Williamwhitmania taraxaci gen. nov., sp. nov., a proteolytic anaerobe with a novel type of cytology from Lake Untersee in Antarctica, description of Williamwhitmaniaceae fam. nov., and emendation of the order Bacteroidales Krieg 2012

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Abstract
The proteolytic bacterium strain A7P-90m T was isolated from Lake Untersee, Antarctica. The anoxic water was collected from a perennally sealed (~100 millennial) glacial ice lake. Gram-stain-negative cells were 0.18–0.3 × 8.0–25.0 μm in size, straight, slender rods with unusual gliding motility by external, not previously reported, organelles named here as antiae. At the end of stationary phase of growth, spheroplasts were terminally formed and the cells resembled dandelions. After death, cells were helical. The isolate was an athalassic, strictly anaerobic and catalase-negative proteolytic chemoorganotroph. It was moderately psychrophilic with a temperature range for growth of 3–26 °C and an optimum at 22–23 °C. The pH range for growth was 5.5–7.8 with an optimum at 6.9. Major cellular fatty acids were branched pentadecanoic and tridecanoic acids, and saturated tetradecanoic acids. The quinone system comprised menaquinone MK-7. The strain was sensitive to all checked antibiotics and ascorbic acid. The G+C content of the genomic DNA was 42.6 mol%. Based on average nucleotide identity, average amino acid identity and phylogenetic analyses, the novel isolate was placed within a unique phylogenetic cluster distant from all eight families in the order Bacteroidales and formed a novel family with the proposed name Williamwhitmaniaceae fam. nov. The description of the order Bacteroidales was emended accordingly. The name Williamwhitmania taraxaci gen. nov., sp. nov. is proposed for the new genus and novel species with the type strain A7P-90m T (=DSM 100563 T=JCM 30888 1). The complete draft genome sequence was deposited at the Joint Genomes Institute (JGI) under number IMG OID 2654581448 and in SRA listed as SRP088197.

The anaerobic microbiota of Lake Untersee, Antarctica, has not been investigated before and the novel isolate is the first extremophilic species cultivated from this lacustral ecosystem. The lake has been perennally sealed by glacial ice for >100 000 years [1, 2], and therefore this ecosystem with its entire biome represents an evolutionary unique isolated ecosystem which has remained unaffected by the modern biosphere. Details of this oligotrophic and athalassic ecosystem, collection of samples and the preliminary results of novel strain isolation were described previously [3–7]. The conical stromatolites in Lake Untersee and the biodiversity of photosynthetics in benthic microbial mats were also reported [8]. In cold environments, decomposition of organic matter is performed by psychrophilic bacteria, archaea and fungi, which remain metabolically active at subzero temperatures. In anaerobic ecosystems, protein decomposition is accompanied by satellite anaerobes which consume inhibiting levels of metabolic end products of the primary anaerobes, such as hydrogen gas and others. Therefore, formation of the consortia with autotrophic homo-acetogens, sulfate-reducers and methanogens is common in marine and cold athalassic environments. In Lake Untersee, despite the high concentrations of sulfide measured in anoxic bottom strata, the low levels of sulfate exclude sulfidogenesis by bacterial sulfate reducers. Thus, the major hydrogen consumers would be expected to be methanogens and/or homo-
acetogens. Two psychrophilic homo-acetogenic strains, V4LU-90mF and LU-96te, were isolated from anoxic samples, but despite the high level of methane measured in Lake Untersee, the isolation of methanogenic cultures has not been reported so far [5]. Filamentous and spherical phages were reported in aerobic bacteria from the lake [9].

Proteolytic metabolism is common among psychrophilic and mesophilic anaerobes in polar ecosystems, and many of these species also possess saccharolytic enzymes. Previously, we described a psychrotolerant, obligately proteolytic anaerobe, *Protocatella sphenisci* PPP21, that is unable to grow on sugars, individual amino acids or perform the Stickland reaction [10]. Proteolytic metabolism was also reported for free-living saccharolytic *Spirochaeta* species [11, 12]. Here, we describe a novel strictly anaerobic, obligately proteolytic and moderately psychrophilic strain, A7P-90m5, isolated from Lake Untersee. Phylogenetic analysis placed the novel isolate within the order *Bacteroidales* with low 16S rRNA sequence similarity (84–85%) to members of existing families. Currently, the order *Bacteroidales* [13, 14] includes eight families (*Bacteroidaceae*, *Marinilabiliaceae*, *Marinilabilaceae*, *Odoribacteraceae*, *Porphyromonadaceae*, *Prevotellaceae*, *Prolixibacteraceae* and *Rikenellaceae*). Our data, as well as others [15, 16], did not support the proposed separation of three families (*Marinilabilaceae*, *Prolixibacteraceae* and *Marinilabiliaceae*) to the order *Marinilabilales* [17]. Based on average nucleotide identity (ANI), average amino acid identity (AAI) and phylogenetic analyses of the 16S rRNA gene, the novel isolate along with five sequences of other ecologically diverse uncultured bacteria represents a unique phylogenetic cluster that is distantly related to eight families of *Bacteroidales* and so forms a novel clade among these taxa. The name *Williamwhitmania taraxaci* gen. nov., sp. nov. is proposed for the new genus and species with the type strain A7P-90m5, which represents a member of the novel family *Williamwhismaniaceae* fam. nov.

Collection of samples of the ice, water and sediments of Lake Untersee (71° 20’ S 13° 27’ E) during the 2008 Antarctica expedition (Figs S1 and S2, available with the online Supplementary Material) was described previously [3, 4]. A Jiffy ice drill was used to bore a 26 cm diameter hole through the 3 m thick ice sheet covering the lake. At the location sampled (S 71° 21’ 21.5”; E 013° 25’ 37.8”), the lake floor was 96 m deep beneath the surface of the ice sheet. The water sample (which gave the novel isolate) was collected in Kemmerer bottles at a depth of 90 m with a H2S maximum zone (20 mM), pH 7.0, salinity <0.5% and temperature of 4°C.

For cultivation, an anaerobic medium (AM) was used with following composition (g l−1): NaCl 5.0, KCl 0.3, KH2PO4 0.3, NH4Cl 1.0, MgSO4·7H2O 0.1, CaSO4·7H2O 0.0125, NaHCO3 0.2, Na2S·9H2O 0.5, resazurin 0.005% (w/v), 2 ml vitamin solution [18], 1 ml trace element solution [19], and yeast extract 0.1. The final pH was 7.0. The gas phase in Hungate tubes was high-purity nitrogen gas. Unless specified, peptone (3 g l−1) was added separately as a growth substrate. The medium was autoclaved at 1 atm, 121°C for 1 h. To obtain a pure culture, serial dilutions and roll tubes were used. Isolation of colonies was performed on 1.5% (w/v) agar AM in roll tubes incubated at 15°C. All other physiological and biochemical tests were performed at 22–24°C. The optimal pH was determined in AM with titration by 2 M H2SO4 and 6 M NaOH at pH increments of 0.5. Dependence upon sodium ions was tested in AM, in which sodium ions were replaced by equimolar amounts of potassium salts and NaCl was added to achieve final concentrations of 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 5.0% (w/v). The culture was incubated within temperatures of −3–30°C at intervals of 1–3°C to determine the optimum and growth range. Growth at negative and near-zero temperatures was monitored for 6 weeks by using an NERLAB RTE-11 circulating chiller with glycerol and an ENDODAL RTE-4DD refrigerated circulating bath (Marshall Scientific). Growth substrates were tested in concentrations of 3–5 g l−1 and were sterilized separately prior to adding to AM. Bacteriolytic capacity was tested on autoclaved biomass of *P. sphenisci* PPP1 cells at a concentration of 30 mg per 10 ml of AM. Potential electron acceptors [S0 (3 g l−1), Na2SO4 (20 mM), Na2SO3 (10 mM), Na2S2O3·7H2O (20 mM), dimethyl sulfoxide (DMSO; 3 ml l−1), NaNO3 (1.2 mM and 10 mM) and Fe(OH)3 (10 mM)] were checked on AM with peptone. Indole production and nitrate/nitrite reduction tests were checked by using crystallized oxalic acid and sulfanilic acid/N-(1-Naphthyl) ethylenediamine dihydrochloride according to methods described previously [20]. Hydrogen sulfide was measured by using the colorimetric method with methylene blue [21]. Metabolic products were measured by using an Agilent 7890A chromatograph (Agilent Technologies) for volatile fatty acids with StabilaWax/Restek columns at the Bioexpression and Fermentation Facility (University of Georgia in Athens), and an SRI 8610 C gas chromatograph (SRI Instruments) as described previously [22]. The gas chromatography method followed the temperature sequence: 85–120°C at 3°C min−1, 120–185°C at 6°C min−1 and 185–220°C at 50°C min−1. Column flow was 1.5 ml min−1. The flame ionization detector was set to 250°C and 50 Hz/0.004 min. The injection volume was 0.2 µl.

Culture growth was estimated by cell counting with a phase-contrast microscope at ×1000 or ×2000 (Fisher Micromaster) and by measurements of the optical density at 510 nm with a spectrophotometer (Genesis 5; Spectronic Instruments).

Cells were imaged by using a Labophot Epifluorescent microscope (Nikon, Japan) with BacLight live versus dead fluorescent stain (Molecular Probes) and phase-contrast microscopy. A charge-coupled device and complementary metal oxide semiconductor microscope digital camera with ToupView 3.7 software (OMAX) was used to record the cellular movement. Additional magnification by using the optics of iPhone ‘touch screen’ technology (Apple) was applied to observe the external organelles involved in
cellular motion. The software Adobe Premiere Pro CS6 version 6.0.5 was used to modify the format of the recorded movies to characterize the specifics of cellular motion. Cell surface features of uncoated cells were imaged with a Quanta 600FEG field emission gun scanning electron microscope-FESEM (FEI) and the element compositions of cells and antiae were measured using energy dispersive x-ray spectroscopy (EDS). The cellular ultrastructure was examined by transmission electron microscopy of negatively stained cells and thin sections, as described previously [23].

Extraction and measurement of quinones and urease activity were performed as described previously [24]. Cellular fatty acid methyl esters were extracted from lyophilized cells grown on AM with peptone for 4 days at 23 °C by using the Sherlock Microbial Identification System (version 6.1) as described previously [25–27]. Analysis was carried out by using an Agilent Technologies 6890 N gas chromatograph equipped with a phenyl methyl silicone fused silica capillary column (HP; 2.25 mm × 0.2 mm × 0.33 µm film thickness) and a flame ionization detector. Hydrogen was used as the carrier gas. The temperature program was initiated at 170 °C and increased at 5 °C min⁻¹ to a final temperature of 270 °C. The relative amount of each fatty acid was expressed in terms of the percentage of total fatty acids using the QTSA1 peak naming library.

DNA was extracted according to the EZNA Bacterial DNA kit’s protocol (OMEGA Bio-Tek GA). The purity of DNA was checked by gel electrophoresis; additional RNAase treatment was conducted. PCR was performed as described previously [23] with the following primers: B16S-F 5’-AGAGTTTGATCMTGGCTCAG-3 and B16S-R 5’-TACG-GYTACCTTGTTACGAC-3. PCR products were purified by using a ZYMO DNA Clean and Concentrator-5 column following the manufacturer’s instructions and prepared for 16S rRNA gene sequencing. Sequencing was done at the Georgia Genomics facility at the University of Georgia in Athens. The sequence was deposited in GenBank under KR072685 and then aligned by using the Ribosomal Database Project (RDP) analysis tools [28]. The processed sequence was subjected to phylogenetic analysis using the FastTree algorithm implemented in Geneious 8 [29, 30]. ANI values were retrieved from Integrated Microbial Genomes (IMG) and AAI values were calculated by using an online tool developed by the Kostas group at the Georgia Institute of Technology [31, 32].

To obtain an enrichment culture, 0.5 ml of the water sample from a 90 m depth of Lake Untersee was anaerobically injected into pre-cooled to 3 °C Hungate tubes with AM. A pure culture was obtained at 3–4 °C. Young colonies (4–7 days) were round or elliptical, 1–2 mm in diameter and white in colour. Aged colonies (10–14 days) had dark yellow colouration (indicative of flavins) with protuberances by perimeter, which are characteristic of proliferating growth on agar, and resembled fuzzy ‘cotton balls’ (Fig. S3a, b). During growth, the agar surface was ulcerated under the colonies forming deep craters; this indicated the agarolytic feature of the strain (Fig. S3a). One of the colonies, from the seventh dilution, was transferred anaerobically to AM. The purity of the culture was confirmed microscopically following growth on different organic substrates.

Strain A7P-90mT had a novel type of cellular morphology. Under light microscopy, the culture comprised elongated, thin, flexible rods with sizes of 0.18–0.3 × 8.0–25.0 µm (Figs 1, 2a and S4a, b). Cells occurred singly as regular rods or chained under a sheath as long filaments. At the stationary phase of growth, cells formed spheroplasts at one of

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**Fig. 1.** Diagnostics of antiae by epifluorescent microscopy of A7P-90mT with live/dead fluorescent stain. (a) Image with a green filter, arrows indicate: white, spheroplasts; black, temporarily stable oval protoplasts reflecting light; and red, erythrocyte-shaped bodies. Glowing tails of antiae are visible at the poles of cells. (b) Dead cells are in green and alive in red. Perpendicular stripes on filamentous cells are the spaces between rings of antiae.
their poles and resembled the seed head of dandelion (*Taraxacum officinale*) flowers. During the logarithmic stage of growth, elongated, thin hair-like rods expressed a trembling, gliding motility. A novel external organelle, named here the *antia*, plural *antiae* [from Latin *antia*, *antiae* – locks (pl.) of hair that hang down in front, forelock] are shown in Figs 2a–c and S5. It had multiple thin fibrils entangled in several larger diameter whip-like extensions. The length of each extended plexus could reach 10–20 times that of the cell’s length. In Video S1 the distant antiae can be seen as vortexes (dark dots, two at top right and one in bottom left corner) resembling tentacles of an octopus.

The whip is composed of smaller diameter entangled braids. The antiae do not absorb stains (as regular flagella) at the negative stain for TEM (Fig. 2b) or the silver-plating stain for flagella (Fig. S4b) but could be observed under epifluorescent microscopy (with live/dead cell viability assays) as glowing tails at the polar regions of cells and/or wrapping cellular cylinder in regularly coiled rings (Figs 1a, S6a, b and S7a). Indirect observation of antiae could be performed by ‘out of focus’ phase contrast microscopy: the rings of antiae wrapping a cell causing a visualization of ‘ribs’ on a cellular surface (Fig. 3a). Such an optical effect is the result of photon absorption by the antiae causing the gradient of refractive index. Thin perpendicular stripes on the cell surface (Fig. 1a, b) are indicative of this effect using ‘in focus’ observations with epifluorescent microscopy. This optical microscopy technique is recommended here as the diagnostic method for antiae.

In phase contrast microscopy, the antiae could be observed as fast spinning whips (Fig. S7b), which dissipate into thin fibrils when motility stops. At a slow-mode rotation, the torn ends of shorter antiae sticking out from entangled braids of the whip (Video S2). Antiae arrangement in different plexa is shown in Fig. S5, the rings of the large diameter whips wrapping the cell body, smaller diameter braids and untangled manes of dissipated thinnest fibres. All these extensions were observed in coherent, unison motion of one controlling force. Dissonance or dissimilar random motility of antiae of one cell was never observed; all of them moved as one organelle. This feature distinguishes it from flagella, which can rotate at different times and in different directions causing tumbling. As a result of the high frequency vibration caused by spinning antiae around the cell, the cellular movement appeared as gliding in a slow, smooth manner (Video S3). Twitching or corkscrew motility typical for spirochetes was not observed.

The ends of the cells were rounded (Fig. 2b, c). Multiplication of cells was by binary division through cell-wall septation; eventually, the process of division was corrupted and chains or long filaments composed of separate cells held within the sheath could be observed (Fig. S8a). At the final stage of growth, cells lost motility, appeared helical and...
were no longer viable (Fig. S8b). TEM of thin sections showed the fundamental differences in the cytology of the novel isolate. The important cytoskeletal components in strain A7P-90mT are 10 pairs of 20 nm diameter columns of secretion systems producing antiae fibres (Fig. S10); the columns are localized in cytoplasm by perimeter. In the genome sequence, proteins of the type IV secretory pathway, VirJ component and genes associated with secretion system type IX proteins are supportive for these cytological structures. Paired large pores (20 nm diameter each) of the secretion system exited along the entire length of the cell surface, and usually concentrated at the poles of cells or near septation regions (Fig. 2b, c and S9a, b). Although periplasmic proteins and murein were annotated in the genome description, a clear image of the periplasmic murein layer was impossible to find; pore perforations with plexa of antiae probably contributed to partially hidden patterns of the murein layer (Fig. 3b). The presence of antiae in periplasmic space explained the helical shape of cells after death. However, the plexa of antiae could be exposed to the cell surface through the one pore, but go back inside the periplasmic space through another (Fig. S11), and, therefore, did not repeat the flagella arrangement of spirochetes. Gram staining did not lead to pink or red colouration of A7P-90mT cells. Instead, dark purple-pink or blue was obtained. Also, no clear border of the outer sheath in TEM thin-section images was demonstrated, the only evident image of the sheath was shown by phase contrast microscopy (Fig. 3a).

FESEM images showed that the antiae resembled flames (Fig. S12) and measured x-ray EDS spectra (Fig. S13) indicated increased amounts of silicon and fluorine in the antiae compare to the cellular surface.

The new isolate grew well within a temperature range of 3–26 °C with an optimum at 22–23 °C. Limits of growth were 27 and 0 °C. The salinity range for growth was 0–2 % (w/v) NaCl, with an optimum at 0.5 %. The pH range for growth was 5.5–7.8 with an optimum at 6.9.

Growth characteristics of the new isolate were typical for secondary anaerobes: the optical density never exceeded 0.3 (λ 510 nm) and well-grown cultures acquired a light whitish opalescence. Culture did not smell of ammonia, putrescine or cadaverine (typical of Gram-positive proteolytic ammonifiers such as Tindallia species). With 3 % (v/v) inoculum, culture grew in 48–72 h.

Table 1. The cellular fatty acid profile of strain A7P-90mT

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>C12:0</td>
<td>1.1</td>
</tr>
<tr>
<td>Iso-C13:0</td>
<td>11.2</td>
</tr>
<tr>
<td>Anteiso-C13:0</td>
<td>8.2</td>
</tr>
<tr>
<td>C13:0</td>
<td>1.5</td>
</tr>
<tr>
<td>Iso-C14:0</td>
<td>1.2</td>
</tr>
<tr>
<td>C14:0</td>
<td>7.7</td>
</tr>
<tr>
<td>Iso-C15:0</td>
<td>3.6</td>
</tr>
<tr>
<td>Isomer-C15:0:3OH/15:1H</td>
<td>17.2</td>
</tr>
<tr>
<td>Anteiso-C15:0</td>
<td>20.3</td>
</tr>
<tr>
<td>C15:1 Δ9c</td>
<td>1.6</td>
</tr>
<tr>
<td>C15:0</td>
<td>3.4</td>
</tr>
<tr>
<td>C16:0</td>
<td>6.0</td>
</tr>
<tr>
<td>Iso-C15:0:3OH</td>
<td>3.9</td>
</tr>
<tr>
<td>C15:0 3OH</td>
<td>3.2</td>
</tr>
<tr>
<td>Cyclo-C18:0/17:0</td>
<td>1.4</td>
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</table>
Strain A7P-90m grew well on peptone, bactotryptone, tryptase peptone, meat extract or yeast extract with production of H₂ gas at concentration of ≤4 mmol per litre of culture and traces of CO₂. In liquid phase the following products were determined: acetate (0.5 mM), propionate (0.1 mM), butyrate (0.2 mM), lactate (3 mM) and ethanol (0.4 mM). Methanol, propanol, butanol, methylbutanol, isobutyrate and valerate were not found. All checked substrates that did not support growth are listed in the species description. Growth on maltose and starch was observed only in the first and second transfers. Three transfers were positive on inulin, but in the third transfer cells formed spheroplasts and were no longer viable. Three transfers on casein were positive, but cells did not maintain integrity. Spheroplasts and were no longer viable. Three transfers on casein were positive, but cells did not maintain integrity. Spheroplasts from the third transfer were injected on peptone, but the revival was negative. Casein and gelatin were hydrolysed.

Negative growth was observed on blood agar. Growth on cells of *P. sphenisci* PPP was negative. Individual amino acids were checked in concentrations of 3 g l⁻¹ on AM (without peptone), on all of them growth was negative (see list in the species description). Stickland reaction was negative in all theoretically possible amino acids pairs (see the species description). H₂:CO₂ (80:20) did not support growth (neither lithoheterotrophic nor autotrophic), but the culture grew faster on peptone in atmosphere containing hydrogen than with nitrogen only. The novel isolate was sensitive to all checked antibiotics, as listed in the species description. Elemental sulfur, sodium sulfate, sulfite, thiosulfate and DMSO did not stimulate or inhibit growth, and hydrogen sulfide was not produced. Iron 3-hydroxide was not reduced and did not affect growth, while nitrate and nitrite reduction tests were both positive. Growth in AM

![Fig. 4. Maximum-likelihood (ML) tree based on nearly full-length 16S rRNA gene sequences from representative species and strains of order Bacteroidales. An average of 1460 positions was aligned using the RDP tool. The tree was built by FastTree 2.1.5 using *Flavobacterium aquatile* as an outgroup. Bootstrap values>0.62 are indicated at nodes and are based on 1000 replicates. Bar, substitutions per site. *Derived from genome sequences CR629627(3205533...3207061) and CP002122(369974...371504). Showing the same topology at the family level, a neighbour-joining (NJ) tree was also reconstructed and bootstrapped with 1000 replicates using GENEious 10.1.2. NJ bootstrap values>68 % are included on the ML tree for clusters at the family or higher level. To achieve unambiguous presentation, they are expressed as percentages and indicated below the corresponding ML bootstrap values.](https://www.microbiologyresearch.org/issue/17/6/4132-4145)
without Na₂S·9H₂O was negative. Pink (by resazurin) AM did not support growth; it defined strain A7P-90mᵀ as being strictly anaerobic. Indole was not produced on peptone, which was confirmed by negative growth on tryptophan. Urease activity was considerably high: 2–3 g l⁻¹ of urea was hydrolysed in 5 days. The cultures grew well with urea concentrations of 1.5–6 g l⁻¹; cells were stable at concentrations of 1.5–3 g l⁻¹, but at 6 g l⁻¹ the biomass level was poor and the integrity of cells was unstable. Ascorbic acid (0.5 g l⁻¹) inhibited growth and could not be used as an alternative reducing agent for anaerobic medium preparation. L-cysteine could be used as an alternative reducing agent.

The major cellular fatty acids were branched pentadecanoic anteiso-C₁₅:₀ (20.3 %) and iso-C₁₅:₀ (17.2 %). In moderate quantities were branched tridecanoic iso-C₁₃:₀ (11.2 %) and anteiso-C₁₃:₀ (8.2 %), and saturated tetradecanoic C₁₄:₀ (7.7 %). Minor levels of branched and saturated pentadecanoic acids as well as saturated hexadecanoic acid were present (Table 1).

Lauric or dodecanoic acid C₁₂:₀ was present in minor quantities (1.1 %) in the novel isolate while it was absent in the most closely related strain Barnesiella viscicola C₄₆ᵀ. However, branched pentadecanoic acids (which are not synthesized by animals) anteiso-C₁₅:₀ and iso-C₁₅:₀ were the major cellular fatty acids in B. viscicola [33] and in strain A7P-90mᵀ.

The partial sequence of the 16S rRNA gene, 1389 bp, possessed only 85 % sequence similarity to the genes from: Macellibacteroides fermentans LIND7Hᵀ, Porphyromonas pogenae MI 10-1288ᵀ, Parabacteroides distasonis ATCC 8503ᵀ, Parabacteroides chartae NS31-3ᵀ, Mariniphaga anaerophila Fu 11-5ᵀ, Digestomonas mossii JCM 16699ᵀ and Bacteroides coprophilus CI 672; and 84 % similarity to the genes from Barnesiella viscicola DSM 18177ᵀ and Duganomonas oryzarvi Dy73ᵀ. According to the 86.5 % cut-off for novel families, strain A7P-90mᵀ could represent a separate family of the Bacteroidales [34]. A preliminary phylogenetic analysis based on strains of the type genera of Bacteroidales clustered strain A7P-90mᵀ with B. viscicola and P. distasonis (Fig. S14). However, A7-90mᵀ represented a deep branch in this cluster, and P. distasonis has been shown by others to group with Tannerella forsythia instead of B. viscicola [35]. In addition, the higher branches concerning the Porphyromonadaceae were not resolved, as shown by the low bootstrap values. Therefore, we concluded that this preliminary tree was not reliable.

To resolve the uncertainties of the phylogenetic tree, the sequences of uncultured environmental clones close to A7P-90mᵀ were also included. Those sequences of high quality (>1250 bp) were derived from various habitats such as subsurface aquifer sediment, a groundwater plume, a bioreactor and Antarctica sediments. They possessed 16S rRNA gene identities of 88–97 % to A7P-90mᵀ, indicating they represented taxa from the same family (Fig. 4). Indeed, phylogenetic analysis grouped all these sequences into one separate cluster that was distant from all other Bacteroidales families (Fig. 4). This new tree also provided strong bootstrap support for most established Bacteroidales families. The only exception was the Porphyromonadaceae, which was grouped into several clades indicating that this family possibly represents a collection of more than one family, as discussed below [15, 16].

To further investigate the taxonomic rank of A7P-90mᵀ, a draft genome sequence was prepared by the Department of Energy (DOE) Joint Genomes Institute (JGI). ANI analysis showed that strain A7-90mᵀ had 67–68 % of ANI compared to the strains of type genera within the order Bacteroidales (Table S1). Those values are far lower than the 95–96 % threshold used for species separation, indicating that A7P-90mᵀ represents a lineage at a higher taxonomic rank. AAI has been considered a more reliable parameter for distinguishing ranks higher than species. Therefore, pairwise AAI values between A7P-90mᵀ and type strains of the Bacteroidales type genera were calculated (Tables 2, S2 and S3).

Strain A7-90mᵀ had 43–49 % of AAI compared to all reference strains, consistent with its classification within a novel family. This value was below 50 % – the AAI threshold proposed for the family [31]. Similarly, the intra-family AAI values for all Bacteroidales families except Porphyromonadaceae were higher than 50 % (Table 2). With the exception of Porphyromonadaceae, those intra-family values were also higher than their corresponding inter-family values, confirming the usefulness of this threshold among the Bacteroidales (Tables S2 and S3). Our results are consistent with recent proposals that the Porphyromonadaceae comprise more than one family [15, 16].

The draft genome sequence of strain A7P-90mᵀ was deposited in JGI under number IMG OID 2654588148, in SRA listed as SRP088197. The sequence comprised 4.15 mbp in 185 scaffolds. The coding density was 87.4 %, and the G+C

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### Table 2. Pairwise AAI between A7P-90mᵀ and Bacteroidales families

<table>
<thead>
<tr>
<th>Family</th>
<th>Most distant within family (AAI, %)</th>
<th>Closest outside family (AAI, %)</th>
<th>A7P-90mᵀ (AAI, %)</th>
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<tr>
<td>Bacteroidales</td>
<td>66.4</td>
<td>58.0</td>
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<td>Martinibacteriaceae</td>
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<td>58.0</td>
<td>47.9</td>
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</table>

Full data set is available in Tables S2 and S3.

*Type genus versus all genera within family.
†Type genus versus all type genera outside family.
‡Type genus versus A7P-90mᵀ.
content of 42.6 %. A total of 3658 protein-coding genes, 2515 with functional annotations and 819 encoding enzymes and 77 RNA-encoding genes were identified. The genome possessed three 16S rRNA genes. The genome encoded three CRISPR, phage integrases and 17 transposases, suggesting that phages and horizontal gene transfers were an important component of the isolate's history. Twenty-two genes from Archaea (of the families Methanomicrobiaceae, Methanosarcinaceae, Methanobacteriaceae, Halobacteriaceae and Natrabilaceae) and eight genes from Eukaryota were found among putative horizontally transferred genes. A large number of dehydrogenases and proteases were indicative of a heterotrophic metabolism. The genome also encoded two haemerythrins, nitratereductase, nitritreductase, cyttochromes c and d, flavoprotein, and superoxide dismutase (Fe-Mn family) genes. Many genes were found encoding the intra-flagellar transport proteins involved in gliding motility, flagellum-specific peptidoglycan hydrodase (Flg), rod shape-determining proteins (MreB, M5eC, MreD and RodA), lipoic acid synthetase, peptidoglycan-associated lipoproteins, gliding motility-associated proteins (GldM_C, GldM_N and GldN) and actin-like ATPase involved in cell morphogenesis, as well as periplasmic proteins participating in ABC type transporters. Proteins of the pore secretion system and secretion systems IV and IX were also annotated, supporting the TEM images of 20 nm cytoskeletal channels (Fig. S10). The genes of Fic-cell filamentation protein and a collagen triple helix repeat-containing protein supported the filamentous cytology of the isolate. Twenty-nine types of protease, chitinase and collagenase were encoded as well, supporting a heterotrophic, proteolytic metabolism. Two genes associated with retrovirus reverse transcriptase and multiple genes of DNA-directed RNA polymerase and DNA-directed DNA polymerase were also listed in the genome.

Genes associated with the proteins involved in the accumulation of silicon and fluorine in antiae were: haloacid dehalogenase (three genes), H⁺/Cl⁻ antiporter CkA, chloride channel protein, chloride peroxidase non-haem and fluoride ion exporter CrCB/FEX. In addition, sialic acid synthase SpsE/N-acetylneuraminamate synthase and MtN3, and saliva-related transmembrane protein (involved in cell differentiation, masking antigenic sites by decreasing the affinity of binding and known for cancer cell metastasis). Multiple genes for tellurium-resistant TerC protein, arsenate reductase, zinc transporter ZupT-Zip, Cu/Ag efflux pump and heavy metal efflux pump, the Co/Zn/Cd efflux system compound transporter, chromate transport protein ChrA, and genes of enzymes involved in the biosynthetic pathway of vitamin B12 (cyanocobalamin) were also found.

Of all known bacteria capable of proliferating growth, the novel isolate A7P-90mT demonstrated an unusual cytological morphotype. It had a filamentous rod shape in exponential phase of growth, and helical cells were observed at the end of the stationary phase of growth, or after death. Helical morphotypes have not been reported in Archaea, among which no pathogens have been reported so far. In Bacteria, the genera which possess a helical shape of cells include pathogens. Proteolytic representatives of pathogenic spirochetes and Helicobacter species are characterized by cell diameters of ≥0.35 µm. The novel isolate had a very thin cell diameter of 0.18–0.27 µm, and its obligate moderately psychrophilic physiology excludes the pathogenicity for warm-blooded animals. In Helicobacter species, the spheroplasts are often falsely named as ‘coccoid forms’ and appear at the stationary phase of growth [36]. In the novel isolate the spheroplasts were found as three different types: the usual spherical unstable form (Figs 1a, b and S4a); light-reflecting bright, spherical bodies, which looked like shiny spores and were temporarily stable (Figs 2d, S6 and S7a); and erythrocyte-shaped bodies that also refracted light (Fig. 1a). The last two types have not been observed in spirochetes.

The motility of the novel isolate was also unusual and is reported here as an explanation for the gliding type of cellular motility in Prokaryotes. In contrast to the typical spirochete corkscrew motility, strain A7P-90mT had a gliding cell motion caused by spinning antiae and whole-cell vibration. The fast spinning of the tremendously long antiae entangled in braids and in larger diameter plexa did not result in efficient motility (see the area occupied by plexa of antiae in Video S4). The major factor here is the material from which these external organelles are built. The antiae fibres are most likely composed of lipo-muco-polysaccharide molecules rather than proteins. This explains the lack of stain absorbency and transparency at TEM. Presence of antiae was also detected for Alkalispirochaeta americana AspG1T (Fig. S15), but they were not as bushy as in A7P-90mT, were transparent when using ‘in focus’ observation and caused a high frequency trembling of cells (see Video S5). The specificity of the antiae arrangement in this spirochete is the mode of its attachment, which is at both ends of the cell as a string.

A common feature of species with a helical cytomorphology is proliferating growth. The organelles responsible for cellular motion are the key factors enabling cells to penetrate and proliferate agar during colonial growth. Because of this feature, the cultures exhibit ‘cotton ball’ colonies on agar that are known for spirochetes and Helicobacter species [36–39]. The new isolate A7P-90mT also had fuzzy ‘cotton ball’ colonies during growth on agar. Despite the absence of an undulatory rotating axial filament composed of several flagella in the periplasmic space, typical for spirochetes, the novel isolate performed proliferating growth by antiae, which had an extracellular spinning rotation around a cell cylinder. Comparative analysis of the genomes of A. americana AspG1T, Helicobacter pylori 26695 and A7P-90mT confirmed the absence of flagellation in the novel isolate (Table S4). A description of the draft genome sequence of Alkalispirochaeta americana AspG1T is presented in the Supplementary Material. Instead of genes associated with
flagella apparatus, genes encoding gliding motility proteins were predominant in genome annotations.

So far, gliding motility has not been comprehensively explained in Bacteria and may result from different mechanisms in different groups [40]. Its mechanism was unknown. Extracellular structures such as pili and fimbriae are known only for their adhesive function, and flagella are the only external organelles known for prokaryotic cell motion. Flagella are cylindrical, tube-shaped, not the flat tape spirals shown in antiae. The molecular composition of flagella is diverse across bacteria, with many proteins only found in some species [41]; the flagellar apparatus is very flexible in evolutionary terms.

Our demonstration by different microscopy techniques (dark field, UV epifluorescent, phase contrast, FESEM and TEM) of the cellular external organelles, antiae along with a cytoskeletal structure possibly involved in their synthesis and monophasic spinning rotation, suggests an explanation for the gliding cellular motion. In summary, the evidence for the novel type of cellular motility is as follows: (1) gliding motility observed with an optical microscope; (2) proliferating growth on agar, typical of gliding or spirochetes; (3) antiae observed and demonstrated with different microscopy techniques; (4) novel type of ultrastructure involved in cellular motion – symmetrical segmentation in the form of four ‘rotor’ wheels in the centre of cell and channelled columns of the secretion system by perimeter; and (5) the absence of flagella-associated structures (such as hooks, basal body, motor, flagellum) in the genome of A7P-90mT. A detailed description of the novel organelle and ultrastructure of A7P-90mT will be the subject of a further cytological study.

It is interesting that antiae were also identified in TEM images of the most virulent and carcinogenic wild strain H. pylori 26 695 (Fig. S16). Table S4 compares several similar features in the genomes of three helical extremophiles: moderately psychrophilic A7P-90mT; halaalkaliphilic hydrogen-producing A. americana ASpG1T [39]; and extreme acidophilic lithoautotrophic H. pylori 26 695, known to increase carcinogenesis by hydrogen stimulation [42–44]. Annotated retron-type reverse transcriptase in the genomes of A7P-90mT and A. americana ASpG1T immediately attracted our attention, and checked TEM and SEM images of human immunodeficiency virus type-1 (available on the Internet) indicated the presence of antiae (Fig. S17). TEM micrographs of Orthopoxvirus Simian virus 40 and Retrovirus avian leucosis virus (not shown), as well as Filoviridae Zaire ebolavirus (Fig. S18) also demonstrated the presence of antiae, which were ignored due to their transparency and poorly contrasted imaging – often they look like cell debris or the side effect of a stain treatment. Finding antiae in viruses alters the view on them as non-living entities, and the presence of organelles responsible for their motility may also contribute to the pathogenesis picture of many deadly diseases. Antiae were determined in the TEM images published by Flint et al. [45] for: Human adenovirus type 5; Arenovirus Lympohytic choriomeningitis; Murine coronavirus; Filovirus Zaire ebolavirus; Flavivirus Dengue virus; Hepadnavirus wood chuck hepatitis virus; Human herpes simplex virus type 1; Influenza virus A; Paramyxovirus; adenovirus-associated virus 4; poliovirus; Orthopoxvirus Simian virus 40; Poxvirus vaccinia virus; Orthoreovirus (Mammalian); Retrovirus avian leucosis virus; vesicular stomatitis virus Indiana; and Alphavirus Ross River virus.

Among Bacteroidales [13], the novel isolate is the only obligately moderate psychrophilic and proteolytic representative. In spirochetes, there are true psychrophilic species Spirochaeta psychrophila and Pleomorphocheta multiformis (former Sphaerochaeta multiformis), both isolated from deep sea sediments [46–48], and they have cellular fatty acid profiles similar to that of A7P-90mT, but they lack menaquinones and are obligately dependent upon marine salinity.

In addition to the aforementioned unique morpho-physiological characteristics that have never been found in the Bacteroidales, substantial pieces of molecular evidence suggest that A7P-90mT represents a novel family within the order Bacteroidales: (1) it only shares about 85 % of 16S rRNA gene similarities with its closest relatives, lower than proposed family cut-off at 86.5 % [34]; (2), 16S rRNA gene phylogenetic analysis placed A7P-90mT among uncultured environmental clones with 88–97 % similarity into a separate cluster distantly from all other Bacteroidales families; and, finally, (3) A7P-90mT shared low ANI (67–68 %) and AAI (43–48 %) values with all species of the seven Bacteroidales families. Updated, genome-based taxonomic classification of Bacteroidetes presented a significant number of newly recombined and emended taxa [16]. Table 3 shows differentiation of the novel family Williamwhitmaniaceae with other families of the order Bacteroidales. It is the only family with an obligate moderately psychrophilic, athalassic representative which possesses a strictly anaerobic and exclusively proteolytic metabolism within the order; and it also has a novel, unique cytological type with gliding motility.

Based on obtained results, we present descriptions of the new taxa.
Table 3. Comparative characteristics of the novel family *Williamwhitmaniaceae* with families of the order *Bacteroidales*

<table>
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<td>Iso/anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;: C&lt;sub&gt;15:0&lt;/sub&gt;-C&lt;sub&gt;16:1&lt;/sub&gt;:9c</td>
<td>Iso- C&lt;sub&gt;15:0&lt;/sub&gt;: C&lt;sub&gt;15:0&lt;/sub&gt;-C&lt;sub&gt;16:1&lt;/sub&gt;:9c</td>
<td>C&lt;sub&gt;15:0&lt;/sub&gt;: C&lt;sub&gt;15:0&lt;/sub&gt;-C&lt;sub&gt;16:1&lt;/sub&gt;:9c</td>
<td>Iso- and anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;: C&lt;sub&gt;15:0&lt;/sub&gt;-C&lt;sub&gt;16:1&lt;/sub&gt;:9c</td>
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<td>Iso- and C&lt;sub&gt;15:0&lt;/sub&gt; anteiso-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
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<td>G+C, mol%</td>
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<td>34–62</td>
<td>42.4</td>
<td>33–45</td>
<td>41–44</td>
<td>55–61</td>
<td>38–55</td>
<td>40–52</td>
<td>42–47</td>
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<td>Type genus</td>
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<td><em>Bacteroides</em></td>
<td><em>Odoribacter</em></td>
<td><em>Marinifilum</em></td>
<td><em>Marinilabilia</em></td>
<td><em>Rikenella</em></td>
<td><em>Porphyromonas</em></td>
<td><em>Prevotella</em></td>
<td><em>Prolixibacter</em></td>
</tr>
</tbody>
</table>

An, anaerobes; Ae, aerobes; FAn, facultative anaerobes.

*Species of all families are not spore-forming and Gram-stain-negative.*
which may occur in two planes. Anaerobes with an organo-
trophic, fermenting metabolism. Psychrophilic or meso-
philic. Respiratory system possesses menaquinones and
cytochromes c and d. The cellular fatty acid profile is predo-
nominated by branched and saturated C<sub>13:0</sub>·C<sub>15:0</sub> chains.

The type species is *Williamwhitmania taraxaci*.

**DESCRIPTION OF WILLIAMWHITMANIA TARAXACI SP. NOV.**

*Williamwhitmania taraxaci* (ta rêve ci. N.L. gen. n. taraxaci
of Taraxacum pertaining to the dandelion seed head, the
shape possessed by cells of the original culture).

Cells have sizes 0.18–0.3 × 8.0–25.0 µm, Gram-stain-negative
and non-spore-forming. Cells are straight, thin, hair-like
rods during the lag, exponential and stationary phases of
growth, and helical at the end of the stationary phase or
after death. Motile by antia entangled at polar regions in
plexa wrapping and spinning around the cell cylinder. The
type of cell motion is gliding. At the end of the stationary
phase of growth, spheroplasts are formed terminally.

Colonies (diameter ~2–4 mm) are round, ellipses or spheri-
cal with fuzzy 'cotton-ball' growth; the cells proliferate and
liquefy agar. Young colonies are white in colour and aged
ones are dark yellow with a dense darker centre.

Strictly anaerobic and catalase-negative. Moderate psychro-
philic with a growth temperature range of 3–26 ºC and optim-
um at 22–23 ºC. No growth at 0 or 27 ºC.

Athalassic with a salinity growth range of 0–2 % (w/v) NaCl,
and optimum at 0.5 %.

The pH range for growth is 6.5–8.0 and optimum at 6.9.

Proteolytic, grows on peptone, tryptone, trypticase peptone,
casamino acids, gelatin, meat extract or yeast extract. In two
transfers (but not on third) growth is observed on inulin
and starch. Casein is hydrolysed, but the culture loses cell
integrity and is sustained in non-revivable spheroplasts.

No growth is observed on H<sub>2</sub>::CO<sub>2</sub> (80:20), formate, acetate,
lactate, pyruvate, propionate, butyrate, citrate, fumarate,
methanol, ethanol, glycerol, acetone, trimethylamine, tri-
ethylamine, chitin, betaine, N-acetylg glucosamine, collagen,
cholesterol, D-arabinose, cellobiose, D-fuctose, D-fucose,
d-galactose, D-glucose, lactose, maltose, D-mannose, D-ribose,
sucrose, trehalose, xylole, D-mannitol, D-alanine, D-arginine,
L-asparagine, L-aspartic acid, L-cystine, L-cysteine, L-gluta-
mic acid, L-glutamine, glycine, L-histidine, L-leucine, L-isole-
ucine, L-lysine, L-methionine, L-phenylalanine, L-proline,
L-serine, L-threonine, trans-4-hydroxy-L-proline, L-tryptoph-
phan, L-tyrosine, L-valine, D-alanine, D-aspartic acid,
D-histidine, D-leucine, D-methionine, D-proline, D-serine,
D-threonine and D-tryptophan. No growth is observed on
blood agar.

The Stickland reaction is negative on L-proline+L-valine, L-
proline+L-leucine, L-proline+L-isoleucine, L-proline+L-alanine,
L-tryptophan+L-valine, glycine+L-alanine, glycine+L-valine,
L-glutamine, glycine+L-leucine and glycine+L-isoleucine.

Metabolic end products following growth on peptone are:
lactate, acetate, propionate, butyrate, ethanol, H<sub>2</sub> and CO<sub>2</sub>.

Ureolytic and does not produce indole. Sulfur compounds
do not stimulate or inhibit growth, H<sub>2</sub>S is not produced
from cysteine, sulfate, thioulsulfate, sulfite, DMSO or S<sup>2-</sup>. Fe
(OH)<sub>3</sub> is not reduced or affect growth. NO<sub>3</sub> and NO<sub>2</sub> reduc-
tase-positive.

The type strain is sensitive to the following antibiotics:
ampicillin, kanamycin, gentamycin, rifampicin, tetracycln
(250 µg ml<sup>–1</sup>) and chloramphenicol (125 µg ml<sup>–1</sup>)

The major cellular fatty acids are: branched pentadecanoic
(anteiso-C<sub>15:0</sub> and iso-C<sub>15:0</sub>) branched tridecanoic (iso-
C<sub>13:0</sub>) and anteiso-C<sub>13:0</sub>) and saturated tetradecanoic C<sub>14:0</sub>
acids.

The quinone system possesses exclusively menaquinone
MK-7.

The G+C content of the genomic DNA is 42.6 mol% (genome
data).

Type strain A7P-90m<sup>T</sup> (=DSM 100563<sup>T</sup>=JCM 30888<sup>T</sup>) was
isolated from anoxic water, Lake Untersee, Antarctica.

The complete draft genome sequence was deposited in JGI
under number IMG OID 2654588148, in SRA listed as
SRP088187.

**DESCRIPTION OF WILLIAMWHITMANIACEAE FAM. NOV.**

*Williamwhitmaniaceae* (Wil.li.am.whit.man.i.a.ce.ae. N.L.
fem. pl. n. *Williamwhitmania* type genus of the family; -
ceae ending to denote family; *Williamwhitmaniaceae* the
*Williamwhitmania* family).

The family is established on the basis of unique cytology,
physiological characteristics, phylogenetic analysis of the
16S rRNA gene and genome sequencing. Cells stain Gram-
negative and do not form spores. Motile cells appear as
straight, flexible, filamentous, thin rods or helical. The
novelty of the taxon’s cytology is determined by external organ-
elles (multiple fibrils of antia entangled in braids and larger
plexa -whips) at the poles. The type of cell motion is gliding
by a mechanism of a whole-cell vibration caused by spinning
around cell antia gathered in whip-like extensions. Cells
multiply by binary division with cell-wall septation;
the septation can appear in two planes. The family includes
anaerobic chemooorganotrophic, psychrophilic or meso-
philic species. The type genus is *Williamwhitmania*.

**EMENDED DESCRIPTION OF THE ORDER BACTEROIDALES KRIEG 2012**

The description of the order is as that given by Krieg [13,
14], with the following modifications. Some families are
characterized by the novel characterized here, type of
external organelles – antia causing a slow gliding motility of cells.

Thalassic and athalassic physiology with pH requirements from neutral to alkaliphilic. Most representatives are mesophilic, but psychrophilic and thermophilic or moderately thermophilic species are present.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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