Chthonomonas calidirosea gen. nov., sp. nov., an aerobic, pigmented, thermophilic micro-organism of a novel bacterial class, *Chthonomonadetes* classis nov., of the newly described phylum *Armatimonadetes* originally designated candidate division OP10

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An aerobic, saccharolytic, obligately thermophilic, motile, non-spore-forming bacterium, strain T49^T, was isolated from geothermally heated soil at Hell's Gate, Tikitere, New Zealand. On the basis of 16S rRNA gene sequence similarity, T49^T is the first representative of a new class in the newly described phylum *Armatimonadetes*, formerly known as candidate division OP10. Cells of strain T49^T stained Gram-negative and were catalase-positive and oxidase-negative. Cells possessed a highly corrugated outer membrane. The major fatty acids were 16:0, i17:0 and ai17:0. The G + C content of the genomic DNA was 54.6 mol%. Strain T49^T grew at 50–73 °C with an optimum temperature of 68 °C, and at pH 4.7–5.8 with an optimum growth pH of 5.3. A growth rate of 0.012 h⁻¹ was observed under optimal temperature and pH conditions. The primary respiratory quinone was MK-8. Optimal growth was achieved in the absence of NaCl, although growth was observed at NaCl concentrations as high as 2% (w/v). Strain T49^T was able to utilize mono- and disaccharides such as cellobiose, lactose, mannose and glucose, as well as branched or amorphous polysaccharides such as starch, CM-cellulose, xylan and glycogen, but not highly linear polysaccharides such as crystalline cellulose or cotton. On the basis of its phylogenetic position and phenotypic characteristics, we propose that strain T49^T represents a novel bacterial genus and species within the new class *Chthonomonadetes* classis nov. of the phylum *Armatimonadetes*. The type strain of *Chthonomonas calidirosea* gen. nov., sp. nov. is T49^T (=DSM 23976^T=ICMP 18418^T).

Abbreviations: EM, electron microscopy; FAME, fatty acid methyl ester; FESEM, field emission scanning electron microscope; NJ, neighbour joining; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Chthonomonas calidirosea* T49^T is AM749780.

A supplementary table and two supplementary figures are available with the online version of this paper.

INTRODUCTION

The expansion of molecular ecology-based research has dramatically increased our understanding of microbial diversity. This knowledge has led to renewed interest in the cultivation of rare and as-yet-uncultivated lineages of bacteria (Mori *et al.*, 2009; Stott *et al.*, 2008; Tamaki *et al.*, 2011). Despite this interest, the proportion of bacterial phyla with cultivated representatives is remarkably low

(Pace, 2009). Of the currently estimated 70–100 bacterial phylum-level divisions (DeSantis *et al.*, 2006; Pace, 2009), only 29 have cultivated representatives (Euzéby, 1997). The remaining ‘candidate divisions’ (Hugenholtz *et al.*, 1998a) represent a large gap in our knowledge of microbial taxonomic and metabolic biodiversity.

Candidate division OP10 was first described in a molecular ecology study of Obsidian Pool, a geothermal hot spring in Yellowstone National Park (Hugenholtz *et al.*, 1998b). This candidate division is large, with the number of separate, near-full-length 16S rRNA gene sequences now attributed to this division being approximately 330 (SILVA v. 102; Pruesse *et al.*, 2007). Representative phylotypes have been detected in a wide range of environments, including mesophilic and geothermal soils (Cruz-Martínez *et al.*, 2009; Elshahed *et al.*, 2008; Lesaulnier *et al.*, 2008; Soo *et al.*, 2009; Stott *et al.*, 2008), bioreactors (Bond *et al.*, 1995; Huang *et al.*, 2008), freshwater environments (Lehours *et al.*, 2007; Urbach *et al.*, 2001; Wu *et al.*, 2007), hypersaline mats (Ley *et al.*, 2006), geothermal springs (Hugenholtz *et al.*, 1998b; Kanokratana *et al.*, 2004; Portillo & Gonzalez, 2009) and contaminated soils and waterways (Dojka *et al.*, 1998; Nogales *et al.*, 2001; von Wintzingerode *et al.*, 1999). The first bacterial representatives of this division (strains T49^T and P488) were isolated from geothermal soils and partially characterized (Stott *et al.*, 2008); the former is the subject of this descriptive paper. More recently, a new strain (YO-36^T) has been isolated from the rhizoplane of the aquatic reed, *Phragmites australis*, and described as belonging to *Armatimonas rosea* within the phylum *Armatimonadetes* (Tamaki *et al.*, 2011).

Here we describe the phenotypic and phylogenetic features of strain T49^T, which was originally cultivated from geothermal soils in New Zealand (Stott *et al.*, 2008), and we propose the name *Chthonomonas calidirosea* gen. nov., sp. nov. to accommodate this strain. We also propose that the observed phylogenetic and phenotypic characteristics of this strain are sufficiently different from those of *Armatimonas rosea* YO-36^T to warrant the description of a novel bacterial class, *Chthonomonadetes* classis nov., within the newly described phylum *Armatimonadetes*.

METHODS

Enrichment and isolation. Strain T49^T was isolated from geothermally heated soil at Hell’s Gate, Tikitere, New Zealand (Stott *et al.*, 2008). The soil was sampled aseptically at a depth of approximately 15 cm from an orange/copper-coloured soil profile directly below the AH (organic-mineral) horizon. The soil sample had a pH of 4.3 (25 °C) and an *in situ* temperature of approximately 55 °C. The soil gas chemistry is detailed elsewhere (Dunfield *et al.*, 2007). Strain T49^T was enriched and isolated by using a variation of the method described for strain P488 (Stott *et al.*, 2008). Briefly, soil crumbs from the selected soil profile were spread aseptically onto solid medium AOM1 (Stott *et al.*, 2008), and the enrichment was incubated at 60 °C in an aerobic atmosphere. Phytagel (gellan) in the solidified AOM1 medium served as both a solidifying agent and an energy source for chemo-organotrophic growth. Pink/rose-coloured colonies were separated

and isolated through three successive rounds of single-colony picking and plating. Colony purity was confirmed via PCR amplification of the 16S rRNA gene sequence using the universal bacterial primers 9f and 1492r (Weisburg *et al.*, 1991). T49^T cells were maintained routinely on a variation of AOM1 solid medium containing 100 mg B vitamins and 100 mg yeast extract l⁻¹ (Stott *et al.*, 2008), and were subcultured weekly.

Metabolic profile. Unless otherwise stated, all metabolic profiling was conducted using FS1V liquid mineral salts medium (Stott *et al.*, 2008) containing an aerobic headspace of >50 % (v/v). Substrate-utilization tests were cultured in triplicate in 125 ml serum bottles and were incubated statically at 60 °C. Sterile FS1V medium was inoculated using 3-day-old strain T49^T colonies picked from plates. Care was taken to restrict transfer of gellan from the solidified AOM1 medium to the liquid medium during inoculation. The following substrates were tested for supporting the growth of strain T49^T (0.5 g l⁻¹): DL-arabinose, D-ribose, D-fructose, D-glucose, D-mannose, D-rhamnose, D-galactose, D-galacturonic acid, D-N-acetylglucosamine, xylose, sucrose, lactose, maltose, trehalose, cellobiose, raffinose, xylan (>90 % xylan from beechwood), sodium alginate, galactomannan (locust bean gum), agarose, pectin (type 115; CP Kelco), chitin, gellan gum (Phytigel), xanthan (CC, CP Kelco), sodium pyruvate, sodium citrate, sodium acetate, sodium fumarate, sodium lactate, malic acid, yeast extract, Casamino acids (Difco), starch, glycogen, dextrin (corn, type II), CM-cellulose (Hercules), pullulan, Whatman filter paper, crystalline cellulose (Avicel), cotton and assorted non-commercial lignocellulosic pulp preparations with different cellulose, hemicellulose and lignin concentrations. Methanol, ethanol, 1-propanol and 2-propanol [0.05 % (v/v)] were tested for the ability to support the growth of strain T49^T. All media were adjusted aseptically to a final pH of 4.8–5.2 using dilute H₂SO₄ or NaOH as required. Growth was also tested using defined complex media including nutrient broth (Sigma), tryptic soy broth (Difco), R2A (Merck) and Luria–Bertani broth (Difco) according to the manufacturers’ instructions, with the pH adjusted to 4.8 (H₂SO₄). A selection of enzymic activities was tested by using API ZYM strips (ref. 25200; bioMérieux).

Aerobic growth using H₂ or CH₄ gas was tested using 1000 ml Schott bottles containing approximately 500 ml FS1V medium. These were capped to be airtight with butyl rubber septa and CH₄ or H₂ gas was added to the headspaces at final mixing ratios of 5, 10 or 20 % (v/v). Carbon dioxide [1 % (v/v)] was also added. The bottles were incubated at 60 °C at 180 r.p.m. for 7 days. The preferred nitrogen source of strain T49^T was determined by culturing cells (60 °C, 180 r.p.m.) with 0.64 g NaNO₃ · 6H₂O l⁻¹, 0.4 g (NH₄)₂SO₄ l⁻¹, 100 mg yeast extract l⁻¹ or 100 mg Casamino acids l⁻¹ (Difco) under an N₂-free atmosphere (75 % Ar, 20 % O₂ and 5 % CO₂, v/v) in N-free FS1 medium (Stott *et al.*, 2008). Nitrogen fixation was tested in a similar manner except that strain T49^T was incubated in N-free FS1 medium containing 100 mg yeast extract l⁻¹ with an air atmosphere. The salt tolerance of T49^T was tested by growing cells in FS1V medium (60 °C, 180 r.p.m.) containing 0, 2.5, 5.0, 10, 20 or 50 g NaCl l⁻¹. Anaerobic growth was tested using elemental sulfur, nitrate or sulfate as terminal electron acceptors in FS1V medium containing an additional 6.5 g S⁰ l⁻¹, 0.5 g Na₂SO₄ · 10H₂O l⁻¹ or 0.5 g NaNO₃ l⁻¹, respectively. Resazurin (0.25 mg ml⁻¹) and Na₂S · 7H₂O (2 mM) were added to the medium with an anaerobic headspace of (85 % N₂, 5 % CO₂ and 10 % H₂, v/v). The optimal growth temperature and pH were determined via spectrometry (OD₆₀₀) and cell counts in FS1V medium (180 r.p.m.) in triplicate for each condition. A citric acid/Na₂HPO₄ variation of the FS1V medium was used to buffer the medium for pH tests. The concentrations of citric acid and Na₂HPO₄ varied depending on the desired end pH, but were both in the 10–20 mM range.

Antibiotic sensitivity was determined by growing strain T49^T on AOM1 solid medium containing either 10 or 100 µg ml⁻¹ of

trimethoprim, polymyxin B, metronidazole, chloramphenicol, streptomycin, lasalocid A, kanamycin, ampicillin or neomycin. Where no or only minimal growth was observed, the original smear area on the solid medium was transferred onto antibiotic-free AOM1 plates to confirm loss of viability.

Morphology. Gram staining was performed as described by Magee *et al.* (1975). Cell morphology was observed using a phase-contrast microscope (Olympus AX70). Cell counts were performed using a haemocytometer (Hawksley BS.748 counting chamber) via phase-contrast microscopy. For electron microscopy (EM) visualization, strain T49^T was grown in 500 ml liquid culture for 3 days. The culture was then centrifuged at 5000 g (4 °C) for 20 min. The pellet was washed three times with 0.1 M Sorensen's phosphate saline buffer (Gomori, 1955) containing 2.5 % (v/v) glutaraldehyde. For transmission electron microscopy (TEM), cells were embedded in 2 % (w/v) agarose gel, fixed in 4 % (v/v) glutaraldehyde in 0.1 M phosphate buffer for 1 h at room temperature and then post-fixed in 1 % (w/v) osmium tetroxide for 1 h. Before embedding the cells in Spurr resin (Kushida, 1980), the agarose blocks were washed in phosphate buffer and then dehydrated via an ethanol and acetone series. The embedding blocks were sectioned with a diamond knife (90 nm thickness), transferred on a carbon/Formvar support film to grids, and stained with lead citrate (6 min) and uranyl acetate (12 min) before being examined on a JEOL 6700F field emission scanning electron microscope (FESEM) equipped with a scanning and transmission electron microscope detector at 25 kV. For scanning electron microscopy (SEM) analysis, cells suspended in water were placed in a droplet on a carbon dot and allowed to air-dry. The specimen was then coated with 12 nm chromium in a sputter coater before being examined on a JEOL 6700F FESEM.

Quinone and DNA G+C content determination. Quinone and DNA G+C contents were analysed by Brian Tindall and the identification service at the DSMZ, Braunschweig, Germany. For DNA G+C content determination (mol%), approximately 0.5 g freeze-dried material was washed in sterile water and lysed by French press. The genomic DNA was then purified on a hydroxyapatite column (Cashion *et al.*, 1977) and DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989). Quinone analysis was performed according to Tindall (2005).

Fatty acid analysis. Cells of strain T49^T were prepared for fatty acid analysis by combining colony biomass from 12 AOM1 plates grown at 60 °C. Fatty acid methyl esters (FAMES) were prepared and analysed without extraction according to the protocol detailed by Svetashev *et al.* (1995), or extracted from wet biomass (Bligh & Dyer, 1959) in subsequent batches for structure-elucidation purposes and methylated according to Carreau & Dubacq (1978). FAMES were analysed using tricosanoic (23:0) acid methyl ester as an internal standard on a TraceGC Ultra instrument (ThermoFinnigan) equipped with a flame-ionization detector, using a 30 m, 0.32 mm i.d. medium-polarity fused quartz BP-20 capillary column at 195 °C, and at 220 °C using a 60 m, 0.32 mm i.d. non-polar fused BP-1 quartz capillary column (both from SGE). GC-MS analysis was performed on an Agilent 5890N gas chromatograph equipped with a 5973 Inert mass-spectrometric detector with a fused quartz HP-5 capillary column (30 m, 0.32 mm i.d.; Hewlett Packard). Helium was used as the carrier gas. The program was started at 100 °C with a temperature-increase rate of 5 °C min⁻¹ up to 160 °C, then at 1 °C min⁻¹ to 240 °C, and left for 25 min. Dimethyldisulfide adducts were used to locate the exact positions of the double bonds in monounsaturated fatty acids (Yamamoto *et al.*, 1991). Cyclopropane ring location was determined according to Saito & Ochiai (1998), and its *cis* configuration was confirmed by ¹H-NMR (in CDCl₃, using a Bruker Avance III 500 MHz spectrometer).

Phylogenetic analysis. DNA was extracted from pure cultures by using a FastDNA SPIN kit (Q-BIOgene) as per the manufacturer's instructions. The 16S rRNA gene was amplified by using the primer pair 9f and 1492r (Weisburg *et al.*, 1991). PCR products were purified by using a PureLink PCR Purification kit (Invitrogen) and then sequenced using BigDye Terminator chemistry (Applied Biosystems) on an ABI 3730XL capillary DNA sequencer. Sequences were subject to a discontinuous MEGABLAST search against the NCBI GenBank database in order to identify the most closely related organisms (Altschul *et al.*, 1990). Potential chimeric sequences were eliminated by comparison of alignments of the 3' and 5' ends, and by the chimera-check program Bellerophon (Huber *et al.*, 2004). The 16S rRNA gene sequence of strain T49^T was aligned by using the ARB-SILVA database (Ludwig *et al.*, 2004). Phylogenetic trees were constructed by using near-full-length 16S rRNA gene sequences falling within the *Armatimonadetes* clade, using neighbour joining (NJ) and 'Randomized accelerated maximum likelihood' (RAxML) (Stamatakis *et al.*, 2005) in the ARB software environment. Maximum-parsimony analysis (1000 resamples) was conducted to determine support for tree branching nodes in the RAxML and NJ trees. Phylogenetic placement of strain T49^T was challenged by using multiple representatives of different phyla as outgroups. For the determination of 16S rRNA gene sequence divergence across the phylum *Armatimonadetes*, the hypervariable regions of 332 near-full-length 16S rRNA gene sequences were masked, and a pairwise distance matrix was calculated with the Jukes-Cantor correction as implemented in ARB (Ludwig *et al.*, 2004). The strain T49^T 16S rRNA gene sequence has been submitted to GenBank/EMBL/DDJB under the accession no. AM749780.

RESULTS AND DISCUSSION

Isolation and morphology

Strain T49^T was isolated from geothermally heated soils on solid gellan-based medium. T49^T formed circular, convex and entire colonies in approximately 3 days. Colonies were pigmented a light-pink colour that often darkened with age to a dark pink/orange colour. Pitting around the margins of the colonies was noted in the solid medium over time (Supplementary Fig. S1a, available in IJSEM Online). This suggested that the heteropolysaccharide solidifying agent gellan was used as an energy source, and that strain T49^T also exhibits some rudimentary cellulolytic activity. Single colonies were approximately 2–4 mm in diameter after 7 days incubation at 60 °C. In order to maintain planktonic T49^T cells in a liquid medium, incubation conditions of >180 r.p.m., a large air headspace (at least an equal ratio of headspace and medium volumes) and optimal pH conditions were required. Sub-optimal conditions or static incubation resulted in T49^T rapidly clumping and forming a pink amorphous pellicle (Supplementary Fig. S1b). Spectrographic analysis of the pigment extract shows a similar spectral peak (493 nm) and shoulders (data not shown) to the profile of *Thermomicrobium roseum*, a pink-pigmented thermophile from the phylum '*Chloroflexi*' (Wu *et al.*, 2009).

Cells were rods and stained Gram-negative, and were approximately 0.5–0.7 µm in diameter and 2.5–3.0 µm in length (Fig. 1). Cells divided by binary fission. Under phase-contrast microscopy, a limited number of cells were

Fig. 1. Electron micrographs of T49^T. (a) Scanning electron micrograph showing a highly corrugated cell membrane (bar, 1 μ m). (b) Transmission electron micrograph showing cross-section of the cell, detailing cell morphology, electron-dense granule structures (gs), interfolding (if) of the plasma membrane and electron-light cytosolic structures (cs) (bar, 0.5 μ m). Insert details plasma membrane (pm), peptidoglycan (pg) and outer membrane (om) structure (bar, 100 nm).

observed to exhibit a tumbling motility. This trait was highly dependent on growth phase, medium pH and substrate, and has been difficult to reproduce. EM confirmed that cells of strain T49^T possess a Gram-negative cell envelope; a thin peptidoglycan layer is readily visible between the cytoplasmic and outer membranes (Fig. 1b). An irregular, corrugated outer membrane was consistently observed despite using different EM fixing protocols and different cell-preparation methods (Fig. 1a). Similar envelope corrugations have been observed in representatives of the genus *Thermus* (Brock & Edwards, 1970; Kristjánsson *et al.*, 1994). Central ‘electron-light’ cavities in the cytosol were consistently observed in the majority of cells and appeared to contain an unknown crystalline substance that was removed during microtoming. In the near-membrane area of the cytosol, especially at the cell poles, small, electron-dense granules were also observed. Dark staining of microbial biomass with osmium tetroxide is often associated with lipids and suggests that these bodies may be liposomes or micelles. Neither spore formation nor flagella were observed.

Physiology and chemotaxonomic characteristics

Strain T49^T grows between 50 and 73 °C with an optimum growth temperature of 68 °C. No growth was observed at 37 or 76 °C after 3 weeks incubation. T49^T has a narrow pH growth range of pH 4.7–5.8 with an optimum of pH 5.3 when growing planktonically in liquid medium. No growth was observed in liquid medium at pH 4.5 or 6.2. This result was unexpected, as T49^T was isolated from geothermal soil with an *in situ* pH of 4.3. Interestingly, T49^T was readily observed to form pellicles in statically grown medium outside this experimentally determined pH range, suggesting that biofilm formation may be a response to an unfavourable pH environment. A maximum growth

rate of 0.012 h⁻¹ was observed under optimal temperature and pH conditions. Optimal growth was observed in NaCl-free medium, although T49^T exhibited growth in NaCl-amended FSV1 medium of up to 2% (w/v) NaCl. No colony growth was observed at 60 °C in solid medium containing 10 and 100 μ g ml⁻¹ of ampicillin, kanamycin or streptomycin, and 100 μ g ml⁻¹ only of polymyxin B or chloramphenicol. Trimethoprim, metronidazole, lasalocid A and neomycin did not inhibit the growth of T49^T at the concentrations tested.

Strain T49^T has an oxidative, carbohydrate-based metabolism and is able to grow on DL-arabinose, D-xylose, D-ribose, D-fructose, D-glucose, D-mannose, D-rhamnose, D-galactose, sucrose, lactose, maltose, trehalose, cellobiose, raffinose and D-N-acetylglucosamine, but not D-galacturonic acid. Growth was also observed on branched or amorphous polysaccharides such as starch, glycogen, dextrin, CM-cellulose, xylan, galactomannan, pectin, gellan, xanthan, chitin and pullulan, but not on linear polysaccharides such as sodium alginate, agarose, cotton, Whatman filter paper, Avicel or any non-commercial lignocellulosic pulp preparations. Strain T49^T was unable to utilize the alcohols or the nutrient-rich media tested, although pH-adjusted and heavily diluted R2A medium weakly supported growth. Malic acid and sodium pyruvate supported aerobic growth, but sodium citrate, sodium acetate, sodium fumarate, sodium lactate, yeast extract and Casamino acids did not. Whilst yeast extract does not support T49^T growth independently, it appears to be obligately required in trace concentrations in the basal nutrient medium for growth on other carbohydrate substrates. When yeast extract in the basal medium was substituted with trace concentrations of Casamino acids, growth was also observed, suggesting that yeast extract could supplement deficiencies in amino acid synthesis. Further analysis will be necessary to determine the

exact nature of this requirement and may be highlighted in the genome-sequencing programme currently under way. Aerobic oxidation of elemental sulfur, methane or dihydrogen gas was not observed, nor was anaerobic growth using nitrate, sulfate or sulfur as terminal electron acceptors. The preferred nitrogen source of T49^T is the ammonium ion, although yeast extract can also exclusively supply the nitrogen requirement. Nitrate, dinitrogen gas and Casamino acids were unable to act as sole nitrogen sources.

Strain T49^T is catalase-positive and oxidase-negative. T49^T shows positive results for the following enzymic activities: acid phosphatase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, α - and β -galactosidases, α - and β -glucosidases, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase (bioMérieux).

Table 1 shows the fatty acid composition of T49^T. The main fatty acids observed were 16:0 (25.8 %), i17:0 (19.3 %) and ai17:0 (13.5 %). Less common fatty acids included *cis*-16:1 Δ 5 (8.8 %), *cis*-i17:1 Δ 5 (6.8 %) and 5,6-methylene 16:0 (5.2 %). The *cis* configuration of double bonds was inferred due to the lack of *trans*-unsaturation absorbance at 960–980 cm⁻¹ in the IR spectrum of the unsaturated fatty acid fraction. Δ 5 monoenes have been detected previously in *Bacillus* spp. (Kaneda, 1971). The significance of the unusual double-bond position is not clear, but has been tentatively linked to aerobic biosynthesis (Kaneda, 1991). Another unusual fatty acid component of T49^T was *cis*-5,6-methylene-16:0. To our

knowledge, this is the first report of a naturally occurring 5,6-methylene hexadecanoic acid, although its hydroxylated homologue, 2-hydroxy-5,6-methylene-18:0, is one of the components of cepaciamide A, a fungicide isolated from *Pseudomonas cepacia* (Jiao *et al.*, 1996).

The quinone system contains MK-8 (70 %), MK-9 (17 %), MK-7 (10 %) and MK-6 (3 %), all of which appear to contain fully unsaturated isoprenoid side chains. Menaquinone MK-8, as the dominant quinone, is moderately rare in the domain *Bacteria* (Collins, 1994). However, members of several thermophilic bacterial genera, including *Caldisericum* (formerly candidate division OP5), *Thermus* and *Meiothermus*, contain MK-8 as the primary quinone (Garrity & Holt, 2001a; Mori *et al.*, 2009).

Comparative phylogenetic analysis of the T49^T near-full-length 16S rRNA gene sequence (1411 bp; GenBank accession no. AM749780) indicated that strain T49^T was not related closely to any known cultivated microbial strains [<81 % pairwise sequence similarity to *Clostridia* spp. and *Thermoanaerobacter mathranii* (GenBank accession no. AY701758)], based on a discontinuous MEGABLAST search. Rather, strain T49^T was associated most closely with environmental clones from various soil and groundwater environments within the newly described phylum *Armatimonadetes*. Strain T49^T shared the closest 16S rRNA gene sequence similarity with the cultivated and incompletely described strain P488 (GenBank accession no. AM749768; Stott *et al.*, 2008) and a clone from prairie grassland soil (GenBank accession no. EF515962; Cruz-Martínez *et al.*, 2009) with 99 and 88 % identity, respectively. T49^T was consistently placed within one (nominally designated group 3) of six monophyletic groupings within the phylum *Armatimonadetes* and was supported by bootstrapping analysis of the maximum-parsimony and ML datasets (Fig. 2). The group 3 subdivision comprises mainly environmental clones from soil ecosystems, including prairie grasslands soils (Cruz-Martínez *et al.*, 2009; Elshahed *et al.*, 2008; Liles *et al.*, 2003), forested wetland soils (Brofft *et al.*, 2002) and contaminated groundwaters (Nogales *et al.*, 2001; Roh *et al.*, 2009; von Wintzingerode *et al.*, 1999).

Strain T49^T is distantly related to the only other strain in the phylum *Armatimonadetes* with a validly published name (*Armatimonas rosea* YO-36^T; Tamaki *et al.*, 2011), sharing a pairwise 16S rRNA gene sequence similarity of approximately 79 %. Both strains group consistently within separate monophyletic clades (nominally groups 1 and 3; Fig. 2). The phenotypic traits of both strains are also substantially dissimilar (Supplementary Table S1, available in IJSEM Online). Both strains exhibit a saccharolytic-type metabolism, although YO-36^T appears limited in the number of saccharides and polysaccharides that will support its growth. Comparatively, most carbohydrates support the growth of T49^T, along with an array of amorphous polysaccharides such as CM-cellulose, xylan and starch. Other similarities include pink pigmentation, aerobicity, Gram-negative staining and a negative oxidase

Table 1. Fatty acid composition of T49^T

Only components present at ≥ 0.5 % are shown; components making up <0.5 % of the total fatty acid content are not shown (sum total of these components, 2.5 %). ai, anteiso-; br, branched; cy, cyclo; i, iso-.

Fatty acid	Percentage of total fatty acids
i15:0	1.0
ai15:0	0.5
i16:0	4.4
16:0*	25.8
16:1 Δ 5	8.8
i17:0	19.3
ai17:0	13.5
i17:1 Δ 5	6.8
ai17:1 Δ 5	0.7
17:0	0.8
5,6-cy-16:0	5.2
i18:0	1.2
br18:1	0.9
18:0*	3.6
18:1 Δ 5	3.2
i19:0	0.9
i19:1	0.9
Total	97.5

*Low concentrations of components also present in media.

Fig. 2. ML dendrogram based on 16S rRNA gene sequences of strain T49^T (highlighted in bold) and environmental clones belonging to the phylum *Armatimonadetes*. Branch points supported (bootstrap values >90 %) by all inference methods are indicated by ○, values >80 % are indicated by ● and values >70 % by ◇. An expanded tree detailing all *Armatimonadetes* phylotypes used and outgroup representatives is presented in Supplementary Fig. S2 (available in IJSEM Online). GenBank/EMBL/DBJ accession numbers are shown. Bar, 0.10 substitutions per compared nucleotide site.

reaction. Conversely, motility, quinone and fatty acid profiles, temperature and pH requirements for growth, DNA G + C content, cell morphology and salinity tolerance are substantially different. The nitrogen source for YO-36^T is not discussed (Tamaki *et al.*, 2011). Interestingly, we note the ability of strain YO-36^T to utilize unknown components of yeast extract and possibly casein- and/or soy-digested tryptone (in the enrichment and isolation medium). This trait is distinctly different from that of T49^T, which has an inability to utilize peptides or components of peptide-digested media for growth.

Taxonomic conclusions

The phylogenetic and phenotypic findings described above demonstrate that strain T49^T is a novel and distinctive bacterium within the phylum *Armatimonadetes* and is clearly differentiable from *Armatimonas rosea* YO-36^T.

Furthermore, based on the consistent monophyletic placement of the subdivision housing T49^T and a relatively distant 16S rRNA gene sequence similarity of approximately 79 % with YO-36^T, we propose that the group 3 subdivision (Fig. 2.) represents a novel class within the phylum *Armatimonadetes*. We therefore propose a novel genus and species, *Chthonomonas calidirosea* gen. nov., sp. nov., to accommodate this isolate, and create a new class, *Chthonomonadetes* classis nov., with strain T49^T proposed as the type strain. The taxonomic rankings between genus and class are proposed according to Garrity & Holt (2001b).

Description of *Chthonomonas* gen. nov.

Chthonomonas [Chtho.no.mo'nas. Gr. n. *Chthōn* Chthonos earth, soil, land; Gr. fem. n. *monas* a unit, monad; N.L. fem. n. *Chthonomonas* a unit (bacterium) from soil].

Gram-negative, catalase-positive, oxidase-negative. Cells are rod-shaped. Spores are not formed. Cells grow under aerobic conditions. Thermophilic, moderately acidophilic. Cells divide by binary fission. DNA G+C content of the type species is 54.6 mol%. Major respiratory quinone is MK-8. Main fatty acids are 16:0, i17:0 and ai17:0. The type species is *Chthonomonas calidirosea*.

Description of *Chthonomonas calidirosea* sp. nov.

Chthonomonas calidirosea (ca.li.di.ro'se.a. L. adj. *calidus* warm, hot; L. adj. *roseus* -a -um rose-coloured, rosy; N.L. fem. adj. *calidirosea* hot and rosy).

Cells are non-spore-forming, highly corrugated rods (approx. 0.5–0.7 µm in diameter and 2.5–3.0 µm in length). Gram-negative, oxidase-negative and catalase-positive; exhibits an oxidative metabolism. Cell division occurs by binary fission. Cells grow readily on mono- and disaccharides and on short, branching or amorphous polysaccharides such as DL-arabinose, D-xylose, D-ribose, xylose, D-fructose, D-glucose, D-mannose, D-rhamnose, D-galactose, sucrose, lactose, maltose, trehalose, cellobiose, raffinose, D-N-acetylglucosamine, starch, glycogen, dextrin, CM-cellulose, chitin, xylan, galactomannan, pectin, gellan gum, xanthan and pullulan. Malic acid and sodium pyruvate also support growth. Ammonium or yeast extract can act as sole nitrogen sources. No growth is observed on linear or crystalline polysaccharides. Optimal growth occurs at 68 °C (range, 50–73 °C) and pH 5.3 (range, pH 4.7–5.8). Tolerates up to 2 % (w/v) NaCl. Growth on solid gellan-based medium produces pink/rose, opaque colonies with circular and entire edges. The type strain generates a rose-pink pellicle when incubated statically or in medium with non-optimal pH. DNA G+C content of the type strain is 54.6 mol% and the quinone system consists of MK-8 (70 %), MK-9 (17 %), MK-7 (10 %) and MK-6 (3 %). Major fatty acids are 16:0 (25.8 %), i17:0 (19 %) and ai17:0 (13.5 %), with minor amounts of 16:1Δ5 (8.8 %), i17:1Δ5 (6.8 %) and 5,6-cy-16:0 (5.2 %).

The type strain, T49^T (=ICMP 18418^T=DSM 23976^T), was isolated from geothermally heated soils at Hell's Gate, Tikitere, New Zealand.

Description of *Chthonomonadaceae* fam. nov.

Chthonomonadaceae (Chtho.no.mo.na.da'ce.ae. N.L. fem. pl. n. *Chthonomonas* type genus of the family; -aceae ending to donate a family; N.L. fem. pl. n. *Chthonomonadaceae* family of the genus *Chthonomonas*).

The description is the same as for the genus *Chthonomonas*.

Type genus: *Chthonomonas* gen. nov.

Description of *Chthonomonadales* ord. nov.

Chthonomonadales (Chtho.no.mo.na.da'les. N.L. fem. pl. n. *Chthonomonas* type genus of the order; -ales ending to

donate an order; N.L. fem. pl. n. *Chthonomonadales* order of the genus *Chthonomonas*).

The description is the same as for the genus *Chthonomonas*.

Type genus: *Chthonomonas* gen. nov.

Description of *Chthonomonadetes* classis nov.

Chthonomonadetes (Chtho.no.mo.na.de'tes. N.L. fem. pl. n. *Chthonomonadales* type order of the class; N.L. fem. pl. n. *Chthonomonadetes* class of the order *Chthonomonadales*).

The class *Chthonomonadetes* is defined on a phylogenetic basis by comparative 16S rRNA gene sequence analysis of one isolated strain and uncultured representatives from multiple terrestrial and aquatic habitats.

Type order: *Chthonomonadales* ord. nov.

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