Phylogenetic characterization of two novel species of the genus Bifidobacterium: Bifidobacterium saimiriisciurei sp. nov. and Bifidobacterium platyrrhinorum sp. nov.

Phylogenetic characterization of two novel species of the genus *Bifidobacterium*: *Bifidobacterium saimiriisciurei* sp. nov. and *Bifidobacterium platyrrhinorum* sp. nov.

Running title: *Bifidobacterium saimiriisciurei* sp. nov. and *Bifidobacterium platyrrhinorum* sp. nov. from *Saimiri sciureus*.

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The GenBank accession number for the 16S rRNA gene sequence of strains SMA1^T, SMB2 and SMA15^T are MN722462, MN722464 and MN722463, respectively. The accession numbers for the genomes are WHZU00000000, WHZT00000000 and WHZV00000000, respectively.

Three bifidobacterial Gram-stain-positive, non-spore forming and fructose-6-phosphate phosphoketolase-positive strains, SMA1^T, SMB2 and SMA15^T were isolated from the faeces of two adult males of the squirrel monkey (*Saimiri sciureus*).

On the basis of 16S rRNA gene sequence similarities, the type strain of *Bifidobacterium primatium* DSM 100687^T (99.3%; similarity) was the closest neighbour to strains SMA1^T and SMB2, whereas the type strain of *Bifidobacterium stellenboschense* DSM 23968^T (96.5%) was the closest neighbour to strain SMA15^T. The average nucleotide identity (ANI) values of SMA1^T and SAM15^T with the closely related type strains were 93.7% and 88.1%, respectively. The *in silico* DNA–DNA hybridization values with the closest neighbours were 53.1% and 36.9 %, respectively. GC contents of strains SMA1^T and SMA15^T were 63.6 and 66.4 mol%, respectively. Based on the phylogenetic, genotypic and phenotypic data obtained, the strains SMA1^T and SMA15^T clearly represent two novel taxa within the genus *Bifidobacterium* for which the names *Bifidobacterium saimiriisciurei* sp. nov. (type strain SMA1^T = BCRC 81223^T = NBRC 114049^T= DSM 106020^T) and *Bifidobacterium platyrrhinorum* sp. nov. (type strain SMA15^T = BCRC 81224^T = NBRC 114051^T= DSM 106029^T) are proposed.

Abbreviations: ANI, average nucleotide identity; MLSA, Multi-locus sequence analysis; *is*DDH *in silico* DNA DNA hybridization.

Keywords: new species; *Bifidobacterium*; *Bifidobacterium saimiriisciurei*; *Bifidobacterium platyrrhinorum* sp. nov.; squirrel monkey; *Saimiri sciureus.*

Members of the genus *Bifidobacterium* are commensal, anaerobic bacteria, and are considered the most ancient representatives of the phylum Actinobacteria [7], adapted to animal intestinal tracts and existed in the times when the Earth's atmosphere contained little oxygen. Today, bifidobacteria are commensal microorganisms that inhabit a wide range of hosts, including insects, birds and mammals. The overview of bifidobacteria ecology suggests a strict association between bifidobacterial species and the animal niches that they occupy [19]. One possible explanation is that species-specific adaptation and long-term co-evolution led to the formation of this relationship. The mechanisms responsible for the adaptation of bifidobacteria to various hosts during the evolutionary process remain poorly understood [7]. Thus, the study of extant primates provides an opportunity to analyse potential coevolutionary relationships between hosts and these microbes.

Since the first evidence of *Bifidobacterium* presence in Callitrichidae [9,10] there has been an increase about the distribution of its species among ancestral primates, such as New World monkeys [5,6, 22–29]. Furthermore, as recently stated [32] digestive tract of non-human primates represents one of the largest reservoir of bifidobacterial diversity. At present, the genus *Bifidobacterium* comprises 70 established species with 10 subspecies according to LPSN (http://www.bacterio.net/bifidobacterium.html).

To the best of our knowledge, there is no research investigating the presence of this genus in squirrel monkey, an extant subfamily of flat-nosed arboreal monkeys in the New World. Therefore, the objective of this study was to describe the presence and diversity of cultivable bifidobacteria in the faecal samples of squirrel monkey (*Saimiri sciureus*).

Saimiri sciureus (Linnaeus, 1758) are small platyrrhines widely distributed in northern South America. This species is found throughout Amazonia, from the Rio Orinoco and the Guianas to the southern limits of the Amazon basin [11].

Squirrel monkeys have an omnivorous diet, being insectivores-frugivores, consuming insects and fruit depending on seasonal abundance of each resource, and supplementing their diets with small vertebrates, nectar, flowers, buds, seeds, leaves, and gum [1,2,17]. The small vertebrates consumed include bats, which they systematically search for in large stands of trees, small birds, and bird eggs [13].

In December 2016, fresh individual faecal samples of two adult specimens of the squirrel monkey housed under semi-natural conditions in Parco Natura Viva - Garda Zoological Park (Bussolengo, Verona, Italy), were collected from the ground using a sterile spoon, put into a sterile plastic tube and stored under anaerobic conditions in an anaerobic jar (Merck) at 4 °C. The anaerobic atmosphere was obtained using the GasPak EZ Anaerobic Pouch system (BD).

Samples of faeces were collected by the animal-care staffs (keepers) during their routine cleaning of the enclosure, and were taken promptly to the laboratory (within 2 h). Animals were free from intestinal infections and did not receive antibiotics or probiotics for two months before samples were collected. The diet consisted mostly of grass but sometimes also insects.

For isolation of bifidobacteria, aliquots of approximately 1–2 g of faecal sample were serially diluted (tenfold) with Peptone Water (Merck) supplemented with cysteine hydrochloride (0.5 g/L). Aliquots of 1 ml from each dilution (from 10^{-1} down to 10^{-9}) were inoculated onto MRS (Difco) agar supplemented with mupirocin (100 mg/L) (Applichem) [34] [20]. Plates were incubated in anaerobic conditions, at 37 °C for 48–72 h. The anaerobic atmosphere was obtained using the GasPak EZ Anaerobic Pouch system (BD). After incubation, morphologically different colonies were randomly picked and re-streaked for several generations in order to obtain purified individual bacterial isolates. Isolates were suspended in a 10% (w/v) sterile skim milk solution, supplied with lactose (3%) and yeast extract (0.3%), and kept both freeze dried and frozen at –120 °C until further analysis.

Fifteen isolates were obtained from two adult males of the squirrel monkeys, i.e. specimens A and B, (SMA1, SMA2, SMA4 and SMA15 were from male A, whereas strains SMB1, SMB2, SMB4, SMB6, SMB7, SMB9, SMB11, SMB13, SMB20, SMB21 and SMB22 were from male B) and for their discrimination, BOX-PCR fingerprinting was carried out by using the BOXA1R primer (5'-CTACGGCAAGGCGACGCTGACG-3') and the conditions previously described [28].

BOX-PCR typing allowed the identification of two clusters: Cluster I (only one isolate, SMA15) and Cluster II (remaining 14 isolates) (Supplementary Fig. S1). Representatives of each cluster (SMA1, SMB2 and SMA15) were selected and further characterized. Therefore, the genome of all selected strains was decoded through a next generation sequencing (NGS) approach, using a MiSeq platform (Illumina) at Istituto Zooprofilattico Sperimentale (Teramo, Italy). The generated data were depleted of low quality reads using FASTQ/A Trimmer in FASTX-toolkit

(http://hannonlab.cshl.edu/fastx_toolkit/), assembled by SPADES version 3.13.0 [3] and annotated through DFAST program [38].

The draft genome size of strains SMA1, SMB2 and SMA15 were reported in Table 1 together with their other genomic features.

In *silico* analysis of all the sequenced genomes allowed the estimation of their G+C content to be in the range of 66.4–63.6 mol% (Table 1), which is coherent with the average G+C content of *Bifidobacterium* spp., i.e., 52–67 mol% [28].

These Whole Genome Shotgun projects have been submitted to GenBank under the BioProject PRJNA578600 and the accessions reported in Table 1.

We investigated the phylogenetic relatedness of the selected strains with the other recognized bifidobacterial taxa by inferring the nucleotide sequences of 16S rRNA (please see Fig. 1), the nucleotide sequences of five housekeeping genes (*hsp*60, *clp*C, *dna*G, *dna*J and *rpo*B genes) (Fig. 2) as well as the genes constituting the core genome of *Bifidobacterium* spp.(Fig. 3).

Furthermore, to perform an exhaustive comparative genomic analysis, three additional bifidobacterial type strains, were submitted to genome sequencing, i.e., *Bifidobacterium aerophilum* DSM 100689^{T}, *Bifidobacterium avesanii* DSM 100685^{T} and *Bifidobacterium ramosum* DSM 100688^{T}, whose genomes have been deposited at DDBJ/ENA/GenBank under accession numbers WHZY00000000, WHZW00000000 and WHZX00000000, respectively [22].

The 16S rRNA gene sequence (1529 bp) of strains SMA1, SMB2, SMA15 and of their closest relatives retrieved from the DDBJ/GenBank/EMBL databases were aligned using MUSCLE in MEGA 6.0 (1328 nt) [8,37]. A phylogenetic tree based on a total of 84 partial 16S rRNA gene sequences, including those of members of the genus *Bifidobacterium* was reconstructed with the maximum-likelihood method [40] and the evolutionary distances were computed by nucleotide

model of GTR CAT. The tree was constructed using RaxML version 8.2.7 [36] and rooted with *Scardovia inopinata* JCM 12537^T. The statistical reliability of the tree was evaluated by bootstrap analysis of 1000 replicates and the algorithm Bootstrap rapid hill climbing was used. The tree was visualized using iTOL (https://itol.embl.de/) [18] (Fig. 1).

Comparative analysis of the 16S rRNA gene sequence revealed that strains SMA1 and SMB2 were closely related to *Bifidobacterium primatium* DSM 100687^T, 99.3 %, whereas strain SMA15 resulted related to *Bifidobacterium stellenboschense* DSM 23968^T, 96.5 %. Similarity values were obtained using the EZBIOCLOUD (https://www.ezbiocloud.net) [16] (Supplementary Table S1).

The maximum-likelihood analysis confirmed the phylogenetic relatedness of strains SMA1, SMB2 and SMA15 with their closest neighbours (Fig. 1).

Strains SMA1 and SMB2 revealed a high 16S rRNA gene sequence homology to *B. primatium* DSM 100687^T. Indeed, obtained value 99.3% was higher than the suggested cut-off for species delineation, (i.e. 98.7%). Therefore, the genetic similarity at genomic level of the isolates SMA1 and SMB2 with respect to their nearest neighbours was evaluated based on average nucleotide identity (ANI) analysis, which was calculated by using JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison (http://jspecies.ribohost.com/jspeciesws) (Supplementary Table S2). The ANI values of the strains SMA1 and SMB2 with respect to their closest neighbour showed values of 93.7 % and 93.6 %, respectively.

Furthermore, due to the high 16S rRNA gene sequence identity between SMA1 and SMB2 and the ANI values obtained, an *in silico* DDH (*is*DDH) was also carried out using GGDC (Genome-To-Genome Distance Calculator (GGDC version 2.1), the most accurate known tool for calculating DDH-analogous values, developed at DSMZ and available at www.ggdc.dsmz.de. The threshold value of ≤70% is generally accepted for separated prokaryote species [21]. The values achieved

with formula two for SMA1 and SMB2 with respect to *B. primatium* DSM 100687^T were 53.1 and 52.8 % respectively (Supplementary Table S3).

To complete the phylogenomic study, the ANI and *is*DDH were also calculated for strain SMA15 with respect to its closest neighbour *B. stellenboschense* DSM 23968^T and the values obtained were 88.1 % and 36.9 %, respectively (Supplementary Table S4).

Based on these results, SMA1^T and SMA15^T were characterized further as type strains.

In order to assess the genetic diversity of SMA1^T, SMB2 and SMA15^T as compared to the other currently recognized *Bifidobacterium* species, multi-locus sequence analysis (MLSA) was also carried out. Therefore, the phylogenetic location of the novel strains was verified by the analysis of five housekeeping genes (*hsp*60, *rpo*B, *clp*C, *dna*J, *dna*G), which have proven to be discriminative for the classification of the genus *Bifidobacterium* [15,39].

For this purpose, a phylogenetic tree for 82 bifidobacterial type strains was constructed by joining the five coding sequences in the following order: *clp*C (720 bp), *dna*G (992 bp), *dna*J (477 bp), *hsp*60 (662 bp) and *rpo*B (500 bp). The resulting in-frame concatenated gene sequences (3351 bp) were aligned with MUSCLE in MEGA 6.0 [8,37]. The evolutionary distances were computed by nucleotide model GTR CAT, and the phylogenetic tree was constructed by RaxML (version 8.2.7, Maximum-Likelihood method) [36] with *Scardovia inopinata* JCM 12537^T as the root (Fig. 2). The statistical reliability of the tree was evaluated by bootstrap analysis (rapid hill climbing) of 1000 replicates. The visualization was performed with iTOL (https://itol.embl.de/) [18].
The MLSA analysis confirmed the phylogenetic positioning of the strains SMA1^T and SMB2, which were related to *B. primatium* DSM 100687^T whereas strain SMA15^T resulted closest to *Bifidobacterium samiri* LMG 30940^T (Figs. 1 and 2).

Furthermore, the level of similarity for the partial housekeeping gene sequences of strains in relation to the type strains of their closest phylogenetic relatives was calculated using EMBOSS Water web-based program (https://www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html). The values of similarity for the *hsp*60, *rpo*B, *clp*C, *dna*J and *dna*G gene sequences were calculated and reported in Supplementary Table S2.

In order to reconfirm the above phylogenetic analysis, we also constructed the phylogenetic tree based on the core genome of the genus *Bifidobacterium*. A total of 83 type strains of *Bifidobacterium* were annotated with the DFAST program [38], and 362 orthologous gene clusters were identified as the core retained in all genomes. The core protein sequences from each genome were concatenated and aligned using the MAFFT program (version 7.313) [14]. The alignments were trimmed using trimAl with -automated1 option [3].

The phylogenomic tree (Fig. 3) based on the concatenated amino acid sequences of 362 core genes confirmed the positioning of the strains SMA1^T, SMB2 and SMA15^T within the genus *Bifidobacterium* as observed in the phylogenetic analyses based on 16S rRNA (Figs. 1 and 3). Thus, these findings clearly supported the genetic diversity of strains SMA1^T and SMA15^T from any other currently recognized bifidobacterial species.

Strains SMA1^T and SMA15^T both showed rod-shaped cells, frequently forming filaments, with irregular contractions along the cells and bifurcations. Cells were cultivated under anaerobic conditions and maintained in TPY broth [35] pH 6.9, at 37 °C, unless indicated otherwise. Morphological, cultural and biochemical characterization of the strains were performed at 37 °C unless otherwise stated, according to Modesto *et al.* [28].
The morphology of cells of strains SMA1^T and SMA15^T, as revealed by phase-contrast microscopy, is shown in Fig. 4.

Optimal growth conditions of the strains were determined in TPY broth after 24 h of incubation at 37 °C in anaerobic condition. Growth at 22, 25, 30, 35, 37, 40, 42, 45 and 48 °C was tested. Sensitivity to low pH was screened at 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 values of pH. The ability of the strains to grow under aerobic and microaerophilic conditions (CampyGen; Oxoid) was also verified in TPY broth after 48 h of incubation at 37 °C. Particularly, best growth conditions were obtained in TPY broth pH 6.5 at 37 °C and studied strains resulted able to survive and grow in microaerophilic and in aerobic conditions. All results are showed in Table 2.

Haemolytic activity was determined in Columbia blood agar (Biolife) at 37 °C under anaerobic conditions for 48 h [33].

Gram staining, motility assay, catalase and oxidase activities were performed according to Modesto *et al*. [28].

Strains SMA1^T, SMB2 and SMA15^T and related species *B. primatium* DSM 100687^T and *B. stellenboschense* DSM 23968^T were also investigated for substrates utilization and enzymes production with API 50 CHL and Rapid ID 32 kits (BioMérieux). Results are summarized in Table 2.

Bifidobacteria and members of related genera possess fructose-6-phosphate phosphoketolase (F6PPK), the enzyme degrading hexose via the F6PPK pathway, which is considered a taxonomic marker for identification of *Bifidobacterium* and related genera [19]. Detection of F6PPK activity was carried out according to the method described by Orban & Patterson [31]. All studied strains possessed F6PPK activity.

For analysis of amino acid composition, the cell-wall peptidoglycan of strains SMA1^T and SMA15^T was prepared and hydrolysed as described previously [12]. Cell-wall amino acids were analysed by HPLC (model LC-20AB; Shimadzu) equipped with a Wakopak wakosil-PTC column (200×4.0 mm i.d.; Wako Pure Chemical Industries), as their phenyl isothiocyanate derivatives (Wako). Amino acid isomers in the cell-wall hydrolysate were analysed as described previously using a liquid chromatograph-mass spectrometer (model LCMS-2020 and LC-20AB; Shimadzu) equipped with a

Shim-Pack FC-ODS column (150 × 2.0 mm i.d.; Shimadzu) [30]. The peptidoglycan of strain SMA1^T contained glutamic acid (Glu), serine (Ser), alanine (Ala) and ornithine (Orn) in a molar ratio of 1:1:2:1.

Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of L-Orn-D-Ser-L-Glu-Glu$_2$.

The peptidoglycan of strain SMA15^T contained Asparagine (Asp) glutamic acid (Glu), alanine (Ala) and Lysine (Lys) in a molar ratio of 1:2:2:1. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of L-Lys-L-Glu-D-Asp.

Whole-cell fatty acids were analysed as fatty acid methyl esters (FAMEs) with the Sherlock Microbial Identification System (MIDI Inc.) as described previously [4]. The cultures were incubated on MRS agar (supplemented with 0.05% cysteine) at 37°C for 24 h under anaerobic conditions. Extracts of the methylated fatty acids were prepared according to the protocol provided by the manufacturer and analyzed with Agilent 6890N gas chromatograph equipped with a flame ionization detector and 7683 Automatic Liquid Sampler (Agilent Technologies, CA, USA). Identification of the peaks was made by comparing the results with the built-in TSBA 50 database (MIDI). Results are shown in Table 3.

On the basis of the phenotypic and chemotaxonomic characterization as well as the molecular-based methods, phylogenetic analysis based on the 16S rRNA gene sequences, MLSA based on the concatenated five housekeeping gene sequences, and the whole-genome-based sequence comparisons in silico, strains SMA1^T and SMA15^T were genetically and phenotypically discernible from the currently recognized species of bifidobacteria; thus, according to Minimal Standard guidelines [20], they represent two novel taxa for which the name *Bifidobacterium saimiriisciurei* sp. nov. and *Bifidobacterium platyrrhinorum* sp. nov. are proposed. The protologues were shown in Tables 4 and 5.

Conflicts of interest

The authors declare that they have no competing interests.

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Figure Legends

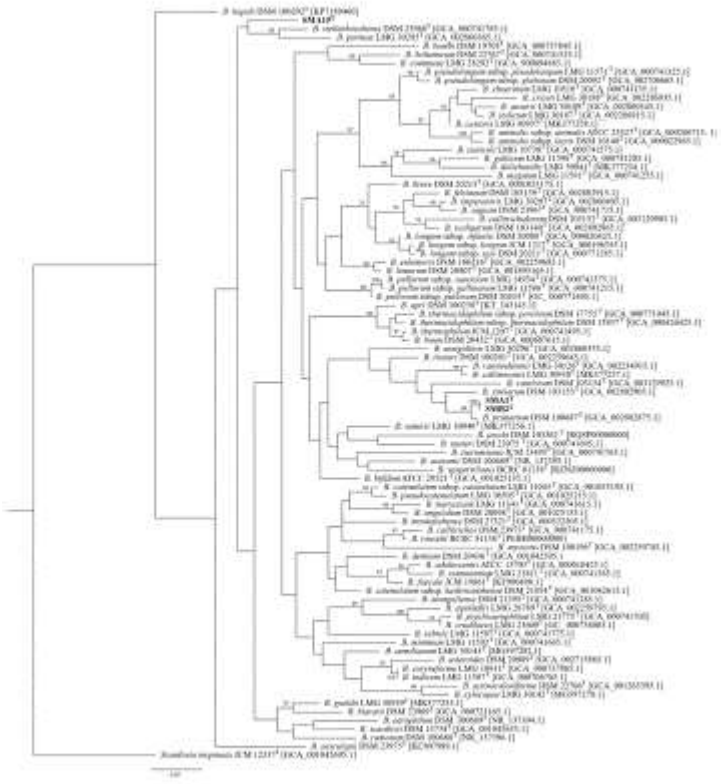

Figure 1. Phylogenetic tree of the genus *Bifidobacterium* based on 16S rRNA gene sequences, showing the relationship between strains SMA1^{T}, SMB2 and SMA15^{T} isolated from the squirrel monkey and other members of the genus *Bifidobacterium*. The 16S rRNA gene-based tree was constructed by the maximum likelihood method; the corresponding sequence of *Scardovia inopinata* JCM 12537^{T} was used as an outgroup. Bootstrap percentages above 70 are shown at node points, based on 1000 replicates of the phylogenetic tree.

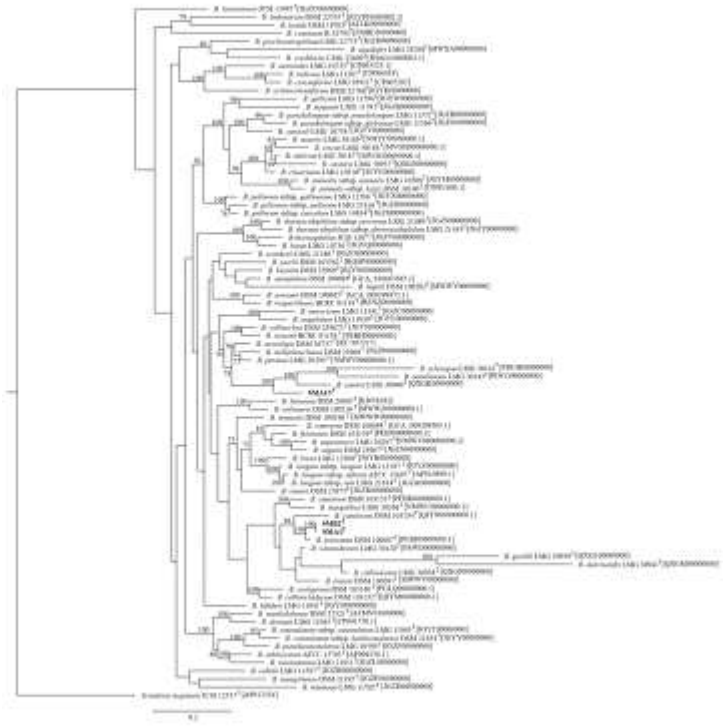

Figure 2. Phylogenetic three based on the concatenation of the housekeeping gene sequences for *hsp*60, *rpo*B, *dna*J, *dna*G and *clp*C, showing the phylogenetic relationships between strains SMA1^{T}, SMB2 and SMA15^{T} isolated from the squirrel monkey and members of the genus *Bifidobacterium*. The housekeeping gene-based tree was constructed by the maximum likelihood method, with corresponding sequences of *Scardovia inopinata* JCM 12537^{T} being used as an outgroup. Bootstrap percentages above 70 are shown at node points, based on 1000 replicates of the phylogenetic tree.

Figure 3. Phylogenetic tree of the genus *Bifidobacterium* based on the concatenation of 362 protein sequences that represent the *Bifidobacterium* core genome sequences.

The phylogenetic tree was built by the maximum likelihood method with corresponding sequences of *Scardovia inopinata* JCM 12537^{T} being used as an outgroup. Bootstrap percentages above 70 are shown at node points, based on 1000 replicates of the phylogenetic tree.

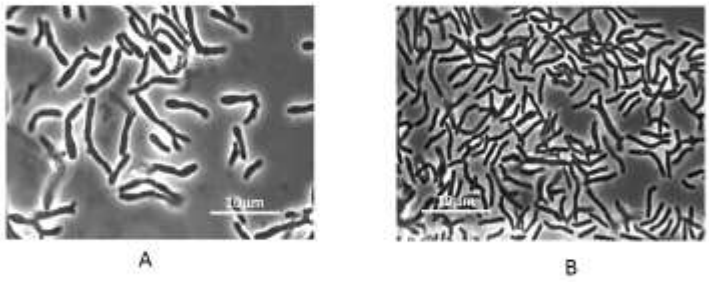

Figure 4. Phase-contrast photomicrographs of cells of *B. saimiriisciurei* sp. nov. SMA1^{T} (A) and *B. platyrrhinorum* sp. nov. SMA15^{T} (B) grown in TPY broth showing cellular morphology. Bar, 10 μm.

Table 1. Genomic and Phylogenetic features of strains SMA1^{T}, SMB2 and SMA15^{T}.

Strain	**SMA1^{T}**	**SMB2**	**SMA15^{T}**
Accession number	WHZU00000000	WHZT00000000	WHZV00000000
GC content	63.6 %	63.6 %	66.4 %
Contigs	94	20	45
Length	2769758	2594580	2617475
ORF	2334	2150	2281
tRNA	57	55	63
rRNA	3	3	3
Coverage	46	290	154

Table 2. Differential phenotypic characteristics of strains SMA1^T, SMB2 and SMA15^T isolated from the squirrel monkey (*Saimiri sciureus*) and their phylogenetic related species *B. primatium* DSM 100687^T and *B. stellenboschense* DSM 23968^T. Phenotypic data were obtained from this study.

	SMA1^T	SMB2	DSM 100687^T	SMA15^T	DSM 23968^T
Fermentation					
D-Ribose	+	+	+	w	+
D-Xylose	+	w	+	w	–
L-Xylose	–	–	–	+	–
D-Galactose	+	+	+	w	–
D-Glucose	+	+	+	w	+
D-Mannose	–	+	+	–	–
Dulcitol	w	w	+	–	–
D-Fructose	w	+	+	w	+
D-Mannitol	w	w	w	–	+
D-Sorbitol	+	+	+	w	+
Methyl-α-D-Mannopyranoside	–	–	–	w	–
Methyl-β-D_Xylopyranoside	w	–	–	–	–
Methyl-α-D-Glucopyranoside	–	–	+	+	+
N-Acetyl Glucosamine	–	–	w	+	+
Amygdalin	w	+	–	–	+
Arbutin	–	–	+	+	+
Salicin	w	w	w	+	+
D-Cellobiose	–	–	w	–	–
D-Maltose	w	w	+	+	+
Lactose	w	+	+	+	+
D-Melebiose	w	w	+	+	+
Sucrose	+	w	+	+	+
D-Melezitose	–	–	–	–	+
Trehalose	w	w	+	+	+
D-Raffinose	w	w	+	+	+
Starch	–	–	w	–	–
Gentiobiose	–	–	+	w	w
Glycogen	w	–	+	–	–
Xylitol	–	+	–	–	–
D-Turanose	w	w	+	w	+
D-Lyxose	–	–	+	–	+
D-Arabitol	+	+	+	–	–
Gluconate	–	–	w	–	w
2-keto Gluconate	–	–	–	–	–
5-keto Gluconate	–	–	w	–	–
Enzymatic activity					
L-arginine dihydrolase	–	–	+	–	–

α-Galactosidase	+	+	+	+	w
β-Galactosidase	–	w	+	+	w
α -Glucosidase	–	+	–	+	w
β- Glucosidase	–	w	–	+	–
α -Arabinosidase	–	–	–	–	+
β-Glucuronidase	–	–	–	–	+
Alkaline Phosphatase	+	+	+	–	+
Leucyl Glycine Arylamidase	–	–	+	–	+
Arginine Arylamidase	+	w	+	–	+
Proline Arylamidase	–	–	+	+	–
Leucyl Glycine Arylamidase	–	–	+	–	+
Phenylalanine Arylamidase	–	–	+	w	–
Leucine Arylamidase	–	–	+	–	+
Pyroglutamic acid Arylamidase	+	+	–	–	+
Glycine Arylamidase	+	+	+	–	–
Tyrosine Arylamidase	–	–	w	w	–
Histidine Arylamidase	+	+	+	–	–
Glutamyl Glutamic acid Arylamidase	+	+	–	–	+
Serine Arylamidase	–	–	+	–	w
Range Temperature for growth	25–46 °C	25–46 °C	25–46 °C	25–46 °C	25–42 °C
Optimum	37 °C	37 °C	37 °C	37 °C	37 °C
pH for growth range	5.5–7.0	5.5–7.0	5.57.0	5.5–7.0	5.0–8.0
Optimum pH	6.5	6.5	6.5	6.5	6.5
DNA GC content (mol%)	63.6 %	63.6 %	63.5 %	66.3 %	65.3%
Peptidoglycan type	L-Orn-D-Ser-L-Glu-Glu_2.	L-Orn-D-Ser-L-Glu-Glu_2.	L-Orn(Lys)-L-Ser*	L-Lys-L-Glu-D-Asp	L-Lys (L-Orn)-D-Asp§

+, positive; −, negative; w, weakly positive. All strains ferment L-Arabinose. No strains ferment glycerol, erithritol, D-arabinose, L-xylose, D-adonitol, methyl-*β*-D-pyranoside, sorbose, inositol, L-rhamnose, inulin, D-tagatose, D-fucose, L-fucose, L-arabitol, inulin and 2-ketoglucoonate. No strains show *β*-galactosidase-6-phosphate, *β*-glucosidase, *N*-Acetyl-*β*-Glucosaminidase, Alanine Arylamidase, *α*-Fucosidase. No reduction of urea, nitrates or indole production.

*Data are from Modesto *et al*. 2018[28]; § Data are from Endo *et al*. 2012 [10].

Table 3. Fatty acid compositions of strains SMA1^{T}, SMB2 and SMA15^{T} isolated from the squirrel monkey *Saimiri sciureus*

Summed feature 7 comprises unknown fatty acid (ECL 18.846), $C_{19:1\ \omega6c}$ and/or cyclo $C_{19:1\ \omega10c}$;

Fatty acid	**SMA1***	**SMB2***	**SMA15***
$C_{16:0}$	**54.3 ± 1.4**	**45.5 ± 0.4**	**45.6 ± 0.2**
$C_{14:0}$	4.0 ± 0.5	5.9 ± 0.6	11.30 ± 0.1
$C_{18:1}$ ω9c	**15.0 ± 1,3**	**12.1 ± 0.4**	8.4 ± 0.3
$C_{18:0}$	**13.1 ± 0,8**	9.6 ± 1.1	7.3 ± 0.8
$C_{17:1}$ w8c	2.1 ± 0,4	1.4 ± 0.2	–
$C_{13:1}$ at 12-13	1.2 ± 0.1	1.3 ± 0.1	–
$C_{17:0}$ 2OH	1.1 ± 0.0	–	–
Summed features			
7	–	**12.4 ± 0.7**	**19.0 ± 0.9**
1	4.2 ± 0.5	4.4 ± 0.4	2.0 ± 0.4
8	2.1 ± 0.1	2.4 ± 0.3	1.6 ± 0.1

Summed feature 1 comprises $C_{15:1}$ iso H/13:0 3OH or $C_{13:0}$ 3OH/15:1 I H; Summed feature 8 comprises $C_{18:1\ \omega7c}$ and/or $C_{18:1\ \omega6c.}$

*Values are percentages of total fatty acids. Analysis are conducted in triplicate. The major components of fatty acid (≥ 10%) are highlighted in bold.

Table 4. Description of *Bifidobacterium saimiriisciurei* sp. nov.

Genus name	*Bifidobacterium*
Species name	*Bifidobacterium saimiriisciurei*
Specific epithet	*saimiriisciurei*
Species status	**sp. nov.**
Species etymology	*Bifidobacterium saimiriisciurei* (sai.mi.ri.i.sci.u're.i. N.L. gen. n. saimiriisciurei of the squirrel monkey *Saimiri sciureus*).
Phenotipic features that differentiate the taxon from its closest relatives	Cells are Gram-stain-positive, non-motile, asporogenous, non-haemolytic, F6PPK-positive, catalase- and oxidase-negative, indole-negative, and when grown in TPY broth are rods of various shapes forming a branched structure with a 'Y' shape at both sides. Well-isolated colonies grown on the surface of TPY agar under anaerobic conditions are white, opaque, smooth and circular with entire edges, while the embedded colonies are lens-shaped or elliptical. Colonies reach 1.0–2.0 mm in diameter after 3 days of incubation. Cells can grow under aerophilic and microaerophilic conditions. Cells can grow in the range 25–46 °C; no growth occurs at 22 °C. Cells grow at pH 5.5–7.0. Optimal conditions of growth occur at pH 6.5 and 37 °C. Using the API 50 CHL test system, acids are produced from glucose, L-arabinose, ribose, D-xylose, D-galactose, mannose, raffinose, sorbitol, sucrose, xylitol, D-arabitol; acids are produced weakly from fructose, dulcitol, D-mannitol, amygdalin, salicin, lactose, melibiose, trehalose, turanose but not from other carbohydrates in API50CH tests. Enzymatic activity was observed for α- and β-galactosidase, α- and β-glucosidase, alkaline phosphatase, pyroglutamic acid arylamidase, glycine arylamidase, histidine arylamidase. Nitrates are not reduced. Cells are negative for urease but positive for aesculin hydrolysis.
Country of origin	Italy
Region of origin	Veneto
Date of isolation (dd/mm/yyyy)	9/12/2016
Source of isolation	Faecal samples of adult male of *Saimiri sciureus*
Sampling date (dd/mm/yyyy)	2/12/2016
Latitude (xx°xx′xx″N/S)	45°28'39.9"N
Longitude (xx° xx′ xx″ E/W)	10°47'38.3"E
Altitude (meters above sea level)	200 mt
16S rRNA gene accession nr.	MN722462
Genome accession number [RefSeq; EMBL; ...]	WHZU00000000
Genome status	Draft
Genome size	2,76
GC mol%	63,6
Number of strains in study	2
Source of isolation of non-type strains	Faecal samples of one adult male of the squirrel monkey (*Saimiri sciureus)*

Designation of the Type Strain	SMA1
Strain Collection Numbers	SMA1^T= BCRC 81223^T, NBRC 114049^T=DSM 106020^T

Table 5. Description of *Bifidobacterium platyrrhinorum* sp. nov.

Genus name	*Bifidobacterium*
Species name	*Bifidobacterium platyrrhinorum*
Specific epithet	*platyrrhinorum*
Species status	**sp. nov.**
Species etymology	*Bifidobacterium platyrrhinorum* (pla.ty.rrhi.no'rum.N.L. gen. pl.n. platyrrhinorum of the Platyrrhini parvorder).
Phenotipic features that differentiate the taxon from its closest relatives	Cells are Gram-stain-positive, non-motile, asporogenous, non-haemolytic, F6PPK-positive, catalase- and oxidase-negative, indole-negative, and when grown in TPY broth are rods of various shapes forming a branched structure with a 'Y' shape at both sides. Well-isolated colonies grown on the surface of TPY agar under anaerobic conditions are white, opaque, smooth and circular with entire edges, while the embedded colonies are lens-shaped or elliptical. Colonies reach 1.0–2.0 mm in diameter after 3 days of incubation. Cells can grow under aerophilic and microaerophilic conditions. Cells can grow in the range 25–46 °C; no growth occurs at 22 °C. Cells grow at pH 5.5–7.0. Optimal conditions of growth occur at pH 6.5 and 37 °C. Using the API 50 CHL test system, acids are produced from glucose, L-arabinose, D-galactose, D-fructose, mannose, D-ribose, sorbitol, sucrose, xylitol, arabitol; acids are produced weakly from dulcitol, mannitol, amygdalin, salicin, maltose, lactose, melibiose, trehalose, turanose but not from other carbohydrates in API50CH tests. Enzymatic activity was observed for α- and β-galactosidase, α- and β-glucosidase, alkaline phosphatase, arginine arylamidase, pyroglutamic acid arylamidase, glycine arylamidase, histidine arylamidase, glutamil glutamic acid arylamidase. Nitrates are not reduced. Cells are negative for urease but positive for aesculin hydrolysis.
Country of origin	Italy
Region of origin	Veneto
Date of isolation (dd/mm/yyyy)	9/12/2016
Source of isolation	Faecal samples of an adult male of *Saimiri sciureus*
Sampling date (dd/mm/yyyy)	2/12/2016
Latitude (xx°xx′xx″N/S)	45°28'39.9"N
Longitude (xx° xx′ xx″ E/W)	10°47'38.3"E
Altitude (meters above sea level)	200 mt
16S rRNA gene accession nr.	MN722463
Genome accession number	WHZV00000000
Genome status	Draft

Genome size	2,769758
GC mol%	63,6
Number of strains in study	1
Source of isolation of non-type strains	Faecal samples of one adult male of the squirrel monkey (*Saimiri sciureus)*
Designation of the Type Strain	SMA15^{T}
Strain Collection Numbers	SMA15^{T}= DSM 106029^{T}= BCRC 81224^{T}, NBRC 114051^{T} = DSM 106029^{T}