Bifidobacterium canis sp. Nov., a novel member of the bifidobacterium pseudolongum phylogenetic group isolated from faeces of a dog (canis lupus f. familiaris)

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# *Bifidobacterium canis* sp. nov., a novel member of the *Bifidobacterium pseudolongum* phylogenetic group isolated from faeces of a dog (*Canis lupus* f. *familiaris*)

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## Abstract

A fructose-6-phosphate phosphoketolase-positive strain (GSD1FS[T]) was isolated from a faecal sample of a 3 weeks old German Shepherd dog. The closest related taxa to isolate GSD1FS[T] based on results from the EZBioCloud database were *Bifidobacterium animalis* subsp. *animalis* ATCC 25527[T], *Bifidobacterium animalis* subsp. *lactis* DSM 10140[T] and *Bifidobacterium anseris* LMG 30189[T], belonging to the *Bifidobacterium pseudolongum* phylogenetic group. The resulting 16S rRNA gene identities (compared length of 1454 nucleotides) towards these taxa were 97.30, 97.23 and 97.09%, respectively. The pairwise similarities of strain GSD1FS[T] using *argS*, *atpA*, *fusA*, *hsp*60, *pyrG*, *rpsC*, *thrS* and *xfp* gene fragments to all valid representatives of the *B. pseudolongum* phylogenetic group were in the concatenated range of 83.08–88.34%. Phylogenomic analysis based on whole-genome methods such as average nucleotide identity revealed that bifidobacterial strain GSD1FS[T] exhibits close phylogenetic relatedness (88.17%) to *Bifidobacetrium cuniculi* LMG 10738[T]. Genotypic characteristics and phylogenetic analyses based on nine molecular markers, as well as genomic and comparative phenotypic analyses, clearly proved that the evaluated strain should be considered as representing a novel species within the *B. pseudolongum* phylogenetic group named as *Bifidobacterium canis* sp. nov. (GSD1FS[T]=DSM 105923[T]=LMG 30345[T]=CCM 8806[T]).

Bifidobacteria are saccharolytic Gram-stain-positive bacteria that colonize different ecological niches connected primarily to the gastrointestinal tract of social mammals, poultry and insects [1, 2]. Their occurrence seems to be common in the gastrointestinal tract of dogs too [3–5]. Over time, the dog diet has changed, starting from carnivorous behaviour with a high protein diet to a carbohydrate-rich diet with the tendency to live an urban lifestyle [6, 7]. Cohabiting dogs and humans share more bacterial operational taxonomic units compared with hosts from separate households [8]. Despite this human–dog co-evolution, multi-host bifidobacterial

species such as *Bifidobacterium animalis* and *Bifidobacterium pseudolongum* are frequently detected in dog faeces [3–5]. Moreover, detailed profiling of the bifidobacterial population of dogs based on ITS-based sequencing approaches has identified the occurrence of putative new bifidobacterial taxa [4].

In total, 49 dog faecal samples (Table 1) were analysed for bifidobacterial occurrence on Wilkins–Chalgren anaerobe agar supplemented with GMO-free soya peptone (5 g l⁻¹; both Oxoid), ʟ-cysteine (0.5 g l⁻¹) and Tween 80 (1 m l⁻¹; both Sigma-Aldrich), mupirocin (100 mg l⁻¹) and norfloxacin

**Table 1.** Strains isolated on bifidobacterial media identified using MALDI-TOF MS and their detected counts

ND, Not detected; NRI, not reliable identification; F, female; M, male; w, weeks; m, month(s); NORF-MUP agar, Wilkins–Chalgren anaerobe agar supplemented with GMO-free soya peptone (5 g l$^{-1}$; both Oxoid), acetic acid (1 ml l$^{-1}$), L-cysteine (0.5 g l$^{-1}$), and Tween 80 (1 ml l$^{-1}$; all Sigma-Aldrich), mupirocin (100 mg l$^{-1}$) and norfloxacin (200 mg l$^{-1}$; both Oxoid).

| No. | Dog breed | Age (years) | Sex | Bacterial isolates from NORF-MUP agar identified by MALDI-TOF MS | NORF-MUP agar (log CFU/g±SD) | | |
|---|---|---|---|---|---|---|---|
| 1 | German Shepherd | 3 w | M | **Bifidobacterium animalis**, NRI (GSD1FS$^T$) | 7.56 | ± | 0.32 |
| 2 | German Shepherd | 3 w | M | **Bifidobacterium animalis**, NRI | 7.48 | ± | 0.79 |
| 3 | German Shepherd | 3 | F | **Bifidobacterium pseudolongum** | 6.37 | ± | 0.12 |
| 4 | Golden Retriever | 1.5 | F | *Clostridium perfringens* | 5.76 | ± | 0.25 |
| 5 | Samoyed | 3 | M | *Clostridium perfringens* | 5.85 | ± | 0.01 |
| 6 | German Shepherd | 10 | M | *Clostridium sordellii* | 5.88 | ± | 0.03 |
| 7 | German Shepherd | 4 | M | **Bifidobacterium pseudolongum** | 5.88 | ± | 0.03 |
| 8 | German Shepherd | 7.5 | M | *Clostridium perfringens, Clostridium sordellii* | 6.66 | ± | 0.06 |
| 9 | German Shepherd | 3 | M | *Clostridium perfringens* | 4.00 | ± | 0.01 |
| 10 | Czechoslovakian Wolfdog | 4.5 | M | *Clostridium perfringens* | 3.62 | ± | 0.06 |
| 11 | Crossbreed | 9 | M | *Clostridium sordellii* | 3.51 | ± | 0.01 |
| 12 | German Shepherd | 3 | F | *Escherichia coli, Clostridium sordellii, Lactobacillus murinus* | 4.52 | ± | 0.07 |
| 13 | Swiss Shepherd | 3 | M | **Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium animalis** | 8.54 | ± | 0.09 |
| 14 | German Shepherd | 5 | M | **Bifidobacterium animalis** | 7.46 | ± | 0.01 |
| 15 | Labrador Retriever | 3 | F | No isolates | <2 | | |
| 16 | Fox Terrier | 7 | F | **Bifidobacterium catenulatum/pseudocatenulatum** | 9.16 | ± | 0.08 |
| 17 | Belgian Shepherd | 4 m | M | **Bifidobacterium catenulatum/pseudocatenulatum, Bifidobacterium pullorum** | 5.21 | ± | 0.00 |
| 18 | Czechoslovakian Wolfdog | 7.5 | M | **Bifidobacterium pseudolongum** | 4.78 | ± | 0.12 |
| 19 | Swiss Shepherd | 4 | M | *Clostridium sordellii* | 6.88 | ± | 0.27 |
| 20 | Crossbreed | 1.5 | M | **Bifidobacterium pseudolongum** | 5.51 | ± | 0.02 |
| 21 | Crossbreed | ND | M | *Clostridium perfringens* | 4.31 | ± | 0.00 |
| 22 | German Shepherd | ND | M | *Clostridium perfringens* | 8.79 | ± | 0.00 |
| 23 | German Shepherd | 5 | F | No isolates | <2 | | |
| 24 | Belgian Shepherd | 6 m | M | No isolates | <2 | | |
| 25 | Havanese | ND | F | **Bifidobacterium catenulatum/pseudocatenulatum, Bifidobacterium pullorum** | 2.58 | ± | 3.64 |
| 26 | Havanese | 2 w | ND | *Clostridium perfringens* | 1.45 | ± | 2.05 |
| 27 | Havanese | 2 w | ND | *Pediococcus acidilactici* | 2.08 | ± | 2.93 |
| 28 | Crossbreed | ND | F | *Clostridium sordellii, Clostridium perfringens* | 6.28 | ± | 0.01 |
| 29 | Crossbreed | ND | M | **Bifidobacterium longum**, *Lactobacillus murinus, Clostridium perfringens* | 8.17 | ± | 0.02 |
| 30 | Crossbreed | ND | M | **Bifidobacterium catenulatum/pseudocatenulatum, Bifidobacterium longum**, *Lactobacillus murinus, Clostridium perfringens* | 7.14 | ± | 0.01 |
| 31 | Crossbreed | ND | F | *Clostridium perfringens* | 6.29 | ± | 0.01 |

**Table 1.** Continued

| No. | Dog breed | Age (years) | Sex | Bacterial isolates from NORF-MUP agar identified by MALDI-TOF MS | NORF-MUP agar (log CFU/g±SD) | | |
|---|---|---|---|---|---|---|---|
| 32 | Golden Retriever | 6 | F | *Propionibacterium acnes* | <2 | | |
| 33 | German Shepherd | 7 | M | *Clostridium perfringens* | 4.89 | ± | 0.01 |
| 34 | German Shepherd | 5 | M | *Escherichia coli* | 7.60 | ± | 0.00 |
| 35 | German Shepherd | 1 | M | **Bifidobacterium longum** | 4.84 | ± | 0.01 |
| 36 | German Shepherd | 7.5 | F | **Bifidobacterium longum** | 5.43 | ± | 0.02 |
| 37 | German Shepherd | 3.5 | F | **Bifidobacterium pseudolongum** | 6.66 | ± | 0.00 |
| 38 | German Shepherd | 1 | F | **Bifidobacterium pseudolongum** | 5.39 | ± | 0.04 |
| 39 | German Shepherd | 8.5 | F | **Bifidobacterium pseudolongum** | 9.03 | ± | 0.01 |
| 40 | German Shepherd | 8.5 | F | **Bifidobacterium pseudolongum**, *Clostridium perfringens* | 7.13 | ± | 0.01 |
| 41 | German Shepherd | 8.5 | F | **Bifidobacterium pseudolongum**, *Clostridium perfringens* | 5.93 | ± | 0.02 |
| 42 | German Shepherd | 8.5 | F | **Bifidobacterium pseudolongum** | 6.81 | ± | 0.01 |
| 43 | German Shepherd | 7 | F | No isolates | 1.65 | ± | 2.33 |
| 44 | German Shepherd | 7 | F | *Clostridium perfringens* | 3.50 | ± | 0.01 |
| 45 | German Shepherd | 6 | F | *Clostridium sordellii* | 5.92 | ± | 0.02 |
| 46 | German Shepherd | 5 | F | **Bifidobacterium pseudolongum, Bifidobacterium animalis** | 8.04 | ± | 0.01 |
| 47 | German Shepherd | 4 | F | **Bifidobacterium pseudolongum, Bifidobacterium animalis**, *Lactobacillus murinus* | 6.32 | ± | 0.07 |
| 48 | German Shepherd | ND | F | *Clostridium perfringens* | 1.81 | ± | 2.56 |
| 49 | German Shepherd | ND | F | *Clostridium perfringens* | 3.69 | ± | 0.02 |

(200 mg l⁻¹; both Oxoid) according to Vlkova *et al.* [9], which was used as an norfloxacin-mupirocin (NORF-MUP) agar. Isolates with variable cultivation characteristics were subcultivated in Wilkins–Chalgren anaerobe broth/agar (Oxoid) supplemented with GMO-free soya peptone (5 g l⁻¹), acetic acid (1 ml l⁻¹), ʟ-cysteine (0.5 g l⁻¹), and Tween 80 (1 ml l⁻¹) under anaerobic conditions, used as Wilkins–Chalgren with soya peptone (WSP broth)/agar. Obtained cultures were identified using MALDI-TOF MS (ethanol–formic acid extraction procedure with an HCCA matrix according to the manufacturer's instructions; Bruker Daltonik) and by the detection of fructose-6-phosphate phosphoketolase (F6PPK) activity [10]. From 285 bacterial isolates, 155 were identified as *Bifidobacterium* species and other isolates belonged to *Clostridium perfringens*, *Clostridium sordellii*, *Lactobacillus murinus*, *Escherichia coli*, *Pediococcus acidilactici* and *Propionibacterium acnes*. The most-detected bifidobacterial species were *Bifidobacterium animalis* and *Bifidobacterium pseudolongum*, followed by *Bifidobacterium catenulatum/pseudocatenulatum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis* and *Bifidobacterium pullorum* (Table 1). An F6PPK-positive strain, GSD1FS^T, was not reliably identified using MALDI-TOF MS and selected for precise identification and characterization.

The 16S rRNA gene of isolate GSD1FS^T was amplified and sequenced from both directions using the Bif285 (5′-GAGG GTTCGATTCTGGCTCAG-3′) and Bif261 (5′-AAGGAG-GTGATCCAGCCGCA-3′) [11] primers. The obtained sequence (MG028631; 1454 nts long) was inserted into the EZBioCloud database [12] to obtain the closest related taxa. Representatives of the *Bifidobacterium pseudolongum* phylogenetic group [13] including *Bifidobacterium animalis* subsp. *animalis* ATCC 25527^T, *Bifidobacterium animalis* subsp. *lactis* DSM 10140^T, *Bifidobacterium anseris* LMG 30189^T, *Bifidobacterium castoris* LMG 30937^T, *Bifidobacterium italicum* LMG 30187^T, *Bifidobacterium pseudolongum* subsp. *globosum* DSM 20092^T, *Bifidobacterium pseudolongum* subsp. *pseudolongum* LMG 11571^T and *Bifidobacterium choerinum* DSM 20434^T were revealed as the closest relatives with pairwise identities in the range of 96.12–97.30%. These results suggested that the examined strain could represent a novel species within the *B. pseudolongum* phylogenetic group. Multilocus sequence analysis on the basis of eight housekeeping genes was used to confirm the status of a novel species. The partial sequences of the *fusA*, *hsp*60, *pyrG*, *thrS* and *xfp* genes were amplified and sequenced using specific primers and PCR conditions, as described in previous studies [14–18]. New primers and PCR conditions for amplification and sequencing of the *argS* (encoding the arginyl-tRNA synthase), *atpA* (encoding the ATP synthase alpha subunit) and *rpsC* (encoding the 30S ribosomal protein S3) gene regions applicable to the entire

family *Bifidobacteriaceae* were designed and optimized in this study (Table S1, available in the online version of this article). Methods for primer design and optimization of PCR conditions were described in our previous studies [17, 18]. The consensus sequences were obtained using Geneious version 7.1.7 software based on the sequences of 12 complete genome representatives belonging to the family *Bifidobacteriaceae* (Table S2).

Sequences of the 16S rRNA gene and eight housekeeping genes were obtained for strain GSD1FS[T] using the methods mentioned above and deposited in the NCBI database using the Banklt application (www.ncbi.nlm.nih.gov/WebSub/?tool=genbank). Sequences of the same genes were retrieved from the complete genomes of the 13 representatives belonging to the *B. pseudolongum* phylogenetic group [13, 19] to provide gene comparative and phylogenetic analyses (Table S3). To ensure better phylogenetic tree topology, two species classified to the *Bifidobacterium boum* phylogenetic group (*B. boum* and *Bifidobacterium porcinum*) and *Aeriscardovia aeriphila* DSM 22365[T] were exploited as a root of trees and included in Table S3.

Gene comparative and phylogenetic analyses were performed using individual and concatenated gene alignments. The 16S rRNA (length of 1425 nt), *argS* (741), *atpA* (642), *fusA* (774),

*hsp60* (588), *pyrG* (798), *rpsC* (288), *thrS* (726) and *xfp* (477) gene alignments, created using the CLUSTAL_W algorithm in the Geneious version 7.1.7 software package, were used for this purpose. Gene pairwise identities of novel strain GSD1FS[T] towards all valid taxa classified into the *B. pseudolongum* group have been automatically computed by the Geneious software package. All phylogenetic trees were reconstructed in MEGA 5.05 software [17] using the maximum-likelihood method, the best fit AIC (Akaike information criterion) ML model and 1000 bootstrap replicates.

The pairwise similarities of strain GSD1FS[T] using the *argS*, *atpA*, *fusA*, *hsp60*, *pyrG*, *rpsC*, *thrS* and *xfp* gene fragments to all valid representatives of the *B. pseudolongum* phylogenetic group were at intervals 77.73–87.72, 83.65–90.65, 87.08–90.70, 82.82–86.91, 79.45–85.09, 85.42–94.79, 81.41–89.53 and 83.23–92.24%, respectively (Table S3). These results markedly suggest that strain GSD1FS[T] should be considered as representing a novel species [14–18].

A separated position and the closest affinity of strain GSD1FS[T] to *B. animalis* subspecies within the *B. pseudolongum* phylogenetic group is obvious from a phylogenetic tree reconstructed using an alignment of concatenated sequences of the *argS*, *atpA*, *fusA*, *hsp60*, *pyrG*, *rpsC*, *thrS* and *xfp* genes (Fig. 1). An almost identical tree topology was obtained based on
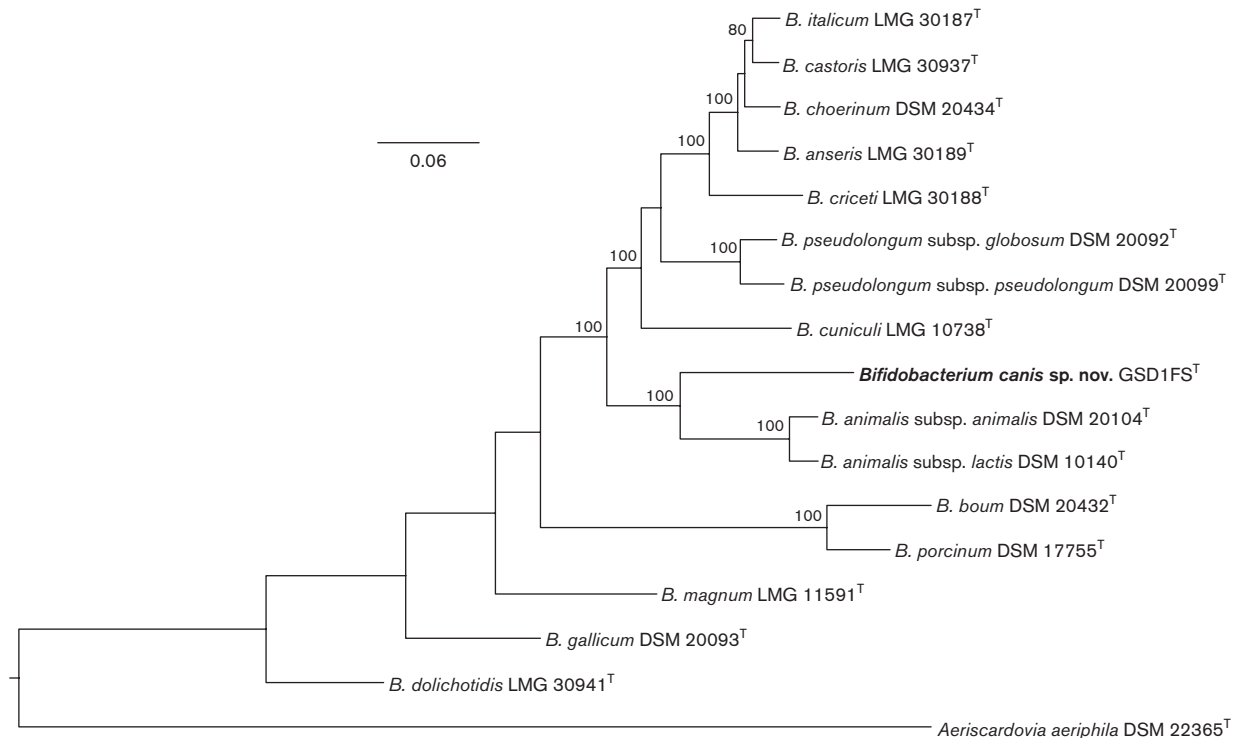


**Fig. 1.** Maximum-likelihood phylogenetic tree reconstructed using an alignment consisting of concatenated sequences of the *argS* (741 nts), *atpA* (642), *fusA* (774), *hsp60* (588), *pyrG* (798), *rpsC* (288), *thrS* (726) and *xfp* (477) genes, respectively (total 5034 nts). The GTR +G+I best fit AIC (Akaike information criterion) model in the MEGA version 5.05 software package was used for the reconstruction. Bootstrap values (≥70), expressed as percentages of 1000 replicates, are given at nodes. The tree was rooted by *Aeriscardovia aeriphila* DSM 22365[T]. Bar, 0.06 substitutions per nucleotide position. The phylogeny clearly documents the close affinity of strain GSD1FS[T] to *B. animalis* subspecies within the *B. pseudolongum* phylogenetic group.

**Table 2.** General genetic features of strain GSD1FS[T]

| Feature | GSD1FS[T] |
|---|---|
| Biological origin | *Canis lupus* f. *familiaris* |
| Average coverage | 58× |
| Number of assembled contigs | 22 |
| Genome length | 2270696 |
| Average G+C content (mol%) | 57.5 |
| Number of predicted ORFs | 1883 |
| tRNA | 56 |
| rRNA* | 4 |
| Similarity of 16S rRNA gene | 97.3% *Bifidobacterium animalis* subsp. *animalis* ATCC 25527[T] |
| ANI value | 88.17% *Bifidobacterium cuniculi* LMG 10738[T] |

*Predicted number of rRNA loci.

amino-acid (aa) alignment derived from the concatenate (Fig. S1). The close relatedness of strain GSD1FS[T] to *B. animalis* subspecies within the *B. pseudolongum* phylogenetic group was revealed in almost all individual phylogenetic trees reconstructed based on 16S rRNA, *argS*, *atpA*, *fusA*, *hsp60*, *pyrG*, *rpsC*, *thrS* and *xfp* gene alignments (Figs S2–S10).

Genomic DNA of strain GSD1FS[T] was isolated using DNeasy UltraClean Microbial DNA kit (Qiagen). The genome was sequenced with Ion Torrent technology on a Proton sequencer at the Seqme.eu company (Czechia). The generated reads were depleted of adapter sequences, quality-filtered and assembled through the MEGAnnotator pipeline [20]. In addition, ORF identification and functional annotation of ORFs were carried out as previously reported [20]. Table 2 shows the basic genome features of strain GSD1FS[T]. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession number WNLP00000000. Genetic similarity at the genomic level of GSD1FS[T] with respect to the other currently recognized bifidobacterial (sub)species was evaluated based on average nucleotide identity (ANI) analysis, and
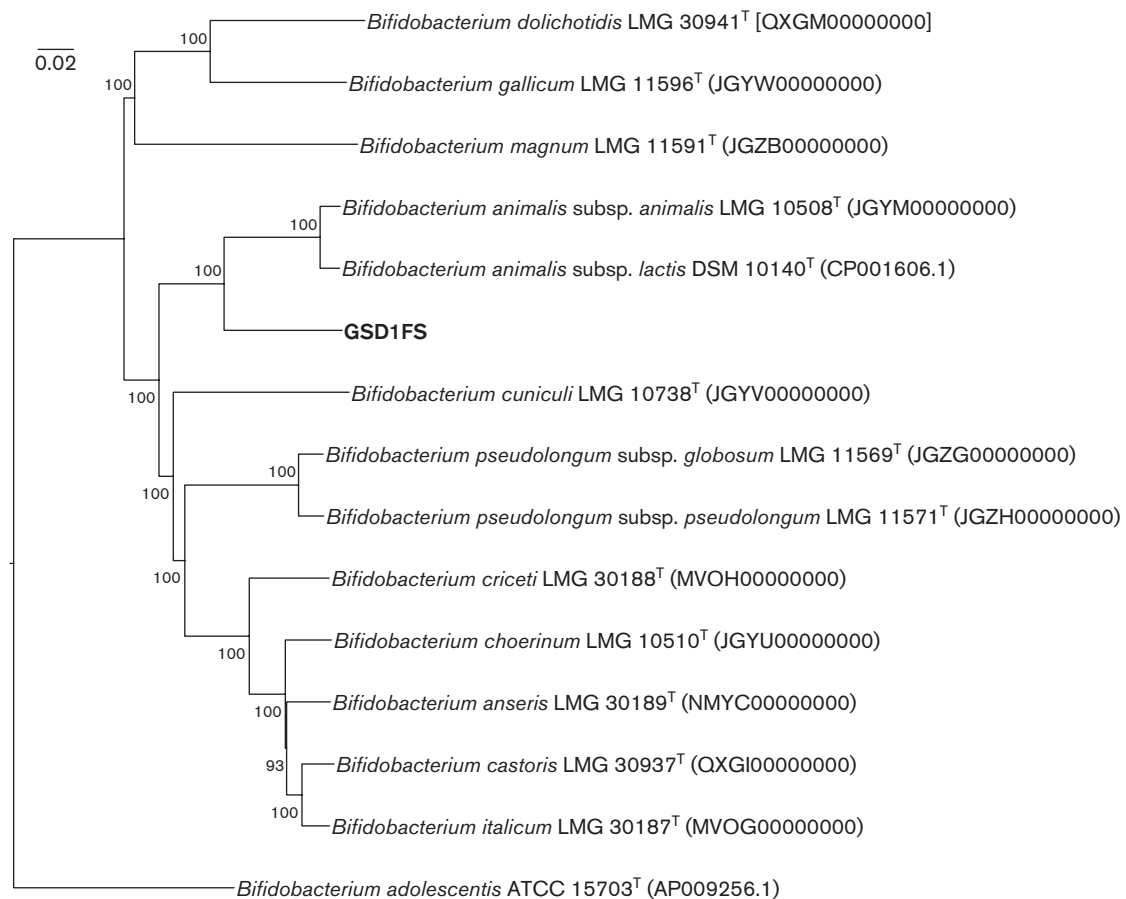


**Fig. 2.** Phylogenetic tree of bifidobacteria based on the concatenation of 619 protein sequences that represent the *B. pseudolongum* phylogenetic group core genome sequences. The phylogenetic tree was reconstructed by the maximum-likelihood method and the gene sequences of *Bifidobacterium adolescentis* ATCC 15703[T] were used as outgroups. Bootstrap percentages above 50% are showed at node points, based on 1000 replicates of the phylogenetic tree.

**Table 3.** Phenotypic characteristic of GSD1FS[T] and species of the closest-related *Bifidobacterium* taxa and species common for dogs

Strain: 1, *B. anseris* LMG 30189[T]; 2, *B. animalis* subsp. *lactis* DSM 10140[T]; 3, *B. animalis* subsp. *animalis* DSM 20104[T]; 4, GSD1FS[T]; 5, *B. cuniculi* DSM 20435[T]; 6, *B. pseudolongum* subsp. *pseudolongum* DSM 20099[T]; 7, *B. pseudolongum* subsp. *globosum* DSM 20092[T]. The results were read after 48 h. Fermentation characteristics were determined using API 50 CHL kit (bioMérieux): +, positive reaction (yellow colour, pH <5); +/−, weak reaction (greenish colour, pH 5.0–5.5); −, negative reaction (purple colour, pH 5.6–6.6), v, variable. Basic media pH 6.5. Other substrates in the API 50 CHL kit were negative for all tested strains.

Growth in variable conditions such as pH level, temperature and presence of oxygen were determined based on optical density: +, good growth $OD_{600}>0.2$; +/−, weak growth $0.1 \leq OD_{600} \leq 0.2$; −, no growth $0.1 < OD_{600}$.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Starch | + | − | − | v | + | + | + |
| Amygdalin | − | + | − | − | − | − | − |
| Cellobiose | − | − | − | +/− | +/− | +/− | +/− |
| D-Fructose | − | +/− | +/− | +/− | +/− | +/− | +/− |
| D-Galactose | − | + | + | + | + | + | + |
| D-Glucose | + | + | + | + | + | + | + |
| Lactose (bovine origin) | + | + | + | +/− | +/− | + | + |
| Maltose | + | + | + | + | + | + | + |
| D-Mannose | − | − | − | − | + | − | − |
| Melibiose | + | + | + | + | − | + | + |
| Raffinose | + | + | + | + | − | + | + |
| D-Ribose | − | + | + | + | + | + | − |
| Sucrose | + | + | + | + | +/− | + | + |
| Turanose | − | − | − | +/− | − | +/− | +/− |
| D-Xylose | − | − | + | + | + | + | − |
| Esculin iron citrate | − | + | + | + | + | + | + |
| Gentibiose | − | − | − | − | + | − | − |
| Glycogen | + | − | − | v | + | + | + |
| L-Arabinose | − | − | + | + | + | + | − |
| L-Fucose | + | − | − | v | − | − | − |
| Xylitol | − | − | − | − | +/− | − | − |
| pH 3.5 | − | − | − | − | − | − | − |
| pH 4 | − | − | − | − | − | − | − |
| pH 4.5 | + | + | − | + | − | − | − |
| pH 5 | + | + | − | + | − | + | +/- |
| pH 6 | + | + | + | + | + | + | + |
| pH 6.5 | + | + | + | + | + | + | + |
| pH 7.5 | + | + | + | + | + | + | + |
| pH 8.5 | + | + | + | + | + | + | + |
| 10 °C | − | − | − | − | − | − | − |
| 15 °C | − | − | − | − | − | − | − |
| 20 °C | − | − | − | − | − | − | − |
| 30 °C | + | + | + | + | + | + | + |

Continued

**Table 3.** Continued

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 37 °C | + | + | + | + | + | + | + |
| 46 °C | + | +/− | +/− | + | +/− | + | + |
| Anaerobic growth | + | + | + | + | + | + | + |
| Microaerophilic growth | + | + | + | + | + | + | + |
| Aerobic growth | − | +/− | +/− | − | − | − | − |

the Genome-to-Genome Distance Calculator (GGDC) was used to estimate the DNA–DNA hybridization (DDH) values (Table S4). The highest sequence identity value of GSD1FS^T was 88.17% when compared to the chromosome sequences of *Bifidobacterium cuniculi* LMG 10738^T, which belongs to the *B. pseudolongum* phylogenetic group. The estimated DDH value below 70% between these two taxa (DDH estimate generalized linear model-based, 27.8%) led to the proposal of GSD1FS^T as representing a novel bifidobacterial species.

In order to evaluate the phylogenetic relationship of GSD1FS^T with other currently recognized bifidobacterial (sub)species, we investigated the core genome of members belonging to the *B. pseudolongum* phylogenetic group, allowing the reconstruction of a genomic-based tree, i.e. phylogenomic tree (Fig. 2) [19, 21, 22]. Accordingly, a comparative genome analysis involving the chromosome sequences of the currently recognized 13 (sub)species belonging to the *B. pseudolongum* phylogenetic group [23], as well as the genome sequences of GSD1FS^T, was carried out. The ORF content of each genome was organized in functional gene clusters using the gene family method of the PanGenome Analysis Pipeline (PGAP) [24], involving the Basic Local Alignment Search Tool (BLAST; E value cutoff of 1×10−10 and 50% identity across at least 80% of sequence lengths). Sequences were then clustered into protein families, using a graph theory-based Markov clustering algorithm [25]. This *in silico* analysis identified 642 clusters of orthologous groups that are shared by the genomes used in this study. Therefore, the *B. pseudolongum* phylogenetic group core genome sequences, based on the concatenation of 619 protein sequences with the exclusion of paralogs, identified in each chromosome sequence were used to build a phylogenomic core tree (Fig. 2).

The rod-shaped cell morphology of strain GSD1FS^T culture was observed using phase-contrast microscopy (Figs S11 and S12). The cell-wall peptidoglycan composition was examined according to Schumann [26] and was found to comprise L-Orn(Lys)–L-Ala(L-Ser)–L-Ala$_2$.

Fermentation characteristics of GSD1FS^T and the most related species, and at the same time the most common species for dogs (*B. animalis* subsp. *animalis* DSM 20104^T, *B. animalis* subsp. *lactis* DSM 10140^T, *B. anseris* LMG 30189^T, *B. pseudolongum* subsp. *pseudolongum* DSM 20099^T, *B. pseudolongum* subsp. *globosum* DSM 20092^T and *B. cuniculi* DSM 20435^T), were determined using API 50 CHL kit (bioMérieux)

according to manufacturer's instructions. Strains were tested in biologically independent triplicates. The obtained results were evaluated based on colour and pH change. The fermentation profile of strain GSD1FS^T corresponded closely to the profiles of the tested strains of *B. pseudolongum* and *B. animalis*; *B. anseris* and *B. cuniculi* differed more (Table 3). Other tested cultivation characteristics were similar between strain GSD1FS^T and the other tested type strains. The cultivation temperature of 37 °C, at pH 6–6.5 and under anaerobic conditions, appeared to be optimal.

Strain GSD1FS^T seemed to be highly genotypically and phenotypically similar to *B. animalis* and *B. pseudolongum*, species which are known to be frequent bifidobacterial species of the dog microbiota.

## DESCRIPTION OF *BIFIDOBACTERIUM CANIS* SP. NOV.

*Bifidobacterium canis* [ca'nis. L. gen. n. *canis* of a dog; common scientific name of a domestic dog (*Canis lupus* f. *familiaris*)].

Cells are Gram-stain-positive, non-motile, non-sporulating and F6PPK-positive. Cells grow under anaerobic and microaerophilic conditions. Colonies grown on the surface of modified Wilkins–Chalgren agar are white and circular, while embedded colonies are white and elliptical. The diameter of each colony ranges from 1.0 to 1.5 mm after 48 h growth on modified Wilkins–Chalgren agar. Cells are able to grow from 30–46 °C, yet are unable to grow at 10, 15, and 20 °C. Moreover, cells grow at pH 4.5–8.5. Optimal conditions of growth occur at pH 6.5 and 37 °C. Cells grown in WSP broth are rods of various shapes, forming a branched structure with 'Y' at both sides.

Fermentation profiles show that strain GSD1FS^T is able to grow well and produce acids on D-galactose, D-glucose, maltose, melibiose, raffinose, D-ribose, sucrose, D-xylose, esculin iron citrate, and L-arabinose. Variable or weak growth was found on cellobiose, D-fructose, lactose, turanose, glycogen, L-fucose and starch. Furthermore, cells are not able to utilize amygdalin, arbutin, D-adonitol, D-arabinose, D-arabitol, D-fucose, D-mannitol, D-mannose, melezitose, D-sorbitol, D-tagatose, trehalose, D-xylanose, dulcitol, erythritol, gentiobiose, glycerol, inositol, inulin, L-arabitol, L-rhamnose, L-sorbose, L-xylose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside,

methyl $\beta$-D-xylopyranoside, *N*-acetylglucosamine, potassium-2-ketogluconate, potassium-5-ketogluconate, potassium gluconate, salicin and xylitol.

The peptidoglycan type is L-Orn(Lys)–L-Ala(L-Ser)–L-Ala$_2$.

The type strain, GSD1FS$^T$ (=DSM 105923$^T$=LMG 30345$^T$=CCM 8806$^T$), was isolated from a faecal sample of a 3 weeks old German Shepherd dog (*Canis lupus* f. *familiaris*). The DNA G+C content is 57.5 mol%. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession number WNLP00000000 and the accession number of the 16S rRNA gene sequence is MG028631.

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

### Ethical statement
The sampling of the dog faeces was made during routine life situations. All procedures involving animals adhered to recommendations in the 'Guide for the Care and Use of Animals' by the Czech University of Life Sciences Prague. The protocol of the experiment was approved by the Czech Central Committee for the Protection of Animals (Permit number: 63479/2016-MZE-17214).

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