly nutrient-limited waters north of the Polar Front (Abell & Bowman, 2005).

In Antarctic marine and coastal zones, members of the marine clade of the family *Flavobacteriaceae* have been isolated and described recently, from a wide range of ecosystems, including sea ice, quartz stone subliths, marine sediment, lake mats and sea water (Bowman, 2000; Bowman *et al.*, 1997a, 1998, 2003; Bowman & Nichols, 2002; Gosink *et al.*, 1998; Van Trappen *et al.*, 2002, 2004). At this stage, molecular surveys provide a good appraisal of the diversity present in these habitats, but well-characterized isolates still only represent a small proportion of this diversity. However, because they can be readily cultured, many new genera of the family *Flavobacteriaceae* from non-polar

marine ecosystems have been described recently (Bruns *et al.*, 2001; Cho & Giovannoni, 2003, 2004; Ivanova *et al.*, 2001, 2004; Nedashkovskaya *et al.*, 2003a, b, 2004a, b, c, 2005a, b, c; Sohn *et al.*, 2004). This expansion of knowledge of cultured members of the family *Flavobacteriaceae* continues in this study, with the description of several novel taxa from various Antarctic maritime samples, including sea ice, crustaceans and quartz stone cyanobacterial communities.

METHODS

Isolates obtained from sea-ice and quartz stone samples and the methods used for isolation have been described previously (Bowman & Nichols, 2002; Bowman *et al.*, 2003). Sea-ice brine drained from sea-ice cores was collected in glass bottles and then plated onto either marine agar (MA) or MA containing 10% NaCl (w/v), and incubated at 4 or 12 °C. Melted sea-ice samples were also filtered through 0.8 µm polycarbonate filters, and the particulate material caught on the filter, mostly algal biomass, was diluted in sterile brine filtrate and plated onto MA and MA + 10% NaCl, and incubated at 4 or 12 °C. MA consisted of 0.5% (w/v) peptone, 0.2% (w/v) yeast extract and 1.5% (w/v) agar, in 1000 ml artificial sea water. The artificial sea water comprised 35 g l⁻¹ of a synthetic sea salts preparation (Aquasonic, Wauchope, NSW, Australia). Strains were also obtained from the surfaces of various crustaceans from various areas of the Vestfold Hills, East Antarctica, collected by using traps and plankton nets. Isolations were made from the calanoid copepod *Paralabidocera antarctica*, collected from Ace Lake, and from sea-ice algae-feeding amphipods collected at the sea ice-sea water interface in Long Fjord. The crustacean samples were rinsed gently several times in small quantities of sterile sea water before being used for isolation. Copepod samples were suspended for 24 h at 4 °C in a dilute marine medium consisting of 0.025% (w/v) peptone and 0.005% (w/v) yeast extract in sea water, before being plated. Material from the surface of the carapaces of the amphipod samples, which was 2–5 mm in length, was plated directly onto MA by gently using forceps that had been flame-sterilized with ethanol. Isolates and reference strains used in this study (Table 1) were maintained and grown routinely on MA at 12 °C, and stored at –70 °C in marine broth containing 30% (v/v) glycerol.

The phenotypic-based methods used have been described previously (Bowman *et al.*, 1997a, 1998, 2003). MA was used as the basal medium, unless specified otherwise. Additionally, tests were performed using API 20E, API 20NE, API AUX and API 32ID AN test strips (bioMérieux–Vitek). For these tests, the inoculating or suspension medium contained 35 g synthetic sea salts l⁻¹. The API 32ID AN test strips were incubated for 24 h at 20 °C, whereas the other test strips were incubated for up to 7 days at 12 °C. Gliding was confirmed by growing cultures for 1–4 days at 12 °C on a dilute marine medium (described above), solidified with 1% (w/v) agar noble, on clean microscope glass slides and viewed by using phase-contrast microscopy (Bowman, 2000).

High molecular mass DNA for DNA G+C content and DNA–DNA hybridization was extracted by using the Marmur technique (Marmur & Doty, 1962). DNA G+C content was determined by using the thermal denaturation procedure involving spectrophotometry (Sly *et al.*, 1986); DNA–DNA hybridization was performed as described previously, by using the spectrophotometric renaturation kinetics approach (Bowman *et al.*, 1998), adapted from Huß *et al.* (1983).

Strains were cultivated on MA at 15 or 20 °C for 3–5 days until good growth was achieved, and harvested for whole-cell fatty acid

Table 1. Strains investigated in this study

Abbreviations: ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia; LMG, BCCM/LMG bacteria collection, Laboratorium voor Mikrobiologie, University of Ghent, Ghent, Belgium; CIP, Collection de l'Institut Pasteur, Institut Pasteur, Paris, France; KMM, Collection of Marine Microorganisms, Vladivostok, Russia; ATCC, American Type Culture Collection, Manassas, VA, USA; DSM, German Collection of Microorganisms, Braunschweig, Germany.

Strain	Species name	Isolation site
G#7 ^T (=ACAM 1070 ^T =CIP 108525 ^T)	<i>Subsaximicrobium wynnwilliamsii</i>	Quartz stone sublithic biofilm
Y5-5 (=ACAM 1073)	<i>Subsaximicrobium wynnwilliamsii</i>	Quartz stone sublithic biofilm
O1-5 (=ACAM 1074)	<i>Subsaximicrobium wynnwilliamsii</i>	Quartz stone sublithic biofilm
O3-5 (=ACAM 1075)	<i>Subsaximicrobium wynnwilliamsii</i>	Quartz stone sublithic biofilm
O5-2 (=ACAM 1076)	<i>Subsaximicrobium wynnwilliamsii</i>	Quartz stone sublithic biofilm
G#4 (=ACAM 1077)	<i>Subsaximicrobium wynnwilliamsii</i>	Quartz stone sublithic biofilm
Y4-5 ^T (=ACAM 1063 ^T =CIP 108526 ^T)	<i>Subsaximicrobium saxinquilinus</i>	Quartz stone sublithic biofilm
P7 ^T (=ACAM 1064 ^T =CIP 108527 ^T)	<i>Subsaxibacter broadyi</i>	Quartz stone sublithic biofilm
O6-2 (=ACAM 1073)	<i>Subsaxibacter broadyi</i>	Quartz stone sublithic biofilm
DJ3 ^T (=ACAM 1055 ^T =CIP 108538 ^T)	<i>Lacinutrix copepodicola</i>	Copepod, coastal saline pond
APA-1 ^T (=ACAM 1056 ^T =CIP 108533 ^T)	<i>Bizionia aloritergicola</i>	Copepod, coastal saline pond
APA-3	<i>Bizionia aloritergicola</i>	Copepod, coastal saline pond
ADA-4 ^T (=ACAM 1058 ^T =CIP 108535 ^T)	<i>Bizionia myxarmorum</i>	Copepod, coastal saline pond
HFD ^T (=ACAM 1059 ^T =CIP 108534 ^T)	<i>Bizionia saleffrena</i>	Coastal fast sea-ice brine
IC134 (=ACAM 1072)	<i>Bizionia saleffrena</i>	Coastal fast sea-ice brine
IC164 ^T (=ACAM 1057 ^T =CIP 108536 ^T)	<i>Bizionia gelidisalsuginis</i>	Coastal fast sea-ice brine
IC158 ^T (=ACAM 1054 ^T =CIP 108531 ^T)	<i>Gelidibacter gilvus</i>	Sea-ice algal assemblage
IC159	<i>Gelidibacter gilvus</i>	Sea-ice algal assemblage
IC163 (=ACAM 1071)	<i>Gelidibacter gilvus</i>	Sea-ice algal assemblage
IC162 ^T (=ACAM 1053 ^T =CIP 108532 ^T)	<i>Gelidibacter salicanalis</i>	Sea-ice algae from brine
IC157 ^T (=ACAM 1062 ^T =CIP 108530 ^T)	<i>Gillisia illustrilutea</i>	Sea-ice algal assemblage
IC144	<i>Gillisia illustrilutea</i>	Sea-ice algal assemblage
IC146	<i>Gillisia illustrilutea</i>	Sea-ice algal assemblage
IC148 ^T (=ACAM 1060 ^T =CIP 108529 ^T)	<i>Gillisia sandarakina</i>	Sea-ice algal assemblage
IC154 ^T (=ACAM 1061 ^T =CIP 108528 ^T)	<i>Gillisia hiemivivida</i>	Sea-ice algal assemblage
Reference strains		
ACAM 536 ^T (=ATCC 700364 ^T)	<i>Gelidibacter algens</i>	Sea-ice algal assemblage
ACAM 550	<i>Gelidibacter algens</i>	Sea-ice algal assemblage
ACAM 551	<i>Gelidibacter algens</i>	Sea-ice algal assemblage
Y1-5	<i>Gelidibacter algens</i>	Quartz stone sublithic biofilm
N1-5	<i>Gelidibacter algens</i>	Quartz stone sublithic biofilm
2SM29 ^T (=DSM 14095 ^T)	<i>Gelidibacter mesophilus</i>	Sea water, Mediterranean Sea
LMG 21470 ^T (=ACAM 1078 ^T)	<i>Gillisia limnaea</i>	Cyanobacterial mat, Lake Fryxell, Antarctica
CIP 107684 ^T (=KMM 3553 ^T)	<i>Formosa algae</i>	Seaweed
ACAM 188 ^T (=CIP 105822 ^T =ATCC 700359 ^T)	<i>Psychroserpens burtonensis</i>	Marine salinity lake
ACAM 74 ^T (=ATCC 23178 ^T =CIP 103822 ^T = LMG 1344 ^T)	<i>Cellulophaga lytica</i>	

extraction. Procedures used for fatty acid analysis were those described previously (Bowman *et al.*, 1997a, 2003). Isoprenoid quinones from lyophilized cell extracts were analysed by using reversed-phase liquid chromatography (Moss & Guerrant, 1983). *Cellulophaga lytica* ACAM 74^T was included as a control for menaquinone-6 (MK-6) content.

Genomic DNA was obtained from small amounts of growth by using a Ultraclean PCR purification kit (Mo-Bio). The 16S rRNA genes from the DNA samples were amplified by PCR as described previously (Bowman *et al.*, 1997b, 2003). Sequences obtained were 1366–1442 nucleotides in length and were aligned manually with sequences obtained from GenBank following N-BLAST searches (<http://www.ncbi.nlm.nih.gov>). The sequence data set was then analysed using the PHYLIP

program package, as described previously (Bowman *et al.*, 2003). The phylogenetic tree (Fig. 1) included the 16S rRNA genes of *Rhodothermus marinus* and *Chlorobium limicola* as outgroup sequences. The 16S rRNA gene sequence of *Gelidibacter gilvus* IC158^T was determined during a previous study (Bowman *et al.*, 1997b).

RESULTS AND DISCUSSION

General characteristics of the isolates

All of the Antarctic isolates investigated had either orange or yellow pigments, were Gram-negative, appeared as

Fig. 1. 16S rRNA gene sequence phylogenetic tree of the family Flavobacteriaceae showing the positions of the new taxa (in bold) described in this study. The tree is based on maximum-likelihood distances and neighbour-joining. Numbers in parentheses indicate the GenBank/EMBL/DDJB accession number. Bootstrap values (from 1000 replicates) are indicated at the branch nodes (●, >90%; ○, 60–90%). Bar, evolutionary distance of 0.05.

coccobacilli or rods, and were strictly aerobic and chemoheterotrophic. Flexirubin pigments were not detected. All strains studied were cold-adapted, with growth occurring at −2 to 20 °C on MA, but not on MA at 30 °C or higher. In MA broth, optimal growth temperatures ranged from 15 to 25 °C; optimal growth yields occurred at 10–15 °C. None of

the strains studied grew at 35 °C or higher in MA broth. The strains were generally typical marine-type halophiles, and all grew well in media containing half- to full-strength seawater-concentration synthetic sea salts (full strength is 35 g l^{−1}), and were tolerant to 1 and 1.25 M NaCl. Common and differential phenotypic properties of the strains

Table 2. Characteristics of the Antarctic isolates belonging to the genera *Subsaximicrobium*, *Subsaxibacter*, *Lacinutrix* and *Gillisia*, compared with the most closely related recognized species

Species: 1, *Subsaximicrobium wynnwilliamsii* (six strains analysed); 2, *Subsaximicrobium saxinquilinus* Y4-5^T; 3, *Subsaxibacter broadyi* (two strains analysed); 4, *Gelidibacter algens* (five strains analysed; data from this study and Bowman *et al.*, 1997a); 5, *Gelidibacter mesophilus* DSM 14095^T (this study and Macián *et al.*, 2002); 6, *Gelidibacter gilvus* (three strains analysed); 7, *Gelidibacter salicanalis* IC162^T; 8, *L. copepodicola* DJ3^T; 9, *Gillisia limnaea* (Van Trappen *et al.*, 2004); 10, *Gillisia mitskevichiae* (one strain; Nedashkovskaya *et al.*, 2005b); 11, *Gillisia illustrilutea* (three strains); 12, *Gillisia sandarakina* IC148^T; 13, *Gillisia hiemivivida* IC154^T. All strains (data for *Gillisia mitskevichiae* not included) formed catalase and were positive for arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, serine arylamidase, alanine arylamidase and glutamyl glutamate arylamidase. No growth occurred by fermentation of D-glucose either with or without sodium nitrate, by anaerobic iron reduction with ferric oxide as the electron acceptor and sodium lactate as the electron donor, or under an anaerobic atmosphere (95 % N₂/5 % CO₂), on either MA containing 0.5 % (w/v) D-glucose or half-strength anaerobe agar (Oxoid), supplemented with sea salts. The strains did not denitrify, produce lipase (with olive oil as the substrate) or tolerate 1 % (w/v) ox bile salts. The strains did not decompose agar, chitin, carboxymethylcellulose, dextran, xylan, xanthine or uric acid. Negative for API 32ID AN tests for α -arabinosidase, β -glucuronidase and pyroglutamate arylamidase; API 20E tests for lysine decarboxylase, ornithine decarboxylase, H₂S production, indole production, Simmons' citrate and carbohydrate fermentation; and API 20NE tests for the utilization of citrate, phenylacetate, caprate and adipate. On minimal or mineral salts media, did not utilize L-rhamnose, D-melibiose, itaconate, salicin, L-fucose, valerate, DL-3-hydroxybutyrate, DL-lactate, suberate, L-histidine or citrate. O, Orange; Y, yellow; +, positive for all strains; v+, type strain is positive, but test result varies among other strains; v-, type strain is negative, but test result varies among other strains; -, negative for all strains; ND, no data available. Results in parentheses indicate a weak or delayed reaction, or resulted in weak growth.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Pigment of cell biomass	O, Y	O	O	Y	Y	Y	Y	Y	Y	Y	Y	O	O
Gliding motility	+	+	+	+	+	+	+	-	-	-	-	-	-
Growth on MA at 25 °C	v+	-	(v+)	(v+)	+	+	+	+	+	+	-	-	+
Requirement for:													
Yeast extract	-	-	+	v-	+	+	-	-	-	-	-	-	-
Divalent cations	-	-	-	-	-	-	-	-	-	-	-	+	+
Growth without salt	-	-	-	-	-	+	+	-	+	-	+	-	-
Tolerance to NaCl:													
1.5 M	+	+	-	+	(+)	+	+	+	+	+	+	+	+
2.0 M	+	+	-	(v-)	-	-	+	+	-	+	-	-	-
2.5 M	-	-	-	-	-	-	(+)	-	-	+	-	-	-
Arginine dihydrolase	-	+	-	-	-	-	+	-	ND	ND	-	-	-
Nitrate reduction to nitrite	-	-	-	v-	-	-	-	-	-	-	-	+	-
Hydrolysis of:													
Gelatin	+	+	+	v-	+	-	+	+	+	+	v-	+	+
Casein	(v+)	+	-	v-	-	-	+	-	-	+	-	-	-
Elastin	v-	-	-	-	-	-	-	-	ND	ND	-	-	-
Starch	v+	+	-	+	+	-	-	-	-	-	-	+	+
Tween 80	+	+	+	v+	-	+	-	+	-	+	-	+	+
L-Tyrosine	+	-	(v+)	+	(+)	-	+	+	-	ND	v-	+	+
Aesculin	+	+	v	+	+	+	+	-	+	ND	-	-	-
DNA	-	+	-	+	-	-	-	-	-	+	+	-	-
Urea	-	-	-	-	-	-	(+)	-	-	+	-	-	+
Egg yolk reaction (lecithinase)	-	-	-	-	-	+	-	-	-	ND	-	-	-
Oxidative acid production from D-glucose	-	-	-	+	+	(+)	(+)	-	(+)	+	+	+	+
API 32ID AN substrates:													
Glutamate decarboxylase	v+	-	v-	+	+	+	+	-	+	ND	v+	+	+
α -Galactosidase	-	-	-	-	-	+	+	-	-	ND	+	-	-
β -Galactosidase	-	-	-	-	-	+	+	-	-	ND	-	-	-
6-Phospho- β -galactosidase	-	-	-	-	-	+	+	-	-	ND	-	-	-
α -Glucosidase	+	+	-	+	+	+	-	-	+	ND	+	+	+
β -Glucosidase	v-	+	-	+	+	+	-	-	(+)	ND	+	+	+
α -Fucosidase	-	-	-	-	-	+	+	-	-	ND	-	-	-
N-Acetyl- β -D-glucosaminidase	(v-)	-	-	v+	+	+	+	(+)	-	ND	-	-	-

Table 2. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Alkaline phosphatase	+	+	+	+	+	—	+	+	+	+	V—	+	+
Proline arylamidase	V—	—	—	—	—	+	—	—	—	ND	—	—	—
Sole carbon and energy sources (API AUX and API 20E tests):													
D-Glucose	+	+	—	+	+	+	+	+	(+)	+	+	+	+
Glycogen	+	+	—	+	+	+	+	+	(+)	ND	V—	—	+
N-Acetyl-D-glucosamine	—	—	—	V+	—	+	+	—	—	ND	V—	—	+
DL-Arabinose	—	—	—	—	—	+	—	—	—	—	V+	+	+
D-Mannose	—	+	—	—	+	—	—	+	—	—	+	+	+
Maltose	+	+	—	V—	+	+	+	+	—	ND	+	—	+
Sucrose	+	—	—	V—	+	+	—	+	—	+	+	—	+
Acetate	V—	—	—	—	—	+	—	—	—	ND	—	—	—
Propionate	—	—	—	—	—	+	—	—	(+)	ND	+	(+)	+
L-Alanine	V—	—	—	—	—	V—	(+)	—	—	ND	+	—	(+)
L-Proline	+	+	—	+	+	+	+	+	(+)	ND	+	+	+
L-Serine	—	—	—	—	—	V—	+	—	—	ND	—	—	—
Mean G + C content (mol%) (T_m)	40	39	35	37	39	39	42	37	38	36	32	36	34

Table 3. Differential characteristics of the Antarctic isolates belonging to the genus *Bizionia* compared with *B. paragorgiae*

Species: 1, *B. saleffrena*; 2, *B. gelidisalsuginis*; 3, *B. algorithergicola*; 4, *B. myxarmorum*; 5, *B. paragorgiae* (data from Nedashkovskaya *et al.*, 2005c). Differential characteristics are as for Table 2, except for the following. All species were positive for yellow pigmentation; requirement for yeast extract; tolerance to 1.5 M NaCl; cytochrome *c* oxidase; hydrolysis of casein, gelatin, Tween 80 and L-tyrosine; and production of alkaline phosphatase. All species were negative for gliding motility; growth in the absence of Na⁺ ions; requirement for divalent cations (sea salts); reduction of nitrate; hydrolysis of starch and aesculin; acid production from carbohydrates; production of glutamate decarboxylase, α -galactosidase, β -galactosidase (API 20NE and API 32ID AN), 6-phospho- β -galactosidase, α -glucosidase, β -glucosidase, α -fucosidase, N-acetyl- β -D-glucosaminidase and proline arylamidase; utilization of D-glucose, DL-arabinose, D-mannose, glycogen, maltose and N-acetyl-D-glucosamine. +, Positive for all strains; V, characteristic varies among different strains within the same species; V—, type strain is negative, but test result varies among other strains; —, negative for all strains; ND, no data available. Results in parentheses indicate a weak or delayed reaction, or resulted in weak growth.

Characteristic	1	2	3	4	5
Growth on MA at 25 °C	+	(V)	(+)	+	+
Requirement for yeast extract	+	+	+	+	+
Tolerance to NaCl:					
2.0 M	+	+	+	+	—
2.5 M	+	+	—	—	—
Arginine dihydrolase	—	+	+	+	—
Hydrolysis of:					
Elastin	+	—	—	—	ND
DNA	—	—	+	+	—
Urea	+	—	+	+	—
Egg yolk reaction (lecithinase)	+	—	—	+	ND
Sole carbon and energy source (API AUX and API 20E tests):					
Acetate	—	+	(V—)	+	ND
Propionate	—	+	—	—	ND
Valerate	—	—	—	+	ND
L-Alanine	—	+	—	+	ND
L-Histidine	(+)	+	(+)	+	ND
L-Proline	—	—	—	+	ND
L-Serine	—	—	—	+	ND
Mean G + C content (mol%) (T_m)	40	39	45	43	38

investigated are given in Tables 2–4. 16S rRNA gene sequence analysis indicated that the strains formed several lineages in the marine clade of the family *Flavobacteriaceae* (Fig. 1).

All strains contained MK-6, the characteristic isoprenoid quinone for the family *Flavobacteriaceae*, and also high levels of branched-chain and 3-hydroxy C₁₅–C₁₇ fatty acids, which are typical for the family *Flavobacteriaceae* (Bowman *et al.*, 1998). However, differences were evident between certain taxa for specific lipid components (Table 5).

Quartz stone strains

Several strains that exhibited gliding motility on dilute marine media were isolated from cyanobacterial biofilms located on the undersides of quartz stones, partially buried in feldfield soils of the Vestfold Hills (Smith *et al.*, 2000), which is an arid, ice-free region on the coast of East Antarctica. The growth of these sublithic (or hypolithic) communities is promoted in this region because it is protected from climatic extremes and includes a relatively diverse set of cold-adapted chemoheterotrophic bacteria

(Smith *et al.*, 2000). Strains from this ecosystem included one group comprising relatively fast-growing, rod-shaped bacteria (G#7^T, G#4, O1-5, O3-5, O5-2, Y4-5^T and Y5-5) (see panel A of Supplementary Figure in IJSEM Online), whereas a second group included slow-growing cocco-bacilli, such as strains P7^T and O6-2 (Table 1, see also panel C of Supplementary Figure in IJSEM Online). Genotypic analysis indicated that the group containing G#7^T included two genospecies. The first consisted of six strains, represented by G#7^T, while the second comprised only strain Y4-5^T. DNA–DNA hybridization analysis indicated that the genomic DNA of strain Y4-5^T showed 47–59 % relatedness with that of strains G#7^T, O1-5 and O3-5. By comparison, the DNA–DNA relatedness of the latter three strains was 89–99 %. Strain Y4-5^T also differed from the other strains in a variety of phenotypic traits (Table 2); however, the fatty acid profiles of the strains were almost identical (Table 5). Both genospecies formed spheroplast-like bodies in old cultures (see panel B of Supplementary Figure in IJSEM Online). Strains P7^T and O6-2 were found to form a single genospecies, with a mean DNA–DNA hybridization value of 88 %. These data concurred with the phenotypic (Table 2) and fatty acid (Table 5) data.

Table 4. Characteristics that differentiate the genera *Subsaximicrobium*, *Subsaxibacter* and *Lacinutrix* from related members of the family *Flavobacteriaceae*

Genera/species: 1, *Subsaximicrobium*; 2, *Subsaxibacter*; 3, *Gelidibacter*; 4, *Lacinutrix*; 5, *Bizionia*; 6, *F. algae*; 7, *A. lectus*; 8, *Psychroserpens*; 9, *Winogradskyella*; 10, *Gillisia*; 11, *Mesonina algae*; 12, *Salegentibacter*; 13, *Psychroflexus*. Data from this study, Ivanova *et al.* (2004), Macián *et al.* (2002), Nedashkovskaya *et al.* (2004c, 2005a, c) and Van Trappen *et al.* (2004). O, Orange; Y, yellow; +, positive for all strains; V, characteristic varies among different species within the same genus; V+, type strain is positive, but test result varies among other strains; –, negative for all strains; ND, no data available. Results in parentheses indicate a weak or delayed reaction, or resulted in weak growth.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Pigment	O or Y	O	Y	Y	Y	Y	O	Y	Y	Y	Y	Y	O
Gliding motility	+	+	+	–	–	+	+	–	+	–	–	–	V
Growth at/in:													
25 °C	V	(V+)	V*	+	+	+	+	–	+	V	+	+	V
30 °C	–	–	V	–	V	+	+	–	+	V	+	+	V
0 NaCl	–	–	V	–	–	+	–	–	–	V	–	–	–
2 M NaCl	+	–	–	+	V	–	–	–	–	–	+	+	V
Requirement for:													
Sea salts†	–	–	–	–	–	–	ND	+	ND	V	ND	–	V
Yeast extract	–	+	–	–	+	–	ND	+	ND	–	ND	ND	V
Hydrolysis of:													
Casein	+	–	V	+	+	–	–	+	V	V	+	–	V
Starch	+	–	V	–	+	(+)	+	–	V	V	–	+	+
Agar	–	–	–	–	–	–	+	–	+	V	–	–	–
Aesculin	+	V	+	–	–	+	+	–	ND	V	ND	+	+
Production of acid from D-glucose	–	–	+	–	–	+	+	–	+	+	–	+	+
Anaerobic growth	–	–	–	–	–	+	+	–	–	–	–	–	–
G + C content (mol%)	38–41	35–36	36–42	37	38–45	34	31–33	27–29	35–36	32–39	32–34	36–37	32–35

*Some strains of *Gelidibacter algens* do not grow at 25 °C. All strains of other *Gelidibacter* species grow at least weakly at 25 °C.

†Sea salts requirement indicates that Na⁺ ions alone do not support growth; instead the strain requires additional cations for growth, such as Mg²⁺ and Ca²⁺, present in sea water.

Table 5. Whole-cell fatty acid profiles of the Antarctic isolates and some closely related reference species

Species: 1, *Subsaximicrobium wynnwilliamsii* G#7^T; 2, *Subsaximicrobium saxinquilinus* Y4-5^T; 3, *Subsaxibacter broadyi* P7^T and O6-2; 4, *Gelidibacter algens* ACAM 536^T; 5, *Gelidibacter mesophilus* 2SM29^T; 6, *Gelidibacter gilyus* IC158^T; 7, *Gelidibacter salicanalis* IC162^T; 8, *L. copepodicola* DJ3^T; 9, *B. saffordii* HFD^T; 10, *B. gelidisalsuginis* IC164^T; 11, *B. alboritergicola* APA-1^T; 12, *B. myxarmorum* ADA-4^T; 13, *B. paragorgiae* KMM 6029^T (data from Nedashkovskaya *et al.*, 2005c); 14, *Gillisia mitskevichiae* (Nedashkovskaya *et al.*, 2005b); 15, *Gillisia illustrilutea* IC157^T; 16, *Gillisia sandarakina* IC148^T; 17, *Gillisia hiemivivida* IC154^T. Values are percentages of total fatty acids. Fatty acid nomenclature: the first number indicates the number of carbon atoms in the molecule. The prefixes 'br', 'i' and 'a' indicate branched (in which branching positions have not been determined), iso-branched and anteiso-branched fatty acids, respectively. The second number following the colon indicates the number of double bonds present. The position of the double bond is indicated by the carbon atom position it follows starting from the methyl (ω) end of the molecule. All mono-unsaturated straight-chain and branched fatty acids were found to be *cis* isomers, indicated by the prefix 'c'. tr, Trace fatty acid (0.01–0.05 %); –, fatty acid below detection levels (<0.01 %).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
13:0	–	–	–	–	–	–	0.2	tr	–	tr	0.2	–	–	–	–	–	0.5
14:0	0.2	0.5	0.6–0.7	0.5	0.3	0.6	0.4	0.4	0.8	0.4	0.4	0.7	–	–	–	tr	0.5
15:0	1.1	0.6	0.7–1.0	2.4	5.3	3.2	4.1	3.7	3.2	2.3	4.1	4.2	4.3	4.4	4.3	4.3	4.3
16:0	1.9	2.7	1.6–2.0	1.6	1.9	2.2	1.2	2.9	1.6	1.6	1.2	1.7	3.4	–	1.7	0.5	1.8
18:0	2.1	1.2	0.0–0.5	0.5	0.8	1.2	0.6	–	–	1.1	0.6	0.5	–	–	0.9	–	0.7
i13:0	–	–	0.0–0.1	–	tr	–	0.2	0.1	3.0	tr	0.2	0.5	–	–	–	–	0.5
a13:0	–	–	0.0–0.2	–	–	–	tr	0.3	–	0.3	0.4	–	–	–	–	–	–
br14:1	0.3	0.5	0.4–0.5	1.7	1.0	0.3	0.9	0.7	–	0.7	1.1	0.8	–	–	tr	0.9	1.0
i14:0	0.7	0.2	0.4–0.6	2.8	0.4	0.6	0.8	1.4	1.2	1.4	1.3	1.8	2.5	1.2	0.6	0.6	1.3
i15:1 ω 10c	13.4	6.7	6.2–6.7	11.4	5.7	5.3	6.7	12.0	3.8	2.1	12.5	10.1	9.4	11.8	14.6	13.2	3.0
a15:1 ω 10c	14.7	5.9	9.9–15.5	15.9	11.8	14.6	16.6	14.2	11.8	7.0	9.6	14.0	5.8	2.4	17.6	12.9	13.1
i15:0	7.4	10.4	6.3–6.4	8.8	7.7	5.0	3.4	19.9	6.8	3.1	13.2	10.6	13.2	7.5	3.2	3.2	3.5
a15:0	17.1	9.9	14.3–14.5	17.7	10.5	13.7	11.5	18.2	17.2	13.9	10.1	20.8	12.3	5.1	10.2	9.1	19.5
br16:1	1.9	4.7	3.5–7.7	10.3	1.4	1.6	5.1	4.6	1.2	1.3	3.9	5.0	5.4	6.0	3.8	6.2	4.2
i16:0	–	–	1.5–1.7	4.4	3.5	1.4	3.5	7.1	1.3	1.7	3.2	4.3	5.8	9.3	14.4	15.8	2.7
i17:1	3.5	5.1	2.3–2.6	2.3	2.1	1.1	1.9	–	3.1	15.1	8.2	9.4	1.7	4.0	2.2	3.4	1.8
a17:1	2.1	3.8	4.0–7.4	3.1	1.9	3.4	2.5	–	3.6	2.0	3.7	4.6	1.5	2.1	3.3	3.0	4.7
15:1	0.2	1.0	1.3–2.0	2.7	3.0	2.8	4.2	1.2	2.3	1.2	1.2	1.9	1.3	1.9	1.4	1.7	2.3
16:1 ω 7c	9.5	13.4	8.3–13.1	4.3	7.4	6.8	9.5	2.8	5.5	3.9	2.6	3.0	6.3	11.1	2.8	4.4	4.0
16:1 ω 5c	–	0.5	1.1–1.2	1.0	0.3	1.1	–	–	–	0.4	0.8	–	–	–	–	–	0.6
18:1 ω 9c	–	–	8.2–18.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3-OH i15:0	5.4	2.4	1.9–2.3	2.2	4.0	6.2	3.5	3.1	9.3	6.2	9.3	1.2	3.4	1.1	1.4	1.5	3.8
3-OH a15:0	3.6	8.7	5.2	0.3	7.9	9.8	10.6	2.1	22.2	22.9	4.7	3.4	–	–	9.9	8.6	14.6
3-OH 15:0	–	0.8	0.0–0.5	tr	1.3	1.5	0.9	0.2	1.2	1.2	1.0	tr	–	–	–	0.7	1.1
3-OH i16:0	8.8	13.7	5.0–6.0	4.1	11.5	12.2	10.7	2.7	1.6	8.5	4.8	1.3	6.8	7.2	4.9	7.0	8.9
3-OH i17:0	3.4	1.0	–	0.4	2.8	3.1	–	2.3	0.5	0.4	1.6	–	4.3	6.7	1.4	1.7	1.4
3-OH 17:0	–	–	–	–	–	–	–	–	–	–	–	–	–	3.8	6.0	5.5	1.9
3-OH a17:0	2.7	5.3	2.3–3.2	1.6	10.5	11.3	1.0	1.7	1.5	2.9	1.6	0.5	–	–	1.6	1.2	0.3
Other	–	–	–	–	1.0	2.3	–	–	–	–	–	–	12.6	6.7	–	–	–

Phylogenetically, the two genospecies represented by strains G#7^T and Y4-5^T were most closely related to the genus *Gelidibacter* (16S rRNA gene sequence similarity of 92–93 %) (Fig. 1). The genospecies differed in various phenotypic traits from *Gelidibacter* species (Tables 2 and 4) and lacked the fatty acid i16:0, which was present in all *Gelidibacter* species (Table 5). Similarly, the genospecies represented by strain P7^T also differed in terms of morphology and various phenotypic characteristics (Table 2) from *Gelidibacter* species, which were the closest relatives (similarity 95 %) (Fig. 1). Strains P7^T and O6-2 contained high levels of the fatty acid 18:1 ω 9c, which was absent from *Gelidibacter* species. The overall taxonomic data suggested

that the quartz stone strains were sufficiently different not to be included in the genus *Gelidibacter*, but represent novel taxa at the genus level.

It is thus proposed that strains G#7^T and Y4-5^T represent a novel genus, named *Subsaximicrobium* gen. nov., containing two novel species designated *Subsaximicrobium wynnwilliamsii* sp. nov. (G#7^T) and *Subsaximicrobium saxinquilinus* sp. nov. (Y4-5^T). *Subsaximicrobium wynnwilliamsii* is the type species. It is also proposed that strain P7^T represents the type strain of a new genus and novel species, named *Subsaxibacter broadyi* gen. nov., sp. nov.

Sea-ice algal isolates

Isolates were obtained from two distinct sea-ice sources: the sea-ice algal assemblage and brine drained from ice cores at the time of sampling. Samples collected from sea-ice communities have been analysed previously by using molecular and culture techniques (Bowman *et al.*, 1997b; Brown & Bowman, 2001; Brinkmeyer *et al.*, 2003) and novel taxa are slowly being described. Isolates studied here were obtained either from algae directly concentrated from basal ice algal assemblages or, in the case of strain IC162^T, from particulate material concentrated from the sea-ice brine. All the strains appeared rod-shaped, and those grouped in the genus *Gelidibacter* exhibited gliding motility. Gliding was only evident for strain IC162^T on dilute media. Some strains also formed short filaments, whereas others contained coccoidal bodies, possibly spheroplasts, in old cultures, as noted in the taxa descriptions below (similar to panel B of Supplementary Figure in IJSEM Online).

Phylogenetic analysis indicated that the sea-ice algae strains could be grouped either within the genus *Gelidibacter* (strains IC158^T, IC159, IC163 and IC162^T) or the genus *Gillisia* (IC157^T, IC144, IC146, IC148^T and IC154^T). The sequences of strains IC158^T, IC163 and IC159, which formed pale, viscid yellow colonies, with spreading margins, were identical, and only IC158^T is shown in the phylogenetic tree (Fig. 1). Sequence analysis revealed that strains IC158^T and IC162^T were clearly distinct from each other and from *Gelidibacter algens* and *Gelidibacter mesophilus* (similarities ~97%) (Fig. 1). In the case of the strains related to the genus *Gillisia*, strains IC157^T, IC144 and IC146 had nearly identical 16S rRNA gene sequences but were quite different from IC148^T and IC154^T, as well as from *Gillisia limnaea* and *Gillisia mitskevichiae* (similarities 96–97%), which are species from Antarctic lake cyanobacterial mats (Van Trappen *et al.*, 2004) and sea water (Nedashkovskaya *et al.*, 2005b), respectively.

The isolates belonging to the genus *Gelidibacter* were compared by DNA–DNA hybridization with *Gelidibacter algens*, *Gelidibacter mesophilus*, and each other. It was found that IC158^T represented a distinct genospecies with strains IC163 and IC159 (hybridization values 93–100%) and were genotypically (hybridization values 12–23%) and phenotypically (Table 2) distinct from other type strains of the genus *Gelidibacter*. Strain IC162^T, like IC158^T, was also genotypically and phenotypically distinct from other *Gelidibacter* strains (hybridization values of 15–32%). Fatty acids produced by the sea-ice strains were similar, and the profiles were qualitatively similar to those of *Gelidibacter algens* and *Gelidibacter mesophilus* (Table 5). The DNA G + C contents of the genospecies represented by strains IC158^T and IC162^T were 39 and 42 mol%, respectively, comparable with data from *Gelidibacter algens* and *Gelidibacter mesophilus* (Table 2). Phenotypically, the isolates were similar to members of the genus *Gelidibacter*, in particular the ability to glide, yellow pigmentation, non-exacting nutritional requirements, the ability to use carbohydrates and growth at

low temperatures. However, with the expansion of the number of ecosystems in which the genus *Gelidibacter* has been found, the genus now exhibits an increased degree of phenotypic heterogeneity (Table 4). Overall, *Gelidibacter* species still possess a number of common traits, which differ from those of closely related taxa, including the novel quartz stone isolates discussed above.

From the DNA–DNA hybridization analysis, it was found that the strains belonging to the genus *Gillisia* clearly formed three genospecies. The bright-yellow-pigmented strains IC157^T, IC144 and IC146 were grouped in one genospecies, whereas the orange-pigmented isolates IC148^T and IC154^T each formed the other two. DNA–DNA hybridization values between the genospecies ranged from 18 to 37%. The DNA–DNA hybridization data also revealed that there was no significant similarity with *Gillisia limnaea* LMG 21470^T, with hybridization values ranging from 11 to 31%. These data were supported by the phenotypic results, which showed that the sea-ice strain groups were distinct from each other and from *Gillisia limnaea* (Table 2). The sea-ice isolates also differed phenotypically (Table 2) from the recently recognized species *Gillisia mitskevichiae*, which is much more halotolerant. The DNA G + C contents of the sea-ice strains were in the same general range as those of *Gillisia limnaea* and *Gillisia mitskevichiae*, with the value for the genospecies represented by strain IC157^T being somewhat lower, with a mean value of 32 mol%. The fatty acid data indicated that the sea-ice strains of the genus *Gillisia* were similar to each other and to *Gillisia mitskevichiae* (Table 5) and *Gillisia limnaea* (data not shown).

Based on the polyphasic taxonomic data, it is proposed that the novel sea-ice algae-derived genospecies constitute novel species. Two of the novel species belong to the genus *Gelidibacter*, for which the names *Gelidibacter gilvus* sp. nov. (type strain, IC158^T) and *Gelidibacter salicanalis* sp. nov. (type strain, IC162^T) are proposed. Three novel species are also proposed for the genus *Gillisia*, with the names *Gillisia illustrilutea* sp. nov. (type strain, IC157^T), *Gillisia sandarakina* sp. nov. (type strain, IC148^T) and *Gillisia hiemivivida* sp. nov. (type strain, IC154^T).

Sea-ice brine and crustacean isolates

As it freezes, sea ice traps and concentrates sea water within the ice matrix. The brine reaches temperatures as low as –20 °C in winter, although it does not freeze due to its high salinity, which can be as high as 150‰. Although an extreme environment, sea-ice brine channels still support psychroactive bacterial and algal communities, which have been found to undergo cell division at –10 °C (Junge *et al.*, 2004). The brine samples examined here were directly plated on MA and on MA containing 10% NaCl, in the hope of finding novel, highly salt-tolerant, but still highly cold-adapted, species. Primary isolation plates of different brine samples included various golden-yellow colonies and, for this study, yielded the strains HFD^T, IC134 and IC164^T. The strains were non-motile, with rod-shaped

or sometimes spiral-shaped or curved cells, similar to the morphology found for the crustacean isolates discussed further below.

Strains HFD^T and IC134 had almost identical 16S rRNA gene sequences, with a similarity of 97.2% with the sequence of strain IC164^T. The brine strains were most closely related to isolates from amphipods (HFD^T, IC134 and IC164^T; similarity 96%) and to *Bizionia paragorgiae* (Nedashkovskaya *et al.*, 2005c) (similarity 96–98%). DNA–DNA hybridization analysis indicated that HFD^T and IC134 belonged to the same genospecies (hybridization value, 83%). However, strain IC164^T appeared to represent a distinct genospecies, sharing only 36% DNA hybridization with the genomic DNAs of the other brine isolates. No significant similarity was found between the brine isolates and the most closely related recognized species, *B. paragorgiae*, or with the epiphytic species *Formosa algae* (values of 4–22%). Phenotypic data for the brine strains are shown in Table 3. The strains required Na⁺ ions and were distinctly salt tolerant, growing best in 1.0 M NaCl and tolerating up to 3.0 M NaCl. Several phenotypic differences were evident between the genospecies represented by strains HFD^T and IC164^T (Table 3).

Sea-salts-containing media onto which crustacean-derived material and bacterial cells were plated also revealed several golden-yellow-coloured colonies that were non-motile, with rod-shaped or sometimes spiral-shaped or curved cells. Four isolates were studied, including DJ3^T, a rod-shaped strain (see panel D of Supplementary Figure in IJSEM Online) that was derived from the copepod sample, and APA-1^T, APA-3 and ADA-4^T, which were each derived from exoskeletal material of individual amphipod samples (Table 1). 16S rRNA gene sequence analysis indicated that strain DJ3^T was phylogenetically quite separate from strains APA-1^T, APA-3 and APA-4^T (similarity 92–93%), whereas the 16S rRNA gene sequences of APA-1^T and APA-3 were identical, and quite similar to that of ADA-4^T (similarity 97%). DJ3^T was most closely related to species of the genera *Psychroserpens* and *Winogradskyella* (similarity 94%). Strains APA-1^T and ADA-4^T were related to the sea-ice brine isolates HFD^T, IC134 and IC164^T discussed above, and like these were most closely related to *B. paragorgiae*. Phenotypically and genotypically, the crustacean strains formed distinct genospecies. DJ3^T did not show significant DNA–DNA hybridization (values 8–28%) with any related strains, including APA-1^T, ADA-4^T, HFD^T and IC164^T, or *Psychroserpens burtonensis* ACAM 188^T. In addition, the mean hybridization value for the genomic DNAs of strains APA-1^T and ADA-4^T was only 38% and there was no genomic similarity with the sea-ice brine strains and *F. algae* (hybridization values 10–17%). Apparent differences in phenotype between the different genospecies, represented by DJ3^T (Table 2), APA-1^T and APA-4^T, supported the phylogenetic and genotypic data (Table 3). The differences between the ecosystems from which the isolates were obtained were also evident, with the sea-ice brine strains being much more salt tolerant than

the crustacean isolates and *B. paragorgiae* (isolated from a coral species), which could not tolerate more than 2.0 M NaCl. However, they had many traits in common, including a requirement for yeast extract and a preference for the metabolism of proteins (such as casein and gelatin) and amino acids (e.g. L-histidine and L-tyrosine), but not carbohydrates.

The fatty acid profiles of HFD^T, IC164^T, APA-1^T and APA-4^T were practically indistinguishable, and were very similar to that of *B. paragorgiae* (Table 5), which was consistent with their phylogenetic relatedness. However, the fatty acid profile of DJ3^T differed from those of the other strains, in the lack of both i17:1 and a17:1. These fatty acids were present in all the other isolates and related species examined in this study. Although strain DJ3^T was in some ways phenotypically similar to the sea-ice brine and amphipod strains (Table 3), there were also a number of key differences (Table 4), including non-exacting nutritional requirements and the ability to utilize carbohydrates. Together with the inability to form branched-chain C₁₇ mono-unsaturated fatty acids (Table 5), various phenotypic characteristics and the DNA G+C content also separated it from the phylogenetically related genera *Psychroserpens* and *Winogradskyella* (Table 4). Thus, based on taxonomic data, strain DJ3^T appears to represent a new genus and a novel species, for which the name *Lacinutrix copepodicola* gen. nov., sp. nov. is proposed.

The sea-ice brine strains and amphipod-derived strains all formed a common phylogenetic cluster with *B. paragorgiae* (Fig. 1). The four genospecies represented by these strains also have many characteristics in common with *B. paragorgiae*, and make up a distinct genus that differs phenotypically from related members of the family *Flavobacteriaceae*, including their closest relatives *F. algae* and *Algibacter lectus* (Table 4). The DNA G+C content values of members of the novel genospecies (40–45 mol%) were also distinctive, and somewhat higher than is typical for many members of the family *Flavobacteriaceae*, but much less than the 55 mol% value recorded for *Robiginitalea biformata* (Cho & Giovannoni, 2004). On the basis of the combined taxonomic data, it is proposed that the sea-ice brine- and amphipod-derived strains should be included within the genus *Bizionia*, comprising four distinct species, *Bizionia saleffrena* sp. nov. (type strain, HFD^T), *Bizionia gelidisalsuginis* sp. nov. (type strain, IC164^T), *Bizionia aloritergicola* sp. nov. (type strain, APA-1^T) and *Bizionia myxarmorum* sp. nov. (type strain, ADA-4^T).

Description of *Subsaximicrobium* gen. nov.

Subsaximicrobium (Sub.sa.xi.mi.cro'.bi.um. L. pref. *sub* below; L. neut. n. *saxum* stone; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Subsaximicrobium* microbe living below stone).

Gram-negative, rod-shaped cells, approximately 0.3–0.5 µm in width and 1–6 µm in length. Older cultures

contain many spherical cells (diameter 1–3 µm). Exhibit gliding motility when grown on dilute agar media. Cell mass is either yellow or orange. Flexirubin pigments are not formed. Do not form resting cells or spores. Strictly aerobic and chemoheterotrophic. Produce catalase. Major fatty acids include i15:1ω10c, a15:1ω10c, i15:0, a15:0, 16:1ω7c, 3-OH a15:0 and 3-OH i16:0. DNA G+C content is 39–40 mol%. A member of the family *Flavobacteriaceae*, class *Flavobacteria*, phylum 'Bacteroidetes'.

The type species is *Subsaximicrobium wynnwilliamsii*.

Description of *Subsaximicrobium wynnwilliamsii* sp. nov.

Subsaximicrobium wynnwilliamsii (wynn.williams'i.i. N.L. gen. n. *wynnwilliams* of Wynn-Williams, named in honour of the late D. D. Wynn-Williams, British Antarctic microbiologist).

Description is as for the genus plus the following. Cell mass is either a bright-orange or golden-yellow colour. Colonies are circular, convex, with an entire edge and a butyrous consistency on MA. Colonies exhibit spreading margins on dilute media. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–20 °C. Growth at 25 °C is weak or negative. Requires Na⁺ ions for growth. Grows in 0.25–2.0 M NaCl, with optimal growth occurring in approximately 0.3–0.4 M NaCl. Grows poorly in media prepared with 2× strength (70 g l^{–1}) sea salts; no growth occurs with 4× strength (140 g l^{–1}) sea salts. Grows well on trypticase soya agar (TSA) containing 1% (w/v) NaCl, but grows poorly on TSA and nutrient agar (NA). Does not require yeast extract or vitamins for growth, and can use inorganic nitrogen sources such as sodium nitrate and ammonium chloride. Grows in mineral salts media with various sole carbon and energy sources, including D-glucose, glycogen, maltose, sucrose and L-proline. Acid production from carbohydrates on Leifson's O–F medium is undetectable. Hydrolyses gelatin, Tween 80, L-tyrosine and aesculin, but not DNA or urea. Most strains also decompose starch and weakly degrade casein. Produces α-glucosidase and alkaline phosphatase. Does not produce arginine dihydrolase or lecithinase, or reduce nitrate. Other phenotypic data are shown in Table 2. Mean DNA G+C content is 40 mol%.

The type strain is G#7^T (=ACAM 1070^T=CIP 108525^T), which was isolated from cyanobacterial biofilms attached to the undersides of partially buried quartz stones, found in the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Subsaximicrobium saxinquilinus* sp. nov.

Subsaximicrobium saxinquilinus (sax.in.qui.li'nus. L. n. *saxum* stone; L. masc. n. *inquilinus* the denizen; N.L. masc. n. *saxinquilinus* the denizen of stone).

Description is as for the genus plus the following. Cell mass is an orange colour. Colonies are circular, convex, with an

entire edge and a butyrous consistency on MA. Colonies exhibit spreading margins on dilute media. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–20 °C. No growth occurs at 25 °C or higher. Requires Na⁺ ions for growth. Grows in 0.1–2.0 M NaCl, with optimal growth occurring in approximately 0.3–0.4 M NaCl. Grows well in media prepared with 2× strength sea salts. No growth occurs with 4× strength sea salts. Grows well on TSA containing 1% (w/v) NaCl, but grows poorly on TSA and NA. Does not require yeast extract or vitamins for growth, and can use inorganic nitrogen sources such as sodium nitrate and ammonium chloride. Grows in mineral salts media with various sole carbon and energy sources, including D-glucose, glycogen, D-mannose, maltose and L-proline. Acid production from carbohydrates on Leifson's O–F medium is undetectable. Hydrolyses gelatin, casein, Tween 80, starch, aesculin and DNA, but not elastin, L-tyrosine or urea. Produces α-glucosidase, β-glucosidase and alkaline phosphatase. Produces arginine dihydrolase. Lecithinase production and nitrate reduction are negative. Other phenotypic data are shown in Table 2. Mean DNA G+C content is 39 mol%.

The type strain is Y4-5^T (=ACAM 1063^T=CIP 108526^T), which was isolated from cyanobacterial biofilms attached to the undersides of partially buried quartz stones, found in the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Subsaxibacter* gen. nov.

Subsaxibacter (Sub.sa.xi.bac'ter. L. pref. *sub* below; L. neut. n. *saxum* stone; N.L. masc. n. *bacter* rod; N.L. masc. n. *Subsaxibacter* bacterial rod living below stone).

Gram-negative, coccobacilli, approximately 0.3–0.5 µm in width and 0.4–1.0 µm in length. Exhibit gliding motility. Cell mass is orange. Flexirubin pigments are not formed. Do not form resting cells or spores. Strictly aerobic and chemoheterotrophic. Produce catalase. Major fatty acids include i15:1ω10c, a15:1ω10c, i15:0, a15:0, 16:1ω7c, a17:1, 18:1ω9c and 3-OH i16:0. A member of the family *Flavobacteriaceae*, class *Flavobacteria*, phylum 'Bacteroidetes'.

The type species is *Subsaxibacter broadyi*.

Description of *Subsaxibacter broadyi* sp. nov.

Subsaxibacter broadyi (broa.dy'i. N.L. gen. n. *broadyi* of Broady, named in honour of P. A. Broady, Antarctic microbiologist from New Zealand).

Description is as for the genus plus the following. Colonies are small (<1 mm), circular and convex, with a spreading or entire edge and a butyrous to viscid consistency on MA. Colonies exhibit spreading margins on dilute media. Growth occurs slowly at –2 °C in marine broth. Psychrophilic. Moderate to good growth occurs on MA at 1–15 °C. Weak growth occurs at 20 °C. No growth occurs at 25 °C or higher. Requires Na⁺ ions for growth. Grows in

0.1–1.25 M NaCl, with optimal growth occurring in approximately 0.3–0.4 M NaCl. Grows well in media prepared with 2× strength sea salts. No growth occurs with 4× strength sea salts. Grows on TSA containing 1% (w/v) NaCl. No growth occurs on TSA or NA. Requires yeast extract for good growth. Peptone also supports poor to moderate growth. Cannot use sodium nitrate or ammonium chloride as sole nitrogen sources. Does not utilize any of the common substrates tested as sole carbon and energy sources (see Table 2 for substrates tested). Acid production from carbohydrates on Leifson's O–F medium is undetectable. Hydrolyses gelatin and Tween 80, but not casein, elastin, starch, DNA or urea. May weakly decompose L-tyrosine or aesculin. Produces alkaline phosphatase. Does not produce arginine dihydrolase or lecithinase, or reduce nitrate. Other phenotypic data are given in Table 2. Mean DNA G + C content is 35 mol%.

The type strain is P7^T (=ACAM 1064^T=CIP 108527^T), which was isolated from cyanobacterial biofilms attached to the undersides of partially buried quartz stones, found in the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Lacinutrix* gen. nov.

Lacinutrix [La.ci.nu'trix. L. n. *lacus* lake; L. fem. n. *nutrix* feeder; N.L. fem. n. *Lacinutrix* lake feeder (in the sense of being basically important for the food chain)].

Gram-negative, straight or slightly curved rods, approximately 0.4–0.5 µm in width and 1–2 µm in length. Non-motile. Cell mass is golden-yellow. Flexirubin pigments are not formed. Do not form resting cells or spores. Strictly aerobic and chemoheterotrophic. Produce catalase. Major fatty acids include i15:1ω10c, a15:1ω10c, i15:0, a15:0, br16:1 and i16:0. A member of the family *Flavobacteriaceae*, class *Flavobacteria*, phylum 'Bacteroidetes'.

The type species is *Lacinutrix copepodicola*.

Description of *Lacinutrix copepodicola* sp. nov.

Lacinutrix copepodicola [co.pe.pod.i'col.a. N.L. neut. pl. n. *copepoda* copepods (small types of crustacea); L. fem. or masc. suff. *-cola* the dweller, inhabitant; N.L. masc. or fem. n. *copepodicola* the inhabitant of copepods].

Description is as for the genus plus the following. Colonies are golden-yellow, circular, convex, with an entire edge and a butyrous consistency on MA. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–25 °C. No growth occurs at 30 °C or higher. Requires Na⁺ ions for growth. Grows in 0.1–2.0 M NaCl, with optimal growth occurring in approximately 0.3–0.4 M NaCl. Grows well in media prepared with 2× strength sea salts. No growth occurs with 4× strength sea salts. Grows poorly on TSA containing 1% (w/v) NaCl. Does not grow on either TSA or NA. Does not require yeast extract or vitamins for growth, and can utilize inorganic nitrogen sources such as sodium nitrate and ammonium chloride. Grows in mineral salts

media with various sole carbon and energy sources, including D-glucose, glycogen, D-mannose, maltose, sucrose and L-proline. Acid production from carbohydrates on Leifson's O–F medium is undetectable. Hydrolyses gelatin, Tween 80 and L-tyrosine, but not casein, elastin, starch, aesculin, DNA or urea. Produces alkaline phosphatase and weakly produces *N*-acetyl-β-D-glucosaminidase. Does not produce arginine dihydrolase and lecithinase, or reduce nitrate. Other phenotypic data are shown in Table 2. Mean DNA G + C content is 37 mol%.

The type strain is DJ3^T (=ACAM 1055^T=CIP 108538^T), which was isolated directly from the calanoid copepod species *Paralabidocera antarctica* dwelling in Ace Lake in the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Bizionia saleffrena* sp. nov.

Bizionia saleffrena [sal.ef.fre'na. L. masc. n. *sal* salt; L. fem. adj. *effrena* unbridled; N.L. fem. adj. *Saleffrena* unbridled by salt (referring to the species' good growth on salt-containing media)].

Cells are rod-like, 0.4–0.5 × 1.5–5 µm. Colonies are golden-yellow, circular and convex, with an entire edge and a butyrous consistency on MA. Flexirubin pigments are not produced. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–20 °C. Growth is poor at 25 °C. No growth occurs at 30 °C or higher. Requires Na⁺ ions for growth. Grows in 0.2–3.0 M NaCl, with optimal growth occurring in approximately 1.0 M NaCl. Grows well in media prepared with 4× strength sea salts. Does not grow on TSA containing 1% (w/v) NaCl, TSA or NA. Requires yeast extract or peptone for growth, and cannot use inorganic nitrogen sources such as sodium nitrate or ammonium chloride. Does not utilize any of the common substrates tested as sole carbon and energy sources (see Table 3 for substrates tested). Acid production from carbohydrates on Leifson's O–F medium is undetectable. Hydrolyses gelatin, casein, elastin, Tween 80, L-tyrosine and DNA, but not starch, aesculin or DNA. Produces lecithinase. Produces alkaline phosphatase. Does not produce arginine dihydrolase or reduce nitrate. Other phenotypic data are shown in Table 3. Mean DNA G + C content is 40 mol%.

The type strain is HFD^T (=ACAM 1059^T=CIP 108534^T), which was isolated from sea-ice brine, drained and collected from fast sea-ice cores obtained from the coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Bizionia gelidisalsuginis* sp. nov.

Bizionia gelidisalsuginis (gel.id.i'sal.su.gin.is. L. adj. *gelidus* icy; L. fem. n. *salsugo* -inis the brine; N.L. gen. n. *geli-disalsuginis* of icy brine).

Cells are rod-like and 0.4–0.5 × 1.5–3.5 µm. Colonies are golden-yellow, circular and convex, with an entire edge and a butyrous consistency on MA. Flexirubin pigments are not

produced. Growth occurs at -2°C in marine broth. Good growth occurs on MA at $1\text{--}25^{\circ}\text{C}$. No growth occurs at 30°C or higher. Requires Na^{+} ions for growth. Grows in $0.2\text{--}3.0\text{ M NaCl}$, with optimal growth occurring in approximately 1.0 M NaCl . Grows well in media prepared with $4\times$ strength sea salts. Does not grow on TSA containing 1% (w/v) NaCl, TSA or NA. Requires yeast extract or peptone for growth, and cannot use inorganic nitrogen sources such as sodium nitrate or ammonium chloride. In minimal growth media can utilize sodium acetate, sodium propionate, L-alanine and L-histidine as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O-F medium is undetectable. Hydrolyses gelatin, casein and L-tyrosine, but not elastin, Tween 80, starch, aesculin, DNA or urea. Produces alkaline phosphatase. Produces arginine dihydrolase. Does not produce lecithinase or reduce nitrate. Other phenotypic data are shown in Table 3. Mean DNA G + C content is $39\text{ mol}\%$.

The type strain is IC164^T (= ACAM 1057^T = CIP 108536^T), which was isolated from sea-ice brine, drained and collected from fast sea-ice cores obtained from the coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Bizionia algorithergicola* sp. nov.

Bizionia algorithergicola (al.go.ri.ter.gi'col.a. L. n. *algor* the cold; L. n. *tergum* outer covering or surface; L. fem. or masc. suff. *-cola* the dweller, inhabitant; N.L. fem. or masc. n. *algorithergicola* the inhabitant of a cold surface/covering).

Cells are rod-like, $0.3\text{--}0.5 \times 1\text{--}3\text{ }\mu\text{m}$. Colonies are golden-yellow, circular and convex, with an entire edge and a butyrous consistency on MA. Flexirubin pigments are not produced. Growth occurs at -2°C in marine broth. Good growth occurs on MA at $1\text{--}20^{\circ}\text{C}$. Poor growth occurs at 25°C . No growth occurs at 28°C or higher. Requires Na^{+} ions for growth. Grows in $0.2\text{--}2.0\text{ M NaCl}$, with optimal growth occurring in approximately $0.3\text{--}0.4\text{ M NaCl}$. Grows well in media prepared with $2\times$ strength sea salts, whereas growth in $4\times$ strength sea salts is very poor. Does not grow on TSA containing 1% (w/v) NaCl, TSA or NA. Requires yeast extract or peptone for growth, and cannot use inorganic nitrogen sources such as sodium nitrate or ammonium chloride. In minimal growth media utilizes L-histidine as a sole carbon and energy source. Acid production from carbohydrates on Leifson's O-F medium is not detectable. Hydrolyses gelatin, casein, Tween 80, L-tyrosine, DNA and urea, but not elastin, starch or aesculin. Produces alkaline phosphatase. Produces arginine dihydrolase. Does not produce lecithinase or reduce nitrate. Other phenotypic data are shown in Table 3. Mean DNA G + C content is $45\text{ mol}\%$.

The type strain is APA-1^T (= ACAM 1056^T = CIP 108533^T), which was isolated from the exoskeletal slime on an unidentified sea-ice, algae-feeding amphipod, collected from the sea ice-sea water interface in coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Bizionia myxarmorum* sp. nov.

Bizionia myxarmorum [myx.ar.mor'um. Gr. n. *myxa* slime; L. gen. pl. n. *armorum* defensive armour; N.L. pl. gen. n. *myxarmorum* of armour slime (of the slime on the carapace of crustacean host)].

Cells are rod-like, $0.3\text{--}0.5 \times 1.5\text{--}3.5\text{ }\mu\text{m}$. Colonies are golden-yellow, circular and convex, with an entire edge and a butyrous consistency on MA. Flexirubin pigments are not produced. Growth occurs at -2°C in marine broth. Good growth occurs on MA at $1\text{--}25^{\circ}\text{C}$. Poor growth occurs at 30°C . No growth occurs above 32°C . Requires Na^{+} ions for growth. Grows in $0.2\text{--}2.0\text{ M NaCl}$, with optimal growth occurring in approximately $0.3\text{--}0.4\text{ M NaCl}$. Grows well in media prepared with $2\times$ strength sea salts, whereas no growth occurs in $4\times$ strength sea salts. Does not grow on TSA or NA. Requires yeast extract or peptone for growth, and cannot use inorganic nitrogen sources such as sodium nitrate or ammonium chloride. In minimal growth media can utilize sodium acetate, sodium valerate, L-alanine, L-histidine, L-proline and L-serine as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O-F medium is undetectable. Hydrolyses gelatin, casein, Tween 80, L-tyrosine, DNA and urea, but not elastin, starch or aesculin. Produces alkaline phosphatase. Produces arginine dihydrolase and lecithinase, but does not reduce nitrate. Other phenotypic data are shown in Table 3. Mean DNA G + C content is $43\text{ mol}\%$.

The type strain is ADA-4^T (= ACAM 1058^T = CIP 108535^T), which was isolated from the exoskeletal slime on an unidentified sea-ice, algae-feeding amphipod, collected from the sea ice-sea water interface in coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Gelidibacter gilvus* sp. nov.

Gelidibacter gilvus (gil'vus. L. masc. adj. *gilvus* pale yellow).

Gram-negative. Cells are rod-shaped, approximately $0.4\text{--}0.6 \times 2\text{--}8\text{ }\mu\text{m}$. Large coccoid bodies ($2\text{--}3\text{ }\mu\text{m}$ in diameter) form in older cultures. Motile by gliding. Cell mass is yellow. Flexirubin pigments are not formed. Colonies are circular and convex, with a thin spreading margin and a viscid consistency on MA. Growth occurs at -2°C in marine broth. Good growth occurs on MA at $1\text{--}25^{\circ}\text{C}$. No growth occurs at 30°C or higher. Halophilic. Grows in $0.1\text{--}1.5\text{ M NaCl}$, with optimal growth occurring in approximately $0.3\text{--}0.4\text{ M NaCl}$. Grows in media prepared with $2\times$ strength sea salts, whereas no growth occurs in $4\times$ strength sea salts. Grows well on TSA containing 1% (w/v) NaCl, and weakly on TSA and NA. Requires yeast extract or peptone for growth, and cannot use inorganic nitrogen sources such as sodium nitrate or ammonium chloride. In minimal growth media can utilize D-glucose, glycogen, N-acetyl-D-glucosamine, DL-arabinose, maltose, sucrose, sodium acetate, sodium propionate and L-proline as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O-F medium is positive for D-glucose,

L-arabinose, D-mannose, D-galactose, D-fructose, D-rhamnose, D-xylose, D-mannitol, sucrose, *N*-acetyl-D-glucosamine, lactose, cellobiose, trehalose, maltose, D-melibiose, inositol and glycerol. Acid is not formed from melezitose, raffinose, dextran, adonitol or D-sorbitol. Hydrolyses Tween 80 and aesculin, but not gelatin, casein, elastin, L-tyrosine, starch, DNA or urea. Produces lecithinase. Produces 6-phospho- β -galactosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, α -fucosidase and *N*-acetyl- β -D-glucosaminidase, but not alkaline phosphatase. Does not produce arginine dihydrolase or reduce nitrate. Other phenotypic data are shown in Table 2. Mean DNA G + C content is 39 mol%.

The type strain is IC158^T (= ACAM 1054^T = CIP 108531^T), which was isolated from sea-ice algae, collected from fast sea-ice cores obtained from the coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Gelidibacter salicanalis* sp. nov.

Gelidibacter salicanalis (sal.i.can.a.l'is. L. n. *sal* salt; L. n. *canalis* channel; N.L. gen. n. *salicanalis* of the salt channel).

Gram-negative. Cells are rod-shaped or filamentous, approximately 0.4–0.6 $\times$ 2–15 μ m. Small coccoid bodies (approximately 1–1.5 μ m diameter) form in older cultures. Motile by gliding (visible on very dilute growth media only). Cell mass is golden yellow. Flexirubin pigments are not formed. Colonies are circular and convex, with an entire edge and a butyrous consistency on MA. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–25 °C. No growth occurs at 30 °C or higher. Non-halophilic. Grows in 0–2.5 M NaCl, with optimal growth occurring at approximately 0.3–0.4 M NaCl. Grows in media prepared with 2 $\times$ strength sea salts, whereas poor growth occurs in 4 $\times$ strength sea salts. Grows on TSA containing 1 % (w/v) NaCl, TSA and NA. Does not require yeast extract for growth, and can utilize inorganic nitrogen sources such as sodium nitrate and ammonium chloride. In mineral salts growth media can utilize D-glucose, glycogen, *N*-acetyl-D-glucosamine, maltose, L-proline and L-serine as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O–F medium is positive for D-glucose, L-arabinose, D-mannose, D-galactose, *N*-acetyl-D-glucosamine, maltose, lactose, cellobiose, sucrose and trehalose. Hydrolyses gelatin, casein, L-tyrosine and aesculin, but not elastin, starch, Tween 80 or DNA. Urea is weakly decomposed. Produces 6-phospho- β -galactosidase, α -galactosidase, β -galactosidase, α -fucosidase, *N*-acetyl- β -D-glucosaminidase and alkaline phosphatase. Produces arginine dihydrolase, but does not produce lecithinase or reduce nitrate. Other phenotypic data are shown in Table 2. Mean DNA G + C content is 42 mol%.

The type strain is IC162^T (= ACAM 1053^T = CIP 108532^T), which was isolated from sea-ice algae collected from pack-ice brine, Southern Ocean.

Description of *Gillisia illustrilutea* sp. nov.

Gillisia illustrilutea (il.lus.tri.lu.te'a. L. adj. *illustris* bright; L. fem. adj. *lutea* yellow; N.L. fem. adj. *illustrilutea* bright yellow).

Gram-negative. Cells are rod-shaped, approximately 0.3–0.5 $\times$ 1–10 μ m. Non-motile. Cell mass is bright yellow. Flexirubin pigments are not formed. Colonies are circular and convex, with an entire edge and a butyrous consistency on MA. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–20 °C. No growth occurs at 25 °C or higher. Non-halophilic. Grows in 0–1.25 M NaCl, with optimal growth occurring in approximately 0.2–0.3 M NaCl. Grows in media prepared with 2 $\times$ strength sea salts, whereas no growth occurs in 4 $\times$ strength sea salts. Grows on TSA containing 1 % (w/v) NaCl, TSA and NA. Does not require yeast extract for growth, and can use inorganic nitrogen sources such as sodium nitrate and ammonium chloride. In mineral salts growth media can utilize D-glucose, D-mannose, maltose, sucrose, sodium propionate and L-proline as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O–F medium is negative for D-glucose. Hydrolyses DNA, but not casein, elastin, L-tyrosine, aesculin, starch, Tween 80 or urea. Produces α -galactosidase, α -glucosidase and β -glucosidase. Does not produce arginine dihydrolase or lecithinase, or reduce nitrate. Other phenotypic data are shown in Table 2. Mean DNA G + C content is 32 mol%.

The type strain is IC157^T (= ACAM 1062^T = CIP 108530^T), which was isolated from sea-ice algae collected from fast sea-ice cores, obtained from the coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Gillisia sandarakina* sp. nov.

Gillisia sandarakina [san.da.ra.kin'a. N.L. fem. adj. *sandarakina* (from Gr. fem. adj. *sandarakinê*) of orange colour].

Gram-negative. Cells are rod-shaped, approximately 0.4–0.5 $\times$ 1–10 μ m. Non-motile. Cell mass is light orange. Flexirubin pigments are not formed. Colonies are small (1 mm diameter), circular and convex, with an entire edge and a butyrous consistency on MA. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–20 °C. No growth occurs at 25 °C or higher. Requires sea salts for growth. Media supplemented only with Na⁺ ions do not support growth. Grows in 0.2–1.5 M NaCl, with optimal growth occurring in approximately 0.3–0.5 M NaCl. Grows in media prepared with 2 $\times$ strength sea salts, but no growth occurs with 4 $\times$ strength sea salts. Does not grow on TSA containing 1 % (w/v) NaCl, TSA or NA. Does not require yeast extract for growth, and can use inorganic nitrogen sources such as sodium nitrate and ammonium chloride. In mineral salts growth media can utilize D-glucose, DL-arabinose, D-mannose, sodium propionate (weak growth) and L-proline as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O–F medium is negative for D-glucose. Hydrolyses gelatin, starch, Tween 80

and L-tyrosine, but not casein, elastin, aesculin, DNA or urea. Produces α -glucosidase, β -glucosidase and alkaline phosphatase. Does not produce arginine dihydrolase or lecithinase. Reduces nitrate to nitrite. Other phenotypic data are shown in Table 2. Mean DNA G+C content is 36 mol%.

The type strain is IC148^T (= ACAM 1060^T = CIP 108529^T), which was isolated from sea-ice algae collected from fast sea-ice cores, obtained from the coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

Description of *Gillisia hiemivivida* sp. nov.

Gillisia hiemivivida [hi.em.i'vi.vi.da. L. fem. n. *hiems* -emis the cold (of winter); L. fem. adj. *vivida* lively; N.L. fem. adj. *hiemivivida* lively in the cold)].

Gram-negative. Cells are rod-shaped, approximately 0.4–0.6 × 1–7 µm. Non-motile. Cell mass is light orange. Flexirubin pigments are not formed. Colonies are circular and convex, with an entire edge and a butyrous consistency on MA. Growth occurs at –2 °C in marine broth. Good growth occurs on MA at 1–25 °C. No growth occurs at 30 °C or higher. Requires sea salts for growth. Media supplemented only with Na⁺ ions do not support growth. Grows in media containing 0.2–1.5 M NaCl, with optimal growth occurring in approximately 0.3–0.5 M NaCl. Grows in media prepared with 2× strength sea salts, but no growth occurs in 4× strength sea salts. Does not grow on TSA containing 1% (w/v) NaCl, TSA or NA. Does not require yeast extract for growth, and can use inorganic nitrogen sources such as sodium nitrate and ammonium chloride. In mineral salts growth media can utilize D-glucose, glycogen, N-acetylglucosamine, DL-arabinose, D-mannose, maltose, sucrose, sodium propionate, L-alanine (weak growth) and L-proline as sole carbon and energy sources. Acid production from carbohydrates on Leifson's O-F medium is negative for D-glucose. Hydrolyses gelatin, starch, Tween 80, L-tyrosine and urea, but not casein, elastin, aesculin or DNA. Produces α -glucosidase, β -glucosidase and alkaline phosphatase. Does not produce arginine dihydrolase or lecithinase, or reduce nitrate. Other phenotypic data are shown in Table 2. Mean DNA G+C content is 34 mol%.

The type strain is IC154^T (= ACAM 1061^T = CIP 108528^T), which was isolated from sea-ice algae collected from fast sea-ice cores, obtained from the coastal areas of the Vestfold Hills, an ice-free region of East Antarctica.

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