Algoriphagus ratkowskyi gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats

John P. Bowman,[1] Carol Mancuso Nichols[1] and John A. E. Gibson[2]

[1]School of Agricultural Science, University of Tasmania, Hobart, Tasmania 7001, Australia

[2]CSIRO Marine Research, Castray Esplanade, Hobart, Tasmania 7001, Australia

Correspondence
John P. Bowman
john.bowman@utas.edu.au

Several cold-adapted strains isolated from a variety of algal-rich Antarctic and Southern Ocean samples formed three distinct groups within the class *Flavobacteria*, phylogenetically distant from other cultivated species. The first taxon, designated *Algoriphagus ratkowskyi* gen. nov., sp. nov., was isolated from sea ice and from saline lake cyanobacterial mats and includes non-motile, strictly aerobic, saccharolytic rod-like or serpentine strains that were most closely related to the genus *Cyclobacterium* according to 16S rDNA sequence analysis (sequence similarity 0·85). The second taxon, designated *Brumimicrobium glaciale* gen. nov., sp. nov., isolated from sea ice and from continental shelf sediment, formed gliding, rod-like cells that were facultatively anaerobic with a fermentative metabolism. The third taxon, designated *Cryomorpha ignava* gen. nov., sp. nov., isolated from Southern Ocean particulates and from quartz stone subliths, included strictly aerobic, pleomorphic rod-like cells. *Brumimicrobium glaciale* and *Cryomorpha ignava* were most closely allied with '*Microscilla aggregans* var. *catalatica*', which, on the basis of its distinctive taxonomic traits, is also proposed as a new genus and species, *Crocinitomix catalasitica* gen. nov., sp. nov. It is proposed that the three genera *Brumimicrobium*, *Cryomorpha* and *Crocinitomix* belong to a new family, *Cryomorphaceae* fam. nov. (type genus *Cryomorpha*), as they possess generally similar morphological and ecophysiological characteristics and form a common and distinct clade within class *Flavobacteria*.

INTRODUCTION

Members of the class *Flavobacteria* (current designation according to Cavalier-Smith, 2002) are widely distributed in nature and are especially common in cold marine environments. Fluorescent *in situ* hybridization analyses suggest that *Flavobacteria* become more common in the Southern Ocean euphotic zone with increasing latitude (Simon *et al.*, 1999) and, in the presence of algal blooms, can at times make up the bulk of the bacterioplanktonic population (Glöckner *et al.*, 1999; Kirchman, 2002). Marine species of the family *Flavobacteriaceae* make up most representatives of the *Flavobacteria* in the Southern Ocean and Arctic Ocean sea-ice and sea-water ecosystems (Bano & Hollibaugh, 2002; Brown & Bowman, 2001). So far, only a small fraction of the polar flavobacteria that have been detected using various molecular analyses such as 16S rDNA clone library and denaturing gradient gel electrophoretic analyses have been cultivated and characterized. Interest in marine flavobacteria has increased relatively recently for two reasons. Firstly, the class contains many species that are probably integral to the flow of carbon and energy in the marine environment (Kirchman, 2002). Over half of the organic matter formed by photosynthetic primary production is broken down by bacteria (Cole *et al.*, 1988) and it has been demonstrated that flavobacteria are major decomposers of high-molecular-mass organic matter in sea water (Cottrell & Kirchman, 2000). Secondly, polar flavobacteria are often cold-adapted (psychrophilic or psychrotolerant), and the group includes substantial concentrations of psychrophilic species. Psychrophilic flavobacteria are noted as excellent sources of cold-adapted enzymes, which have many industrial applications (as reviewed by e.g. Nichols *et al.*, 1999; Bull *et al.*, 2000).

The systematics of class *Flavobacteria* is currently in a state of rapid expansion, with many new genera being described within the last few years, in particular from the marine environment. Additions in the last 5 years within the family

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains IC156^T, 1-22^T, IC025^T and NCIMB 1418^T are AF521195, AF170738, U85891 and M58791.

Flavobacteriaceae include the genera *Gelidibacter*, *Psychroserpens*, *Polaribacter*, *Psychroflexus*, *Cellulophaga*, *Salegentibacter*, *Arenibacter*, *Zobellia*, *Muricauda*, *Tenacibaculum* and *Aequorivita*. In addition to these new taxa, generically misclassified species are being steadily re-evaluated, with several species so far assigned to different or novel genera following polyphasic taxonomic analysis (Bernardet *et al.*, 2002). With the development and implementation of minimal standards for the classification of *Flavobacteria* (Bernardet *et al.*, 2002), it is expected that novel species and genera can be added readily so that a robust and reliable taxonomy of the *Flavobacteria* can eventually be realized.

In this study, several novel *Flavobacteria* strains from disparate polar marine and polar terrestrial habitats were studied. All form distinct branches within the class *Flavobacteria* and represent novel genera, as indicated by polyphasic taxonomic analysis.

METHODS

Samples. Sublithic material was sampled from the undersides of quartz stones collected from feldfield areas of the Vestfold Hills, as described previously (Smith *et al.*, 2000). Surface sea-water and sea-ice samples were collected in August 1999 over a short transect through the Mertz Polynya, an ice formation zone near the Antarctic coast (66–67°S 142–143°E). In a later marine cruise (January 2000), sediment from the continental shelf region (depth 710–940 m) in the Mertz Glacier Polynya region was sampled using either a Shipek sediment grabber or a gravity corer. Sea-ice samples were collected using a SIPRE ice corer and processed as described by Bowman *et al.* (1997a). Sea-water samples were collected either by bucket or from an inline shipboard water sampler, which takes samples directly from a 7 m water depth. Water samples (1·0–3·0 l sample volumes) and melted sea ice (0·3–2·0 l sample volumes) were filtered through a 1·0 μm filter and then through a 0·2 μm filter. Material present on the two filter types was then resuspended separately in 10–20 ml 0·2 μm-filtered natural sea water. In addition, ice-cover material, shoreline sediment and algal clumps, consisting mostly of filamentous cyanobacteria, were collected from the surface edge zone of Ace Lake, Vestfold Hills, Antarctica (68°S 78°E). Most samples were stored at 2–4 °C for 2–12 weeks before addition to growth media.

Bacterial isolation. Some strains were isolated during previous studies of sea ice (Bowman *et al.*, 1997a, c) or from quartz stone sublith material (Smith *et al.*, 2000) using either marine 2216 agar medium or trypticase soy agar supplemented with sea salts (Sigma; 35 g l^{-1}). Additional strains were obtained from samples that were first suspended at various dilutions in 0·1× or 1× sea-water nutrient (SWN) medium (Bowman *et al.*, 2003) or in marine 2216 broth (Difco) that had been pre-chilled to 2 °C. Two strains were isolated from continental shelf sediment using 0·1× SWN supplemented with (l^{-1}) 0·1 g L-cystine, 0·25 g sodium thioglycollate, 0·25 g sodium formaldehyde sulphoxylate and 2 mg methylene blue and incubated anaerobically at 10 °C using Oxoid anaerobic gaspaks in airtight jars. All variants of the SWN medium were prepared in artificial sea water (Sigma sea salts, 35 g l^{-1}) (Bowman & Nichols, 2002). For isolation of strains from solid medium, agar plates were typically incubated at 2–4 °C for at least 4–6 weeks. Colonies were selected and subcultured on to marine 2216 agar to obtain pure cultures. SWN agar was not used for routine cultivation as strains tended to lose viability relatively quickly. Strains were best maintained as viable cultures on marine 2216 agar slants or plates at 2 °C (survival at least 12 months) and were also cryopreserved frozen at −80 °C within marine 2216 liquid medium supplemented with 30 % glycerol.

Phenotypic analysis. Tests used have been detailed previously by Bowman *et al.* (1998a). Motility was tested using the hanging-drop method, while gliding motility was tested on strains grown for 1–2 days on 0·1× SWN (solidified with 1 % agar noble) and, after 1–2 days incubation at 10–12 °C, observed using phase-contrast microscopy. In addition to the Antarctic strains, phenotypic analysis was also performed on '*Microscilla aggregans* var. *catalatica*' NCIMB 1418^T.

Fatty acid analysis. All strains were cultivated on marine 2216 agar at 10–12 °C for 3–5 days and then harvested. Whole-cell fatty acid profiles were determined quantitatively using GC and GC-MS procedures (Nichols *et al.*, 1993). The positions of double bonds in monounsaturated fatty acids were determined by the dimethyl disulphide addition method of Nichols *et al.* (1986).

DNA base composition and DNA–DNA hybridization. Genomic DNA was extracted and purified from cells using the procedure of Marmur & Doty (1962). The DNA G+C content was then determined from thermal denaturation profiles (Sly *et al.*, 1986). Genomic DNA–DNA hybridization was performed using the spectrophotometric renaturation kinetics procedure (Bowman *et al.*, 1998a).

16S rDNA sequence analysis. The 16S rRNA gene was amplified from all of the isolates obtained using PCR, as described previously (Bowman *et al.*, 1997b). The amplicons were purified using the Prep-A-gene kit (Bio-Rad) and sequences were generated by using ABI Prism BigDye terminator cycle sequencing kits with sequence reactions separated and analysed on an ABI377 automated DNA sequencer. Sequences generated ranged from 1420 to 1430 bp in length. Sequences were initially analysed first by gapped BLAST analysis operated through the National Center of Biotechnology Information website (http://www.ncbi.nlm.nih.gov). From these searches, selected sequences were downloaded and nearly complete sequences were aligned manually to the Antarctic strains. The region of the 16S rDNA aligned and analysed extended from position 31 to 1470 (*Escherichia coli* equivalent). Subsequent phylogenetic analyses of the sequence datasets utilized PHYLIP version 3.57c (Felsenstein, 1993), applying the maximum-likelihood algorithm (DNADIST) option to calculate distances. Phylogenetic trees were then constructed from the distance data by employing the neighbour-joining method (NEIGHBOR). Bootstrap analysis used the programs SEQBOOT and CONSENSE using 250 replicate analyses.

RESULTS AND DISCUSSION

Morphological characteristics of isolates

Nine Antarctic strains were investigated. Colonial morphology (Difco 2216 marine agar, 7 days, 10 °C) clearly separated the strains into three groups.

The first group, including strains IC025^T, IC172a, IC026 and A230 (the IC025 group) (Table 1), produced salmon-pink, smooth, circular, convex colonies (1–3 mm diameter) with an entire edge and a butyrous consistency on marine 2216 agar incubated at 10 °C for 7–10 days. Serpentine filamentous cells (8–20 × 0·4–0·6 μm) (Fig. 1a) occurred occasionally within cultures, appearing more frequently in low-nutrient media. As cultures aged past 10–14 days, these filamentous cells fragmented completely into small

Table 1. Strains investigated in this study

Strain	Isolation site	Isolation location	Medium used for isolation*
Algoriphagus ratkowskyi gen. nov., sp. nov.			
IC025^T (=ACAM 646^T=LMG 21435^T=CIP 107452^T)	Sea-ice algal assemblage	Prdyz Bay, Antarctica	Marine 2216
IC172a	Sea-ice algal assemblage	Mertz Glacier Polynya, Antarctica	Marine 2216
IC026	Sea-ice algal assemblage	Prdyz Bay, Antarctica	Marine 2216
A230	Cyanobacterial mat	Ace Lake, Antarctica	SWN
Brumimicrobium glaciale gen. nov., sp. nov.			
IC156^T (=ACAM 645^T=LMG 21434^T=CIP 107451^T)	Sea-ice algal assemblage	Ellis Fjord, Antarctica	Marine 2216
MGP-8AN	Continental shelf sediment (depth, 709 m)	Mertz Drift, Antarctica	0·1× SWN/anaerobic
MGP-18AN	Continental shelf sediment (depth, 943 m)	Mertz Drift, Antarctica	0·1× SWN/anaerobic
Cryomorpha ignava gen. nov., sp. nov.			
1-22^T (=ACAM 647^T=LMG 21436^T=CIP 107453^T)	Quartz stone sublith	Vestfold Hills, Antarctica	TSA+sea salts
O1-14	Sea-water particulates	Mertz Glacier Polynya, Antarctica	SWN
Cyclobacterium marinum ATCC 25205^T	Sand dollar (*Poro triacanthus*) coelomic fluid	Newport Beach, CA, USA	–
'*Microscilla aggregans* var. *catalatica*' NCIMB 1418 (=ATCC 23190)	Under frozen sand, upper littoral zone	Auke Bay, AK, USA	–

*Medium formulations are detailed in Methods.

coccobacilli (0·4–0·9×0·3–0·5 μm) (Fig. 1a). The strains were non-motile.

The second group included strains IC156^T, MGP-8AN and MGP-18AN (the IC156 group), which produced intensely orange-pigmented colonies (1–2 mm diameter) that were smooth, circular, convex, with an entire edge and a somewhat gelid consistency when grown on marine 2216 agar (7–10 days at 10 °C). Cells formed were single, slender, slightly curved or straight rods, 0·3–0·4 μm wide and 1–3 μm long (Fig. 1b). Cells were capable of gliding, though this trait was only clearly apparent on dilute media such as 0·1× SWN.

The third group included strains 1-22^T and O1-14 (the 1-22 group), which produced pinpoint to small (0·5–1 mm diameter), orange-pigmented colonies that were smooth, circular, convex in elevation, with an entire edge and a viscid consistency when grown on marine 2216 agar (7–10 days at 10 °C). Cells in either liquid or solid culture appeared as non-motile, slightly curved or straight rods that ranged in size from 0·5 to 3·0 μm in length and 0·3 to 0·5 μm in width (Fig. 1c).

Ecophysiological characteristics

The strains possessed ecophysiological traits characteristic of many Antarctic marine bacterial species. All strains were cold-adapted, growing optimally at temperatures below 22 °C, exhibiting growth at −1 to −2 °C, and all required Na^+ ions at sea-water concentrations for optimal growth. None of the strains studied was halotolerant, as they were unable to grow in the presence of NaCl concentrations greater than 0·8–1·0 M. All strains were neutrophilic, growing best between pH 6·0 and 8·0.

The IC025 group strains grew optimally at 16–19 °C (μ_{max} 0·14–0·17 h^{-1}) with a growth T_{max} (maximum temperature for growth) of about 23–27 °C. IC156 strains possessed very similar temperature optima and possessed a similar growth rate (0·12–0·14 h^{-1}). Strains of both the IC025 and IC156 groups could grow in a medium in which sea-water salts were replaced with 0·1–1·0 M NaCl. By comparison, strains of the 1-22 group grew more slowly, with a growth rate of 0·092–0·098 h^{-1}, and possessed lower cardinal temperatures, with an optimal temperature of 15 °C and a growth T_{max} of 20–22 °C. Strains of the 1-22 group exhibited an absolute requirement for sea water and were unable to grow in medium supplemented with Na^+ ions alone.

Biochemical and nutritional characteristics

Strains of both the IC025 and 1-22 groups were strictly aerobic. Strains of these groups were unable to reduce nitrate or grow anaerobically by fermentation with D-glucose or other carbohydrates in Leifson's O/F medium. In addition, the strains could not grow anaerobically by respiration with a variety of electron acceptors including ferric iron, nitrate, elemental sulphur, trimethylamine *N*-oxide and anthroquinone sulphate in a medium that included DL-lactate and acetate as the electron donors and

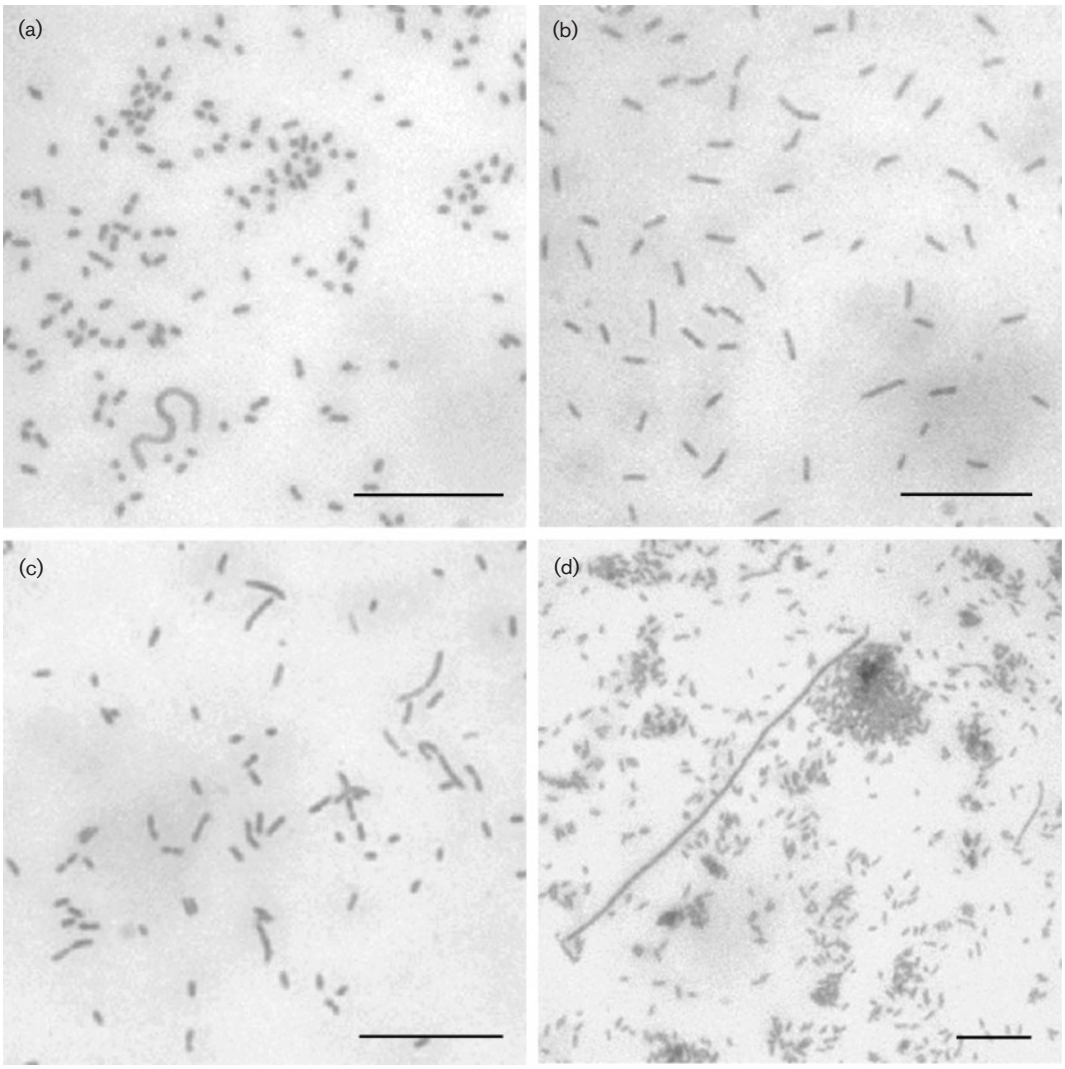

Fig. 1. Photomicrographs of cells of *Algoriphagus ratkowskyi* gen. nov., sp. nov. IC025^T (a), *Brumimicrobium glaciale* gen. nov., sp. nov. IC156^T (b), *Cryomorpha ignava* gen. nov., sp. nov. 1-22^T (c) and *Crocinitomix catalasitica* gen. nov., sp. nov. NCIMB 1418^T (d). Strains were grown on marine 2216 agar at 10 °C for 7 days and observed under phase-contrast microscopy. Bars, 10 μm.

supplemented with 1 g yeast extract l^{-1} and vitamins. IC156 strains, however, could ferment D-glucose in the absence of nitrate. IC156 strains could also reduce nitrate to nitrite but, under anaerobic conditions, did not use nitrate as an electron acceptor for growth when DL-lactate was provided as the electron donor.

Strains of the IC025 group could grow in a defined mineral salts medium containing nitrate but not ammonia as the sole nitrogen source. Strains of this group could also use L-glutamate as a nitrogen source, but not as a sole carbon source. For good growth, strains of the IC156 and 1-22 groups required yeast extract (>0·025 %) and a vitamin solution for growth when 0·1 % peptone (or 0·1 % Casamino acids) was supplied as a nitrogen source. Poor or no growth occurred if yeast extract was omitted.

Individual carbon compounds were found to be readily utilizable as sole sources of carbon and energy by the IC025 strain group only. Strains of the IC025 group exhibited most active growth on carbohydrates, especially maltose and sucrose. Growth of strains of the IC156 group was stimulated markedly by 0·2 % D-glucose. No growth or stimulation of growth occurred otherwise on 74 substrates tested for the IC156 or 1-22 groups using a mineral salts medium supplemented with 0·025 % yeast extract, a vitamin solution (Balch & Wolfe, 1976) and Tween 80 (at 0·005 %). In addition, for these strains, no growth occurred in auxanographic carbon assimilation test strips (bioMérieux-Vitek) in which the growth medium was supplemented with sea salts (25 g l^{-1}) and yeast extract (0·025 %).

Most strains of the IC025 group could hydrolyse casein,

starch and chitin and also produced a wide range of glycosidic enzymes (Table 2), but catalase activity was very weak or absent. Both groups IC156 and 1-22 were relatively inert biochemically, though some strains produced esterases (Tween 80 hydrolysis) and, in the case of the IC156 group, alkaline phosphatase and arginine arylamidase. Strains of the 1-22 group exhibited a very strong catalase reaction.

Fatty acid profiles

Whole-cell fatty acid profiles clearly distinguished representative strains of each of the strain groups from each other (Table 3) as well as from other members of the class *Flavobacteria* for which fatty acid profiles are available (data from various publications). Like many flavobacteria, the Antarctic strains contained high levels of C_{13}–C_{17} branched fatty acids (Bowman *et al.*, 1998b). Indeed, strain IC156^T contained mostly branched fatty acids (summed total 89·3 %), including i15 : 0 (36·4 % of total fatty acids) and a15 : 1ω10*c* (45·0 %), and possessed only low levels of other fatty acid types. The branched-chain fatty acid i13 : 0 (5·6 %) was the only other major fatty acid component found in the strain. Strain IC025^T contained large amounts of straight-chain monounsaturates (summed total 46·1 %), including 16 : 1ω7*c* (34·1 %) and 16 : 1ω5*c* (11·2 %), as well as several branched and hydroxy fatty acid components that are commonly found amongst members of the *Flavobacteria* (e.g. 15 : 0, i15 : 0, a15 : 0, i15 : 1ω10*c*; Bowman *et al.*, 1998b). The strain was also rich in α-hydroxy (2-OH) components. Normally, β-hydroxy (3-OH) fatty acid components are produced by members of the *Flavobacteria*, with relative levels of hydroxy fatty acids varying widely between different genera (Bowman *et al.*, 1998b). Strain 1-22^T was also rich in α-hydroxy fatty acids (Table 3) and, unlike the other strains, possessed high levels of iso-branched C_{14} fatty acids. The fatty acid profile of NCIMB 1418^T contained high levels of the common lipids 15 : 0 and i15 : 0 as well as an interestingly distinct and unusual sequence of branched and straight-chain monounsaturates including i15 : 1ω10*c*/15 : 1ω11*c*, i16 : 1ω11*c*/16 : 1ω12*c* and i17 : 1ω12*c*/17 : 1ω13*c*.

Genotypic comparisons

DNA G+C content and DNA hybridization analysis confirmed that each of the three morphological groups were distinct and coherent genospecies. Strains of the IC025 group possessed common hybridization values of 89–97 % with a mean G+C content of 36 mol% (range 35·4–35·9 mol%). Strains of the IC156 group possessed a mean G+C content of 39 mol% (range 38·4–39·5 mol%) and hybridization values for the three strains ranged from 82 to 101 %. Strains 1-22^T and O1-14 hybridized at a level of 65 % and possessed similar DNA G+C contents, of 36·8 and 37·4 mol%.

16S rDNA sequence analysis

16S rDNA sequence analysis revealed that the strain groups were distinct from each other and all known cultured members of the class *Flavobacteria*. Strain IC025^T was most closely related to the species *Cyclobacterium marinum*, with a sequence similarity of only 0·85 (Fig. 2). Strains IC156^T and 1-22^T formed a common deep branch along with strain NCIMB 1418^T, which is known as '*Microscilla aggregans* var. *catalatica*' (Lewin, 1969; Lewin & Lounsbery, 1969; Mandel & Lewin, 1969; Reichenbach, 1989), though, in culture collection catalogues, it is also referred to as '*Microscilla aggregans* subsp. *catalatica*' or '*Flexibacter aggregans* subsp. *catalaticus*', sharing a sequence similarity of only 0·82–0·84 (Fig. 2).

Taxonomic comparisons

On the basis of a variety of taxonomic criteria, the three strain groups analysed were distinct and coherent taxonomic entities. At the phenotypic level, the IC025 group of strains was distinct in that it formed pink-pigmented colonies, was strongly saccharolytic and produced a negative catalase reaction. The closest related species, *Cyclobacterium marinum* (Raj & Maloy, 1990), has a similar pigmentation and is also saccharolytic, but is catalase-positive. Though other ecophysiological and nutritional differences are evident between these taxa (Table 2), they differ most obviously in terms of cellular morphology. The morphology of the IC025 strain group is quite unlike that of *Cyclobacterium marinum*, which forms highly curved, coiled and circular cells. In addition, 16S rDNA sequence similarity is quite low between the taxa (similarity 0·85), a level that clearly indicates that they belong to separate genera. Compared with other marine flavobacteria, the IC025 strain group is readily separated by a combination of phenotypic traits (Table 4). Thus, it is proposed the IC025 strain group represents a novel bacterial genus of the class *Flavobacteria* and has the proposed name *Algoriphagus ratkowskyi* gen. nov., sp. nov.

The IC156 and 1-22 strain groups possessed superficial similarity in terms of morphology, pigmentation, ecophysiology and nutrition and are related, albeit distantly, by 16S rDNA sequence data (Fig. 2). The IC156 strain group differs from the 1-22 strain group in that it is facultatively anaerobic, is able to reduce nitrate and is able to utilize D-glucose both oxidatively and fermentatively. The groups also differ radically in terms of their fatty acid profiles (Table 3). Together, these characteristics strongly suggest that the two strain groups form distinctly different taxa equivalent to genera.

On the basis of general phenotypic characteristics, the IC156 strain group is most likely to be confused with the genus *Polaribacter* (Gosink *et al.*, 1998) (Table 4). However, *Polaribacter* strains have lower temperature optima (no growth at 20 °C), often produce gas vesicles, tend to form short filamentous cells and have a lower DNA G+C content. In addition, the fatty acid profiles of *Polaribacter* strains are considerably different, lacking the major fatty acid a15 : 1ω10*c* that makes up nearly half of the fatty acid in strain IC156^T. Strain group IC156 is represented by a

Table 2. Phenotypic characteristics that differentiate the Antarctic strain groups and most closely related members of class *Flavobacteria*

Taxa: 1, IC025 group (*Algoriphagus ratkowskyi* gen. nov., sp. nov.); 2, *Cyclobacterium marinum* (data from Raj & Maloy, 1990); 3, IC156 group (*Brumimicrobium glaciale* gen. nov., sp. nov.); 4, 1-22 group (*Cryomorpha ignava* gen. nov., sp. nov.); 5, '*Microscilla aggregans* var. *catalatica*' NCIMB 1418 (*Crocinitomix catalasitica* gen. nov., sp. nov.). Abbreviations: +, positive for all strains; W, only weakly positive or growth is poor; V, result varies between strains; V/W, variable and weak; −, negative for all strains; L-DOPA, 3,4-dihydroxyphenyl-L-alanine; ND, no data available. The following tests were negative for all strains: indole production, arginine dihydrolase, Voges–Proskauer test, hydrolysis of cellulose (filter paper), gelatinase, agar hydrolysis and hydrolysis of tributyrin.

Characteristic	1	2	3	4	5
Filaments > 100 μm	−	−	−	−	+
Coiled, ring-shaped, spiral cells	−	+	−	−	−
Gliding motility	−	−	+	−	+
Colour of cell mass	Pink	Pink	Orange	Orange	Yellow
Salinity range for growth (multiples of sea water)	0·5–2	0·5–3	0·5–2	0·5–1·5	0·5–2
Growth on/at:					
2·5 % NaCl	+	+	+	−	+
10 % NaCl	−	+W	−	−	−
25 °C	+W	+	V/W	−	+
35 °C	−	+	−	−	−
1 % Peptone/sea-water agar	+	+	V/W	−	+
Trypticase soy agar	+W	+	−	−	+W
Nutrient agar	−	+W	−	−	−
Survives 55 °C, 10 min	−	+	−	−	−
Sole nitrogen source:					
Peptone	+	+	+	V/W	+
Casamino acids	+	+	V	−	−
L-Glutamate	+	+	−	−	−
Sodium nitrate, ammonium sulphate	−	+	−	−	−
Yeast extract and vitamin requirement	−	−	+	+	+
Acid from carbohydrates (aerobic)	+	+	−	−	−
Utilization of glucose	+	+	+	−	−
Fermentation of glucose, nitrate reduction	−	−	+	−	−
H_2S production from L-cysteine	−	−	−	−	+
Hydrolysis of:					
Casein	+	−	−	−	−
Starch, chitin	V	−	−	−	−
Tween 80	−	−	+	V	+
Urea	−	−	−	V/W	−
DNA	−	−	V	−	−
L-Tyrosine, L-DOPA	−	ND	−	−	+
Carboxymethylcellulose, urate	−	ND	−	−	−
Production of:					
Catalase	−	+	+	+	+
Oxidase	+	+	−	−	−
Alkaline phosphatase	+	+	+	−	+
β-Galactosidase	+	+	V	−	−
α-Galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, *N*-acetyl-β-glucosaminidase	+	ND	−	−	−
β-Glucuronidase	V/W	ND	−	−	−
Arginine arylamidase	+	ND	+	−	+
Glutamyl glycine arylamidase	+	ND	V	−	−
Utilization as sole carbon source:					
D-Gluconate, malonate, succinate	+	+	−	−	−
Fumarate, malate	V	+	−	−	−
Glycerol phosphate, propionate, L-serine	+	−	−	−	−
Acetate, citrate, DL-tartrate, pyruvate, oxaloacetate	−	+	−	−	−
L-Glutamate	−	V	−	−	−

Table 3. Whole-cell fatty acid profiles of Antarctic strain groups and strain NCIMB 1418^T

Values are percentages of total fatty acids. Fatty acids are designated as total number of carbon atoms : number of double bonds, followed by the position of the double bond from the aliphatic end of the molecule. The prefixes i, a and OH respectively indicate iso-branched, anteiso-branched and hydroxy fatty acids. The suffix *c* indicates the *cis* isomer. tr, Trace levels (<0·1 %) detected.

Fatty acid	IC025^T	IC156^T	1-22^T	NCIMB 1418^T
Saturated fatty acids				
13 : 0	–	0·5	–	–
14 : 0	0·4	1·5	–	0·3
15 : 0	0·2	2·9	0·8	29·3
16 : 0	1·5	1·9	0·9	1·8
17 : 0	–	–	–	–
18 : 0	0·4	0·3	–	–
Sum	2·5	7·1	1·7	31·4
Saturated branched-chain fatty acids				
i13 : 0	–	5·6	–	–
a13 : 0	–	tr	–	–
i14 : 0	0·9	0·8	13·8	–
i15 : 0	13·4	36·4	7·8	36·3
a15 : 0	3·8	tr	5·8	–
i16 : 0	2·7	0·6	7·8	0·4
i17 : 0	0·2	–	–	–
a17 : 0	0·3	–	–	–
Sum	21·3	43·4	35·2	36·7
Unsaturated branched-chain fatty acids				
i14 : 1ω9*c*	–	0·4	6·8	0·7
i15 : 1ω10*c*	8·1	–	7·1	10·9
a15 : 1ω10*c*	0·2	45·0	8·2	–
i16 : 1ω11*c*	–	–	2·9	0·3
i16 : 1ω6*c*	6·9	0·5	–	–
i17 : 1ω13*c*	–	–	–	0·2
i17 : 1ω12*c*	–	–	–	0·7
i17 : 1ω7*c*	3·9	–	–	–
i17 : 1ω5*c*	1·2	–	–	–
a17 : 1ω13*c*	–	–	–	tr
Sum	20·3	45·9	25·0	12·8
Monounsaturated fatty acids				
14 : 1ω5*c*	tr	–	–	–
15 : 1ω11*c*	0·2	–	–	8·3
15 : 1ω8*c*	–	1·4	–	–
15 : 1ω6*c*	0·1	–	–	–
15 : 1ω4*c*	–	–	–	tr
16 : 1ω12*c*	–	–	–	4·4
16 : 1ω7*c*	34·1	1·5	–	–
16 : 1ω5*c*	11·2	–	–	tr
17 : 1ω13*c*	–	–	–	2·1
17 : 1ω6*c*	0·5	–	–	–
18 : 1ω9*c*	–	0·4	–	–
18 : 1ω7*c*	–	0·3	–	–
Sum	46·1	3·6	0	14·8
Hydroxy fatty acids				
2-OH i14 : 0	–	–	6·7	–
2-OH i15 : 0	4·2	–	1·6	–
2-OH a15 : 0	3·6	–	18·4	–
3-OH 15 : 0	–	–	–	tr
3-OH i15 : 0	0·3	–	–	1·0

Table 3. cont.

Fatty acid	IC025^T	IC156^T	1-22^T	NCIMB 1418^T
3-OH 16:0	–	–	2·9	–
3-OH i16:0	0·4	–	6·8	tr
3-OH i17:0	1·1	–	–	3·3
3-OH 17:0	–	–	–	tr
Sum	9·6	0	36·4	4·3

single species, as indicated by DNA–DNA hybridization, and is thus designated *Brumimicrobium glaciale* gen. nov., sp. nov.

In the process of phenotypic identification, the 1-22 strain group is most likely to be confused with orange-pigmented species of the genus *Aequorivita* (Bowman & Nichols, 2002) (Table 4), including *Aequorivita antarctica* and *Aequorivita sublithincola*, which occur in the same Antarctic habitats and which also happen to be rather biochemically inert. These species can still be differentiated from 1-22^T and related strains by various phenotypic characteristics as well as fatty acid patterns. *Aequorivita antarctica* is able to hydrolyse starch and aesculin and to utilize D-glucose aerobically as a sole carbon and energy source, unlike 1-22 group strains, while both *Aequorivita* species form comparatively much longer cells. The fatty acid components i14:0, i14:1ω9*c*, 2-OH i14:0 and 2-OH a15:0, which occur at high levels in 1-22^T, are absent or at trace levels in *Aequorivita* species. Strain group 1-22 is represented by a single species and is designated *Cryomorpha ignava* gen. nov., sp. nov.

The genera *Brumimicrobium* and *Cryomorpha* are most closely related to '*Microscilla aggregans* var. *catalatica*' (strain NCIMB 1418=ATCC 23190), and the three groups form a deep common branch (bootstrap support 92%) on the periphery of the family *Flavobacteriaceae*. Strain NCIMB 1418^T was originally isolated by Lewin (1969), who included it within the species '*Microscilla aggregans*' (ATCC 23162) (name not validly published) but, as it was the only strain of the species to form catalase, it was designated var. *catalatica*. Reichenbach (1989) suggested the strain did not belong to '*Microscilla aggregans*' (Lewin 1969) as it differed in a number of traits, including an inability to utilize carbohydrates and several tested nitrogen sources and possession of psychrotolerance. The G+C content of NCIMB 1418^T is also lower than that of '*Microscilla aggregans*' (35 compared with 37–42 mol%). Most importantly, 16S rDNA sequence data indicated that NCIMB 1418^T was quite remote from the proposed type strain of '*Microscilla aggregans*', ATCC 23162 (sequence similarity 0·79). In general terms, the phenotypic traits of NCIMB 1418^T have superficial similarity to those of the IC156 and 1-22 groups, including the ability to grow at low temperatures, general lack of nutritional versatility and requirement for several growth factors and organic nitrogen sources. However, the strain is quite readily distinguishable

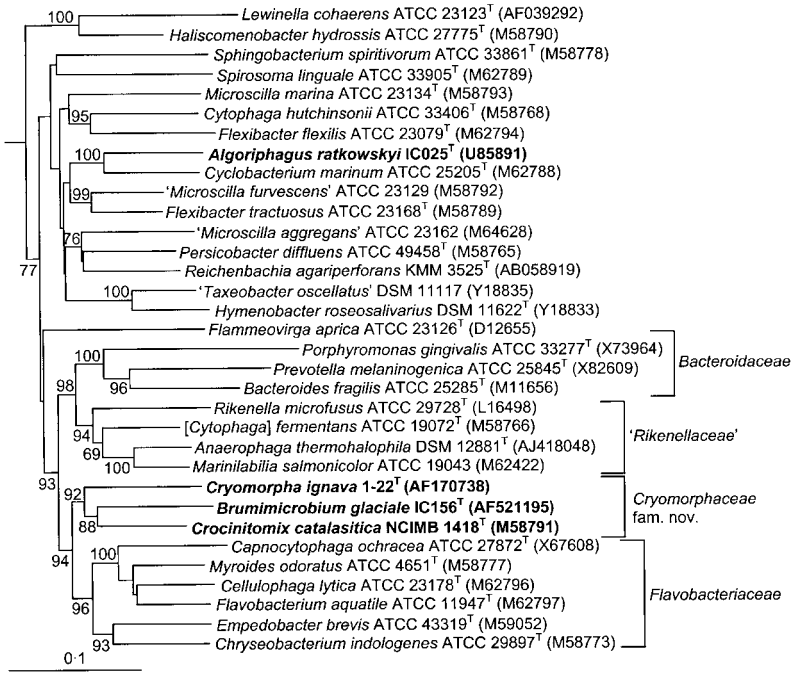

Fig. 2. Phylogenetic relationships of the novel Antarctic and related species amongst class *Flavobacteria*, showing the position of *Cryomorphaceae* fam. nov. The tree is based on 16S rDNA sequences (positions 31–1470, *E. coli* equivalent) and was created using maximum-likelihood distances clustered by the neighbour-joining method. The most significant bootstrap support values (>60%) are indicated next to branch nodes. GenBank nucleotide accession numbers are given in parentheses. The outgroup sequence used for the analysis was from *Chlorobium limicola*.

Table 4. Differentiation of the genera *Algoriphagus*, *Brumimicrobium*, *Cryomorpha* and *Crocinitomix* from other marine genera of class *Flavobacteria*

Genera: 1, *Algoriphagus* gen. nov.; 2, *Crocinitomix* gen. nov.; 3, *Brumimicrobium* gen. nov.; 4, *Cryomorpha* gen. nov.; 5, *Cyclobacterium*; 6, *Marinilabilia*; 7, *Polaribacter*; 8, *Aequorivita*; 9, *Persicobacter*; 10, *Microscilla sensu stricto* (*Microscilla marina* only); 11, '*Microscilla aggregans*'; 12, *Gelidibacter*; 13, *Arenibacter*; 14, *Salegentibacter*; 15, *Cellulophaga*; 16, *Zobellia*; 17, *Psychroserpens*; 18, *Psychroflexus*; 19, *Tenacibaculum*; 20, *Muricauda*; 21, *Flammeovirga*; 22, *Lewinella*; 23, *Flexithrix*. Cold-adapted is defined here as being able to grow at 4 °C or lower. Abbreviations: +, most strains positive; −, most strains negative; V, varies widely between species and/or strains; ND, not determined or uncertain data. Data from this study, Nakagawa & Yamasato (1996), Nakagawa *et al.* (1997), Reichenbach (1989), Bowman *et al.* (1997b, 1998b), Ivanova *et al.* (2001), McCammon & Bowman (2000), Bowman (2000), Barbeyron *et al.* (2001), Suzuki *et al.* (2001) and Bruns *et al.* (2001).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Pigmentation*	P	Y	O	O	P	P	O	Y/O	P	O	Y	Y	O	Y	Y	Y (F)	Y	O	Y	Y	O	Y/O	Y
Filaments always present	−	+	−	−	−	+	V	−	+	+	+	+	−	−	−	−	+	V	V	−	+	+	+
Helical, circular cells	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Sheath formation	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Gliding motility	−	+	+	−	−	+	−	−	+	+	+	+	−	−	+	+	−	V	+	+	+	+	+
Anaerobic growth	−	−	+	−	−	+	V	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Utilizes carbohydrates	+	−	+	−	+	+	+	V	+	+	+	+	+	+	+	+	−	+	V	+	+	−	+
Growth factors required	−	+	+	+	−	−	+	+	ND	−	+	−	−	−	−	−	+	V	ND	−	ND	−	−
Catalase produced	−	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−
Cold-adapted	+	+	+	+	+	−	+	+	−	ND	−	V	−	+	+	V	+	+	V	−	ND	ND	−
G+C content (mol%)	35–36	35	38–40	36–37	35	37–41	31–33	33–39	40–42	42	37–42	36–38	37–38	37–38	32–38	42–44	27–29	32–36	30–33	41	35–37	45–53	37

*O, Orange; Y, yellow; P, pink; F, flexirubins.

from the Antarctic strains in terms of cellular and colonial morphology, as the taxon can form gliding, non-sheathed, filamentous cells (>20 μm in length; Fig. 1d) and forms yellow-pigmented colonies and the fatty acid profile differs dramatically (Table 3). Though represented only by a single strain, the 16S rDNA lineage is quite distant from its closest phylogenetic neighbours (Fig. 2). On this basis, it is proposed that strain NCIMB 1418^T represents the type strain of a new genus and species, designated *Crocinitomix catalasitica* gen. nov., sp. nov.

Together, the genera *Brumimicrobium*, *Cryomorpha* and *Crocinitomix* form a common clade (Fig. 2) and possess the following common traits: rod-shaped cells formed during most of the growth phase (filamentous cells appear to be a minority of cells); possess a cold-adapted, stenohaline chemoheterotrophic ecophysiology and metabolism; isolated from marine ecosystems or from coastal saline habitats (e.g. quartz stone subliths exposed to sea spray); and a requirement for organic compounds for nitrogen. In combination, these traits cannot be regarded as unique, as they also apply to some members of the *Flavobacteriaceae* (e.g. genus *Aequorivita*); however, the basic phenotypic similarities and distinct phylogenetic location support the grouping of the three genera into a new family. The proposed family, *Cryomorphaceae* fam. nov. (type genus *Cryomorpha*), is most closely allied phenotypically and phylogenetically to the now well-defined family *Flavobacteriaceae* (Bernardet *et al.*, 1996).

Description of *Algoriphagus* gen. nov.

Algoriphagus (Al.gor.i.pha′gus. L. masc. n. *algor* cold; Gr. masc. n. *phagos* glutton; N.L. masc. n. *Algoriphagus* the cold eater).

Morphology ranges from coccobacilli to short filaments with rounded ends. Do not form microcysts or gas vesicles. Do not form vibrioid, circular, coiled or helical cells. Non-motile. Cold-adapted, marine, strictly aerobic chemoheterotroph. Nutritionally non-exacting and saccharolytic, able to grow on a range of carbohydrates as sole carbon and energy sources in mineral salts/sea-water media. Fatty acids contain a large proportion of monounsaturated and α-hydroxy components, with major fatty acids including i15 : 1ω10*c*, i15 : 0, i16 : 1ω6*c*, 16 : 1ω7*c*, 16 : 1ω5*c*, 2-OH i15 : 0 and 2-OH a15 : 0. A member of class *Flavobacteria* according to 16S rDNA sequence analysis. The type species is *Algoriphagus ratkowskyi*.

Description of *Algoriphagus ratkowskyi* sp. nov.

Algoriphagus ratkowskyi (rat.kow′sky.i. N.L. gen. n. *ratkowskyi* of Ratkowsky, in honour of David A. Ratkowsky, who made significant contributions to growth-modelling of bacteria, including psychrophilic bacteria).

Exhibits the following properties in addition to those given in the genus description. Cells appear as small coccobacilli (0·3–0·4 × 0·3–0·9 μm). Serpentine filamentous cells (10–20 × 0·4 μm) may also occur. Strains grow at

−2 to 25 °C, with a temperature optimum of 16–19 °C. Can tolerate up to 1 M NaCl (growth range 0·1–1·0 M NaCl). Catalase-negative and cytochrome-*c* oxidase-positive. Can hydrolyse casein. Some strains can also hydrolyse starch and chitin. Produces alkaline phosphatase, β-galactosidase, α-galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, *N*-acetyl-β-glucosaminidase, arginine arylamidase and glutamyl glycine arylamidase. Some strains form β-glucuronidase weakly. Acid is produced oxidatively from D-glucose, DL-arabinose, D-mannose, D-galactose, D-fructose, L-rhamnose, D-xylose, *N*-acetylglucosamine, lactose, cellobiose, trehalose, maltose, sucrose, melibiose and raffinose, but not from sugar alcohols. The following substrates are utilized as sole carbon and energy sources: L-rhamnose, D-fructose, D-galactose, D-mannose, D-xylose, cellobiose, lactose, D-maltose, D-melibiose, sucrose, trehalose, salicin, *N*-acetylglucosamine, D-sorbitol, *m*-inositol, D-mannitol, β-glycerophosphate, D-gluconate, propionate, isobutyrate, malonate, succinate, pimelate, azelate, L-proline, 2-aminobutyrate and L-serine. Best growth occurs on maltose and sucrose as sole carbon sources. L-Ornithine, glycogen, *n*-butyrate, glutarate, aconitate, L-malate, fumarate and hydroxy-L-proline are used by some but not all strains. The following carbon sources are not utilized: DL-arabinose, L-fucose, 2-ketogluconate, adonitol, D-arabitol, dulcitol, i-erythritol, glycerol, methanol, itaconate, formate, acetate, *n*-valerate, suberate, 3-DL-hydroxybutyrate, citrate, oxaloacetate, benzoate, DL-lactate, DL-tartrate, pyruvate, methylamine, isovalerate, heptanoate, caproate, nonanoate, adipate, 2-oxoglutarate, L-alanine, L-aspartate, L-asparagine, L-phenylalanine, L-glutamate, L-histidine, L-threonine, L-tyrosine, L-leucine, putrescine and urate. Produces salmon-pink colonies that are smooth, circular, convex, with an entire edge and a butyrous consistency when grown on marine 2216 agar (at 10 °C). Other characteristics are listed in Table 2. The DNA G+C content of the type strain is 35–36 mol% (thermal denaturation procedure).

The type strain, IC025^T (=ACAM 646^T=LMG 21435^T =CIP 107452^T), and other strains were isolated from cold marine and marine-derived habitats including sea ice and algal mats of saline lakes.

Description of *Brumimicrobium* gen. nov.

Brumimicrobium (Bru′mi.mi.cro′bi.um. L. fem. n. *bruma* winter; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Brumimicrobium* winter microbe).

Cells are slightly curved or straight rods with rounded ends. Do not form microcysts or gas vesicles. Do not form circular, coiled or helical cells. Motile by gliding. Cell mass is pigmented intensely orange. Cold-adapted, marine, facultatively anaerobic, fermentative chemoheterotrophs that require Na^+ ions, yeast extract and vitamins for growth. Fatty acids consist mostly of branched-chain components including i13 : 0, a15 : 1ω10*c* and i15 : 0. A member of class *Flavobacteria* according to 16S rDNA sequence analysis. The type species is *Brumimicrobium glaciale*.

Description of *Brumimicrobium glaciale* sp. nov.

Brumimicrobium glaciale (gla′ci.al.e. L. neut. adj. *glaciale* icy, frozen).

Exhibits the following properties in addition to those given in the genus description. Strains grow at −2 to 25 °C, with temperature optima of about 16–19 °C. Can tolerate up to 1 M NaCl (growth range 0·1–1·0 M NaCl). Catalase is produced. Cytochrome-*c* oxidase test is negative. Utilizes peptone as a nitrogen source but not L-glutamate, nitrate or ammonia salts. Some strains can also use Casamino acids as a nitrogen source. Can hydrolyse Tween 80 and some strains form an extracellular DNase. Does not hydrolyse casein, starch, chitin or urea. Produces alkaline phosphatase and arginine arylamidase. Some strains also produce β-galactosidase and glutamyl glycine arylamidase. Detectable levels of acid are not produced oxidatively from carbohydrates. Utilizes D-glucose aerobically in the presence of 0·05 % yeast extract and a vitamin solution; however, no other substrates tested (see description of *Algoriphagus ratkowskyi* above) are utilized or stimulate growth significantly. Produces intensely orange-pigmented colonies that are smooth, circular, convex, with an entire edge and possessing a somewhat gelid consistency when grown on marine 2216 agar (at 10 °C). Other characteristics are listed in Table 2. The DNA G+C content of the type strain is 38–40 mol% (thermal denaturation procedure).

The type strain, IC156^T (=ACAM 645^T=LMG 21434^T =CIP 107451^T), and other strains were isolated from sea ice and continental shelf sediment.

Description of *Cryomorpha* gen. nov.

Cryomorpha (Cry.o.mor′pha. N.L. *cryo* from Gr. neut. n. *kryos* cold; Gr. fem. n. *morphe* shape or form; N.L. fem. n. *Cryomorpha* cold shape).

Appear as straight to slightly curved rods with rounded ends. Non-motile. Do not form microcysts or gas vesicles. Do not form circular, coiled or helical cells. Cell mass is pigmented orange. Cold-adapted, marine, strictly aerobic chemoheterotrophs that require sea-water salts, yeast extract and vitamins for growth. Do not utilize carbohydrates for growth. Isolated from marine and marine-derived habitats. Fatty acids include large amounts of α-hydroxy and C_{14} branched components including i14 : 1ω9*c*, i14 : 0, i15 : 1ω10*c*, a15 : 1ω10*c*, i15 : 0, a15 : 0, i16 : 0, 2-OH i14 : 0, 2-OH a15 : 0 and 3-OH i16 : 0. A member of class *Flavobacteria* according to 16S rDNA sequence analysis. The type species is *Cryomorpha ignava*.

Description of *Cryomorpha ignava* sp. nov.

Cryomorpha ignava (ig.na′va. L. fem. adj. *ignava* lazy, pertaining to the biochemically and nutritionally inert nature of the species).

Exhibits the following properties in addition to those given

in the genus description. Strains grow at −2 to 25 °C, with temperature optima of about 15 °C. Can tolerate up to 1 M NaCl (growth range 0·1–1·0 M NaCl). Strong catalase reaction. Cytochrome-*c* oxidase test is negative. Requires yeast extract for growth, though some strains can grow weakly on peptone as a sole nitrogen source but not Casamino acids, L-glutamate, nitrate or ammonia salts. Does not hydrolyse DNA, casein, starch or chitin. Some strains produce urease and esterase (Tween 80 hydrolysis). Does not produce alkaline phosphatase, arginine arylamidase or glycosidic enzymes. Acid production from carbohydrates not detected. Does not utilize and is not stimulated by any substrate tested (see description of *Algoriphagus ratkowskyi* above). Produces pinpoint to small orange-pigmented colonies that are smooth, circular, convex in elevation, possess an entire edge and have a viscid consistency when grown on marine 2216 agar (10 °C). Other characteristics are listed in Table 2. The DNA G+C content of the type strain is 36–37 mol% (thermal denaturation procedure).

The type strain, 1-22^T (=ACAM 647^T=LMG 21436^T=CIP 107453^T), and another strain were isolated from quartz stone sublithic environments of the Antarctic continent, Southern Ocean sea-water particulates and superficial marine sediment.

Description of *Crocinitomix* gen. nov.

Crocinitomix (Cro.cin.i.to′mix. L. adj. *crocinus* of or pertaining to saffron; L. fem. n. *tomix* a string or thread; N.L. fem. n. *Crocinitomix* saffron-coloured thread).

Appear as rods or filaments, with filaments more common in young cultures. Do not form microcysts or gas vesicles. Do not form vibrioid, circular, coiled or helical cells. Motile by gliding. Cell mass is pigmented yellow. Cold-adapted, marine, strictly aerobic chemoheterotrophs that require Na^+ ions and organic compounds such as peptone or tryptone for growth. Do not utilize carbohydrates for growth. Major fatty acids include 15 : 0, i15 : 1ω10*c*, i15 : 0, 15 : 1ω11*c*, 16 : 1ω12*c* and 3-OH i17 : 0. Isolated from marine environments. A member of class *Flavobacteria* according to 16S rDNA sequence analysis. The type species is *Crocinitomix catalasitica*.

Description of *Crocinitomix catalasitica* sp. nov.

Crocinitomix catalasitica (cat.a.la.si′ti.ca. N.L. neut. n. *catalasum* catalase; L. suff. *-icus* relating to; N.L. fem. adj. *catalasitica* relating to catalase, pertaining to the ability of this species to produce catalase).

Basonyms: '*Microscilla aggregans* var. *catalatica*', '*Microscilla aggregans* subsp. *catalatica*', '*Flexibacter aggregans* subsp. *catalaticus*'.

Exhibits the following properties in addition to those given in the genus description. Strains grow at 0–30 °C, with temperature optima of about 25 °C. Can tolerate up to 1 M NaCl (growth range 0·1–1·0 M NaCl). Catalase is produced. Cytochrome-*c* oxidase test is negative. Utilizes peptone as a nitrogen source but not Casamino acids, L-glutamate, nitrate or ammonia salts. Can hydrolyse Tween 80. Does not hydrolyse DNA, casein, starch, chitin or urea. Decomposes tyrosine and DL-dihydroxylphenylalanine, with no soluble pigments formed. Produces alkaline phosphatase and arginine arylamidase. Some strains also produce β-galactosidase and glutamyl glycine arylamidase. Detectable levels of acid are not produced oxidatively from carbohydrates. Forms hydrogen sulphide from L-cysteine. Acidification, clotting or redigestion of curd do not occur in Litmus milk (Lewin & Lounsbery, 1969). No growth occurs on acetate, lactate, glycerol, D-glucose, D-galactose or sucrose as sole carbon sources (Lewin & Lounsbery, 1969). Produces yellow (saffron) colonies that are smooth, circular, convex, with a slightly irregular spreading edge and a butyrous consistency when grown on marine 2216 agar. Other characteristics are listed in Table 2. The DNA G+C content of the type strain is 35 mol% (thermal denaturation procedure).

The type strain, NCIMB 1418^T (=ATCC 23190^T), was isolated from under frozen sand of the upper littoral zone of Auke Bay, Alaska, USA.

Description of *Cryomorphaceae* fam. nov.

Cryomorphaceae (Cry.o.mor.pha′ce.ae. N.L. fem. n. *Cryomorpha* the type genus of the family; *-aceae* ending to denote a family; N.L. neut. pl. n. *Cryomorphaceae* the *Cryomorpha* family).

Includes species with a mostly rod-like to filamentous morphology. Cells are non-motile or move by gliding and normally contain carotenoid pigments. Strictly aerobic or facultatively anaerobic (fermentative) chemoheterotrophic metabolism. Species can have complex growth requirements requiring sea-water salts, organic compounds as sole nitrogen sources, yeast extract and vitamins for growth. The type genus is *Cryomorpha*. Also contains the genera *Brumimicrobium* and *Crocinitomix*. Located within class *Flavobacteria*, branching phylogenetically between the families *Flavobacteriaceae* and *Bacteroidaceae*.

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