Chimaereicella boritolerans sp. nov., a boron-tolerant and alkaliphilic bacterium of the family *Flavobacteriaceae* isolated from soil

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A non-motile, Gram-negative, boron-tolerant and alkaliphilic bacterium was isolated from soil of the Hisarcik area in the Kutahya Province of Turkey that was naturally high in boron minerals. The novel isolate, designated T-22^T, formed rod-shaped cells, was catalase- and oxidase-positive and tolerated up to 300 mM boron. The strain also survived on agar medium containing up to 3% (w/v) NaCl. The pH range for growth of this strain was 6.5–10.0 (optimum pH 8.0–9.0) and the temperature range was 16–37 °C (optimum 28–30 °C). Phylogenetic analysis based on 16S rRNA gene sequences revealed a clear affiliation with the genus *Chimaereicella*, with 97.4% sequence similarity to *Chimaereicella alkaliphila* AC-74^T, which was the highest similarity among cultivated bacteria. The DNA–DNA relatedness with *C. alkaliphila* AC-74^T was 28.3%. The major respiratory quinone system was MK-7 and the predominant cellular fatty acids were iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{17:0} 3-OH and summed feature 3 (iso-C_{15:0} 2-OH and/or iso-C_{16:1}ω7c). The DNA G + C content was 42.5 mol%. Based on the phylogenetic analysis and physiological, chemotaxonomic and genetic data, we concluded that strain T-22^T should be classified in the genus *Chimaereicella*, and we propose the name *Chimaereicella boritolerans* sp. nov. for this novel species. The type strain is strain T-22^T (=DSM 17298^T = NBRC 101277^T = ATCC BAA-1189^T).

Several classes of micro-organisms have been recognized as extremophiles, which inhabit environments with extreme levels of salinity, pH, pressure, heat, cold and even radiation. These extremophiles provide a source of discoveries in the basic and applied sciences. We present the boron tolerance of bacteria as a new frontier in extremophiles. The toxic effects of high concentrations of boric acid on living cells are well established (Nable *et al.*, 1997). The biological functions that require boron in microbes and plants are not clear, despite the fact that boron is required by some organisms as an essential nutrient (Warrington, 1923; Rowe & Eckhart, 1999). Although boron salts have often been used in microbial growth media (Stanier *et al.*, 1966), boron essentiality and tolerance to boron toxicity in bacteria has only been reported recently (Ahmed *et al.*, 2007a, b).

Recently, the genus *Chimaereicella* was described in the family *Flavobacteriaceae*; only one species, *Chimaereicella alkaliphila*, which was isolated from a non-saline but highly alkaline aquatic environment, has currently been characterized (Tiago *et al.*, 2006). Most members of the family *Flavobacteriaceae* are Gram-negative and originate from marine and marine-derived surface waters (Bowman & Nichols, 2005). These organisms play a major role in the remineralization of organic matter (Kirchman, 2002).

In the course of exploring the bacterial diversity of boron tolerance, we isolated a novel strain of Gram-negative, alkaliphilic and highly boron-tolerant bacterium from soil sampled from the Hisarcik area in the Kutahya Province of Turkey. This area is naturally high in boron minerals (Çöl & Çöl, 2003). Based upon the data obtained, we propose that the strain represents a novel species of the genus *Chimaereicella*.

Strain T-22^T was isolated from soil according to previously described isolation and enrichment procedures (Ahmed *et al.*, 2007a). After isolation, the strain was cultured

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the strain T-22^T is AB197852.

Maximum-likelihood and maximum-parsimony trees based on 16S rRNA gene sequence data are available as supplementary material in IJSEM Online.

routinely on R3V-A medium (see composition in Tiago *et al.*, 2006) or marine agar 2216 medium (MA; Difco) (pH 8.0) at 30 °C or stored as glycerol stocks (35 %, w/v) at –80 °C.

The motility and morphology of cells were observed using phase-contrast microscopy and scanning electron microscopy (SEM). Gram staining was performed according to Hucker's modified method (Cowan & Steel, 1974). Colonial morphology was observed on isolated colonies grown on R3V-A or MA medium (pH 8.0). For SEM observation, cells grown on agar plates for 5, 10 and 22 days were removed, washed with PBS and fixed with OsO₄. After dehydration by sequential washing with ethanol (50–100 %) that was exchanged to 100 % isoamyl acetate, the sample was treated with platinum before observation using SEM (JEOL model JSM-6700F). Young cells of strain T-22^T in mid-exponential phase were non-motile and rod-shaped, with a length of 1.2–3.4 µm and width of 0.3–0.4 µm; however, long filamentous cells (up to 15 µm) were also observed (Fig. 1a). Older cells (22 days) produced coccoid cyst-like structures or fruiting-body-like structures (Fig. 1b, c), with a diameter of 0.76–0.90 µm. The colonies produced red pigmentation that was diffusible into the medium.

The pH range for growth of the strain was determined on RV3-A or MA media with pH ranging from 4.0 to 10.0 (in pH increments of 0.5), adjusted as described by Tiago *et al.* (2006) at 30 °C. The temperature range for growth of the strain was determined on agar medium with pH 8.0 by incubation at various temperatures (4, 10, 16, 20, 25, 28, 30, 32, 37, 45 and 50 °C). Growth of strain T-22^T was also examined at levels of NaCl ranging from 0 to 7 % (w/v, in 1 % increments) on RV3-A medium at pH 8.0 and 30 °C. Strain T-22^T grew on agar with a pH range of 6.5–10.0; however, it exhibited optimum growth at an alkaline pH of 8.0–9.0, so it was characterized as alkaliphilic according to the definition of Jones *et al.* (1994). The intensity of red pigmentation decreased at pH 10.0, whereas it was maximal at neutral pH. The optimum temperature for growth was 28–30 °C. Slight growth was observed at 16 or 37 °C after several days; no growth was observed at 10 or 40 °C. Strain T-22^T tolerated up to 3 % NaCl.

Catalase and oxidase tests were performed following previously described procedures (Ahmed *et al.*, 2007a). The strain was positive for catalase and cytochrome oxidase tests. Carbon source use was investigated using API 50 CH strips (bioMérieux), using 0.1 M Tris/HCl buffer (pH 8.0) supplemented with 0.3 % (w/v) agar, 0.7 % yeast nitrogen base and 0.05 % yeast extract as a minimal medium. Acid production on various substrates was determined using 50CHB/E medium with or without adding 0.02 M Tris/HCl buffer (pH 8.0) following the manufacturer's instructions and as described by Tiago *et al.* (2006). Results were recorded after 24 and 48 h and 5 days of incubation at 30 °C. Other physiological tests were performed using the API 20NE system (bioMérieux) using the minimal medium described by Tiago *et al.* (2006). Resistance to antibiotics and enzyme activities were evaluated using ATB-VET and

Fig. 1. Scanning electron micrographs showing vegetative and filamentous cells (a) and coccoid cyst-like structures or fruiting bodies (b, c) of strain T-22^T grown on MA for 5 (a), 10 (b) or 22 (c) days at 30 °C. Bars, 1 µm.

API ZYM strips (bioMérieux), respectively, according to the manufacturer's instructions. Physiological experiments using the API system, the determination of antibiotic resistance using ATB-VET and catalase and oxidase tests were repeated several times. Strain T-22^T showed many characteristics that differentiated it from the closely related species *C. alkaliphila* (Table 1); additional features are included in the species description.

The major respiratory quinone system in strain T-22^T was MK-7, as determined using the protocol described by Xie &

Table 1. Physiological characteristics of the members of the genus *Chimaereicella*

Data for *C. alkaliphila* AC-74^T were taken from Tiago *et al.* (2006) unless indicated. Both strains are positive for catalase, oxidase, aesculin hydrolysis, β -galactosidase, production of acid from D-galactose, D-glucose, D-cellobiose, D-maltose, D-lactose, potassium 5-ketogluconate, gentiobiose and D-turanose and resistance to kanamycin. Both strains are negative for urease, indole production from L-tryptophan, acid production from glycerol, erythritol, D- and L-arabinose, D-ribose, D- and L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, amygdalin, D-melezitose, xylitol, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and methyl β -D-xylopyranoside and utilization of glycerol, erythritol, D-arabinose, D-ribose, D- and L-xylose, methyl β -D-xylopyranoside, D-galactose, D-glucose, L-sorbose, L-rhamnose, inositol, *N*-acetylglucosamine, D-lactose, D-melibiose, D-trehalose, D-raffinose, D-turanose, potassium gluconate, L-fucose and D-tagatose. Both species are sensitive to amoxicillin, colistin, doxycycline, ofloxacin, streptomycin and tetracycline. +, Positive; –, negative; (+), weakly positive.

Characteristic	Strain T-22 ^T	<i>C. alkaliphila</i> AC-74 ^T
Cell size [length (filamentous) $\times$ width] (μ m)	1.2–3.4 (15) $\times$ 0.3–0.4	2.1–3.3 $\times$ 0.5
Maximum boron-toxicity tolerance (mM)	300	50*
pH range (optimum) for growth	6.5–10 (8–9)	7.5–11 (8)
Hydrolysis of gelatin	–	+
Nitrate reduction	–	+
Acid from:		
D-Fructose	–	+
D-Mannose	(+)	–
L-Rhamnose	(+)	–
Methyl α -D-mannopyranoside	(+)	–
<i>N</i> -Acetylglucosamine	(+)	–
Aesculin	+	–
Salicin	–	+
D-Melibiose	–	+
Sucrose	–	+
D-Trehalose	–	+
Inulin	+	–
D-Raffinose	+	–
Starch	+	–
Glycogen	(+)	–
D-Lyxose	(+)	–
Use of:		
L-Arabinose	–	+
D-Adonitol	–	+
D-Fructose	–	(+)
D-Mannose	–	(+)
Dulcitol	(+)	–
Inositol	–	–
D-Mannitol	+	–
D-Sorbitol	–	+
Methyl α -D-glucopyranoside	–	+
Amygdalin	+	–
Arbutin	+	–
Aesculin	+	–
D-Cellobiose	–	+
D-Maltose	(+)	–
Inulin	+	–
D-Melezitose	+	–
Starch	(+)	–
Glycogen	(+)	–
Xylitol	(+)	–
Gentiobiose	–	+
D-Lyxose	(+)	–
D-Fucose	(+)	–
L-Arabitol	–	+

Table 1. cont.

Characteristic	Strain T-22 ^T	<i>C. alkaliphila</i> AC-74 ^T
Potassium 2-ketogluconate	–	+
Potassium 5-ketogluconate	–	+
Resistance to: (µg ml ^{–1})		
Cephalothin (8)	–	+
Chloramphenicol (30)	–	+
Gentamicin (4)	(+)	–
Penicillin (0.25)	(+)	–
DNA G+C content (mol%)	42.5	43.5

*Data from this study.

Yokota (2003). To analyse the fatty acid profile, methyl ester derivatives were prepared by saponification, methylation and extraction from 10 mg freeze-dried cell material harvested from either R3V-A or marine broth (MB; Difco). The fatty acid methyl esters were analysed using the GC-based Microbial Identification system (MIDI) following the manufacturer's instructions. The predominant cellular fatty acids detected in strain T-22^T were branched-chain fatty acids, with the major entities iso-C_{15:0}, iso-C_{17:1}ω9c, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:0}ω7c), iso-C_{17:0} 3-OH and iso-C_{16:0} (Table 2).

Genomic DNA of the strain was isolated from cells grown on agar plates according to the method of Marmur (1961), with the slight modification of the use of RNase T₁ in addition to RNase A. The DNA G+C content was determined as described previously (Ahmed *et al.*, 2007a). The DNA G+C content of strain T-22^T was 42.5 mol%.

An almost-complete 16S rRNA gene fragment (1489 nt) of strain T-22^T was amplified by PCR and sequenced as described by Ahmed *et al.* (2007a) using an ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems). The consensus sequence obtained using the DNASIS Pro software package (Hitachi Software Engineering) was compared with sequences of closely related type strains retrieved from the DDBJ/EMBL database using BLAST searches. The multiple-sequence alignment was produced by CLUSTAL X (1.8 msw) software (Thompson *et al.*, 1997). Ambiguous positions and gaps were discarded and sequence identities were calculated using BioEdit software (Hall, 1999). Evolutionary distances and *K*_{nuc} values (Kimura, 1980) were generated from unambiguous alignable data of 1342 nt and phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony and maximum-likelihood algorithms (Felsenstein, 2005). To assess the stability of relationships, a bootstrap analysis (confidence values estimated from 1000 replications of each sequence) was performed using PHYLIP software (Felsenstein, 2005) for the tree topology of the neighbour-joining data. Phylogenetic trees were plotted using the NJPlot software.

Comparison of the 16S rRNA gene sequence with those of closely related species showed that strain T-22^T belonged to the genus *Chimaereicella*, with the highest similarity in the 16S rRNA gene sequence of 97.4 % with the sequence of *C. alkaliphila* AC-74^T. Sequence similarity calculations indicated that strain T-22^T was equidistant from species of the genera *Algoriphagus* (similarity 94.0–95.4 %) and *Hongiella* (similarity 93.6–94.6 %) and occupied the phylogenetic position of *C. alkaliphila* AC-74^T, achieving a 100 % bootstrap value, indicating high reliability of the tree topology (Fig. 2). The topologies of the phylogenetic trees generated using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the tree constructed using the neighbour-joining method (Supplementary Figs S1 and S2 available in IJSEM Online), especially for the clade comprising species of *Chimaereicella*. These analyses strongly support the affiliation of strain T-22^T with the genus *Chimaereicella*.

For DNA–DNA hybridization experiments, Qiagen Genomic-tips 500/G were used to isolate genomic DNA of the novel strain and *C. alkaliphila* AC-74^T following the manufacturer's protocol, with the minor modification that RNase T₁ was used in addition to RNase A. DNA–DNA hybridization was performed at 42 °C with photobiotin-labelled DNA using microplates, as described by Ezaki *et al.* (1989). The DNA–DNA relatedness of strain T-22^T with *C. alkaliphila* AC-74^T was 28.3 %, indicating that strain T-22^T represents a distinct species (Stackebrandt & Goebel, 1994).

Strain T-22^T differed from the type species of the genus, *C. alkaliphila*, in that the latter cannot grow at neutral pH (Tiago *et al.*, 2006). No growth of strain T-22^T was observed at temperatures of 10 or 40 °C. This feature also differentiates the strain from *C. alkaliphila* and strains of the genera *Algoriphagus* and *Hongiella*, which can grow at or below 10 °C (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004; Yoon *et al.*, 2004; Tiago *et al.*, 2006). Strain T-22^T survived on agar medium containing up to 300 mM boron, whereas the closely related strain *C. alkaliphila* AC-74^T could not tolerate 100 mM boron. In

Table 2. Cellular fatty acid compositions of strain T-22^T and *C. alkaliphila* AC-74^T

Data for *C. alkaliphila* AC-74^T grown in R3V-A medium were taken from Tiago *et al.* (2006); the remaining data were obtained in this study. Values are percentages of total fatty acids; only fatty acids comprising >0.50 % in at least one of the strains are included.

Fatty acid	Strain T-22 ^T		<i>C. alkaliphila</i> AC-74 ^T	
	R3V-A	MB	R3V-A	MB
Straight-chain fatty acids				
C _{15:0}	2.63 ± 0.18	0.62	1.60	1.67
C _{16:0}	3.53 ± 0.07	3.16	0.40	0.88
C _{19:0}	—	—	—	0.50
Branched-chain fatty acids				
iso-C _{11:0}	0.68 ± 0.11	0.17	0.90	0.21
anteiso-C _{11:0}	1.49 ± 0.17	0.43	2.60	1.63
Unknown 13.565	2.13 ± 0.51	1.33	—	1.22
Unknown 13.566	—	—	3.67	—
iso-C _{14:0}	0.36 ± 0.01	2.09	0.30	1.37
iso-C _{15:0}	21.32 ± 0.13	16.64	32.40	21.01
anteiso-C _{15:0}	6.99 ± 0.81	6.43	3.40	5.14
iso-C _{15:1} G	0.54 ± 0.25	0.22	1.40	0.26
iso-C _{16:0}	6.49 ± 0.09	20.66	3.20	11.13
iso-C _{16:1} H	3.29 ± 0.71	10.89	3.80	11.55
Unknown 16.580	—	—	1.30	—
Unknown 16.582	0.64 ± 0.01	0.40	—	0.41
iso-C _{17:0}	3.23 ± 0.63	0.77	0.80	0.77
anteiso-C _{17:0}	0.97 ± 0.25	0.29	—	0.18
C _{17:0} cyclo	—	2.41	—	—
iso-C _{17:1} ω9c	13.33 ± 0.74	5.04	14.60	8.01
anteiso-C _{17:1} ω9c	1.88 ± 0.31	1.61	—	0.64
iso-C _{18:1} H	0.64 ± 0.15	1.57	—	0.55
Unsaturated fatty acids				
C _{15:1} ω6c	0.28 ± 0.11	—	1.90	1.34
C _{16:1} ω5c	—	0.47	1.20	2.82
C _{17:1} ω6c	2.50 ± 0.56	1.30	1.70	1.95
C _{17:1} ω8c	0.84 ± 0.08	0.29	0.40	0.39
C _{18:1} ω5c	0.53 ± 0.21	0.71	—	—
Hydroxy fatty acids				
C _{15:0} 2-OH	0.63 ± 0.02	0.14	0.50	0.27
C _{17:0} 2-OH	2.06 ± 0.13	1.09	—	0.71
iso-C _{15:0} 3-OH	2.20 ± 0.11	0.55	3.20	1.35
iso-C _{16:0} 3-OH	0.95 ± 0.02	4.48	1.30	2.95
iso-C _{17:0} 3-OH	7.46 ± 0.33	4.93	7.40	4.64
Summed features*				
3	9.99 ± 0.65	8.94	9.20	12.79
4	0.44 ± 0	—	3.90	1.14

*Summed features 3 and 4 represent the fatty acids C_{16:1} ω7c and/or iso-C_{15:0} 2-OH and iso-C_{17:1} I and/or anteiso-C_{17:1} B, respectively, which cannot be separated reliably by the MIDI system.

addition to boron tolerance, the major differentiating characteristics of strain T-22^T from the closely related type strain *C. alkaliphila* AC-74^T were its negative results for gelatin hydrolysis and nitrate reduction tests and sensitivity to cephalothin and chloramphenicol.

The fatty acid profile of strain T-22^T was similar to that of *C. alkaliphila* AC-74^T (Table 2). Strain T-22^T produced a

significant amount of the β-hydroxy fatty acids iso-C_{17:0} 3-OH, iso-C_{16:0} 3-OH and iso-C_{15:0} 3-OH (Table 2), which are commonly found among members of the *Flavobacteri*a (Bowman *et al.*, 1998). The strain also contained an α-hydroxy (2-OH) fatty acid that is absent from the fatty acid profile of *C. alkaliphila* AC-74^T. *C. alkaliphila* AC-74^T has a DNA G + C content of 43.5 mol%, which was slightly higher than that of the novel strain. However, the highest 16S rRNA

Fig. 2. Phylogenetic tree showing the inter-relationships of strain T-22^T and its closest relatives inferred from 16S rRNA gene sequences. Data with gaps were removed during alignment using BioEdit software, and the tree was constructed using the neighbour-joining method with PHYLIP software (Felsenstein, 2005) based on a comparison of 1342 nt and plotted with NJPlot. Bootstrap values > 70 %, expressed as percentages of 1000 replications, are given at branching points. The sequence of an unknown strain of *Escherichia coli* is used as the outgroup. Bar, 2 % sequence divergence.

gene sequence similarity occurred between strain T-22^T and *C. alkaliphila* AC-74^T (Fig. 1). Critical analysis of other physiological (Table 1) and chemotaxonomic (Table 2) data also suggests that strain T-22^T belongs to the genus *Chimaereicella*.

Phylogenetic analysis based on the 16S rRNA gene sequence, the DNA base composition and chemotaxonomic analyses clearly suggest that strain T-22^T belongs to the genus *Chimaereicella*. However, DNA–DNA relatedness and morphological and phenotypic characteristics (pH range for growth, boron tolerance, gelatin hydrolysis and nitrate reductase activity) suggest that it represents a novel species in the genus, for which we propose the name *Chimaereicella boritolerans* sp. nov.

Description of *Chimaereicella boritolerans* sp. nov.

Chimaereicella boritolerans (bo'ri.to'le.rans. N.L. n. *borum* boron; L. part. adj. *tolerans* tolerating; N.L. part. adj. *boritolerans* boron-tolerating).

Cells are non-motile and rod-shaped, 1.2–3.4 µm long and 0.3–0.4 µm in diameter, occurring singly and occasionally in pairs. Gram staining is negative. Colonies are red-pigmented, circular with entire margins, convex, butyrous in texture and small in diameter after several days of growth at 30 °C. The red pigment loses its intensity at pH 10.0 on agar medium and is diffusible in liquid medium. Grows optimally at 28–30 °C; very slight growth at 37 and 16 °C after several days. The optimum pH for growth is 8.0–9.0, with a range of pH 6.5–10.0. Tolerates up to 300 mM boron, but grows optimally without boron supply. NaCl is tolerated up to 3 % (w/v). Oxidase, catalase, hydrolysis of aesculin and β-galactosidase tests are positive, whereas tests for production of indole, nitrate reduction and hydrolysis of gelatin and urea are negative. Produces acid from the following substrates: D-galactose, D-glucose, aesculin, inulin, D-raffinose, starch, D-cellobiose, D-maltose, D-lactose, potassium 5-ketogluconate, gentiobiose and D-turanose. Can use

the following substrates as carbon sources: D-mannitol, amygdalin, arbutin, aesculin, inulin and D-melezitose. Additional physiological characteristics are provided in Table 1. Major cellular fatty acids are iso-C_{15:0}, iso-C_{17:1ω9c}, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}), iso-C_{17:0} 3-OH and iso-C_{16:0}; traces of some other fatty acids are apparent. The respiratory lipoquinone system is MK-7. Strong enzyme activity is observed for alkaline phosphatase, α-chymotrypsin, N-acetyl-β-glucosaminidase and valine arylamidase, whereas weak activity is observed for leucine arylamidase, naphthol-AS-BI-phosphohydrolase, esterase (C8), esterase lipase (C8), trypsin, acid phosphatase, β-galactosidase and α-glucosidase (API ZYM strip). Resistant to kanamycin, oxacillin, sulfamethizol and metronidazol and weakly sensitive to penicillin, gentamicin, rifampicin and cefoperazon (ATB-VET strip). The G + C content of the type strain is 42.5 mol%.

The type strain is strain T-22^T (=DSM 17298^T=NBRC 101277^T=ATCC BAA-1189^T), isolated from soil that naturally contains boron (Hisarcik, Kutahya, Turkey).

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References

- Ahmed, I., Yokota, A. & Fujiwara, T. (2007a). A novel highly boron tolerant bacterium, *Bacillus boroniphilus* sp. nov., isolated from soil, that requires boron for its growth. *Extremophiles* **11**, 217–224.
- Ahmed, I., Yokota, A. & Fujiwara, T. (2007b). *Gracilibacillus boracitolerans* sp. nov., a highly boron-tolerant and moderately halotolerant bacterium isolated from soil. *Int J Syst Evol Microbiol* **57**, 796–802.
- Bowman, J. P. & Nichols, D. S. (2005). Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats, including *Subsaximicrobium wynnwiliamsii* 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*. *Int J Syst Evol Microbiol* **55**, 1471–1486.

Bowman, J. P., McCammon, S. A., Lewis, T., Skerratt, J. H., Brown, J. L., Nichols, D. S. & McMeekin, T. A. (1998). *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson *et al.* 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**, 1601–1609.

Bowman, J. P., Nichols, C. M. & Gibson, J. A. E. (2003). *Algoriphagus ratowskyi* 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. *Int J Syst Evol Microbiol* **53**, 1343–1355.

Çöl, M. & Çöl, C. (2003). Environmental boron contamination in waters of the Hisarcik area in the Kutahya Province of Turkey. *Food Chem Toxicol* **41**, 1417–1420.

Cowan, S. T. & Steel, K. J. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. London: Cambridge University Press.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Jones, B. E., Grant, W. D., Collins, N. C. & Mwatha, W. E. (1994). Alkaliphiles: diversity and identification. In *Bacterial Diversity and Systematics*, pp. 195–230. Edited by F. G. Priest, A. Ramos-Cormenzana & B. J. Tindall. New York: Plenum.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kirchman, D. L. (2002). The ecology of *Cytophaga*–*Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* **39**, 91–100.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Nable, R. O., Bañuelos, G. S. & Paull, J. G. (1997). Boron toxicity. *Plant Soil* **193**, 181–198.

Nedashkovskaya, O. I., Vancanneyt, M., Van Trappen, S., Vandemeulebroecke, K., Lysenko, A. M., Rohde, M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Swings, J. (2004). Description of *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., from sea water and algae, transfer of *Hongiella halophila* Yi and Chun 2004 to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. and emended descriptions of the genera *Algoriphagus* Bowman *et al.* 2003 and *Hongiella* Yi and Chun 2004. *Int J Syst Evol Microbiol* **54**, 1757–1764.

Rowe, R. I. & Eckhert, C. D. (1999). Boron is required for zebra fish embryogenesis. *J Exp Biol* **202**, 1649–1654.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tiago, I., Mendes, V., Pires, C., Morais, P. V. & Veríssimo, A. (2006). *Chimaericella alkaliphila* gen. nov., sp. nov., a Gram-negative alkaliphilic bacterium isolated from a nonsaline alkaline ground-water. *Syst Appl Microbiol* **29**, 100–108.

Van Trappen, S., Vandecandelaere, I., Mergaert, J. & Swings, J. (2004). *Algoriphagus antarcticus* sp. nov., a novel psychrophile from microbial mats in Antarctic lakes. *Int J Syst Evol Microbiol* **54**, 1969–1973.

Warrington, K. (1923). The effect of boric acid and borax on the broad bean and certain other plants. *Ann Bot* **37**, 629–672.

Xie, C. & Yokota, A. (2003). Phylogenetic analysis of *Lamprospira hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.

Yoon, J.-H., Yeo, S.-H. & Oh, T.-K. (2004). *Hongiella marincola* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1845–1848.