

Supporting Information

Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut

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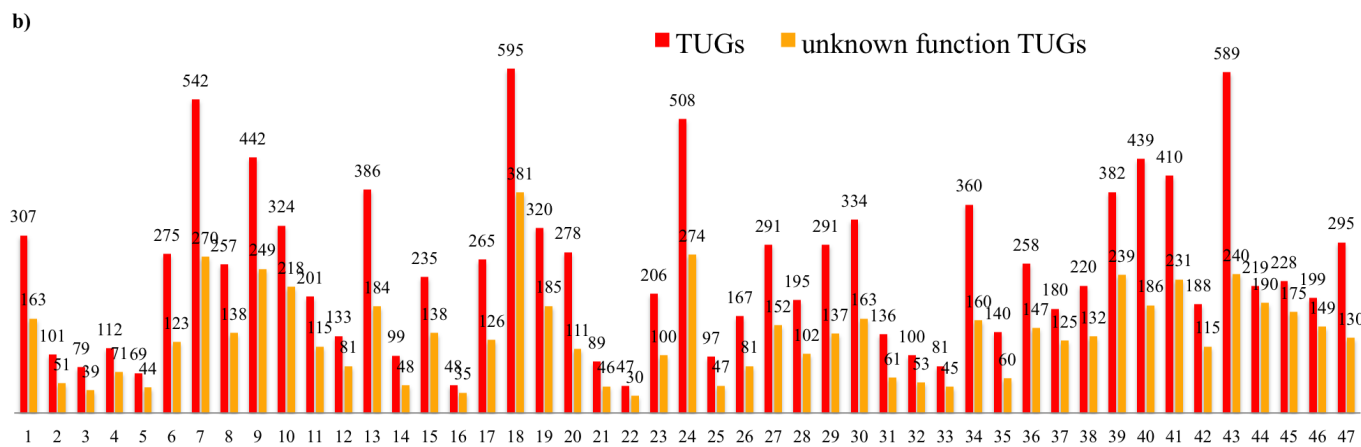
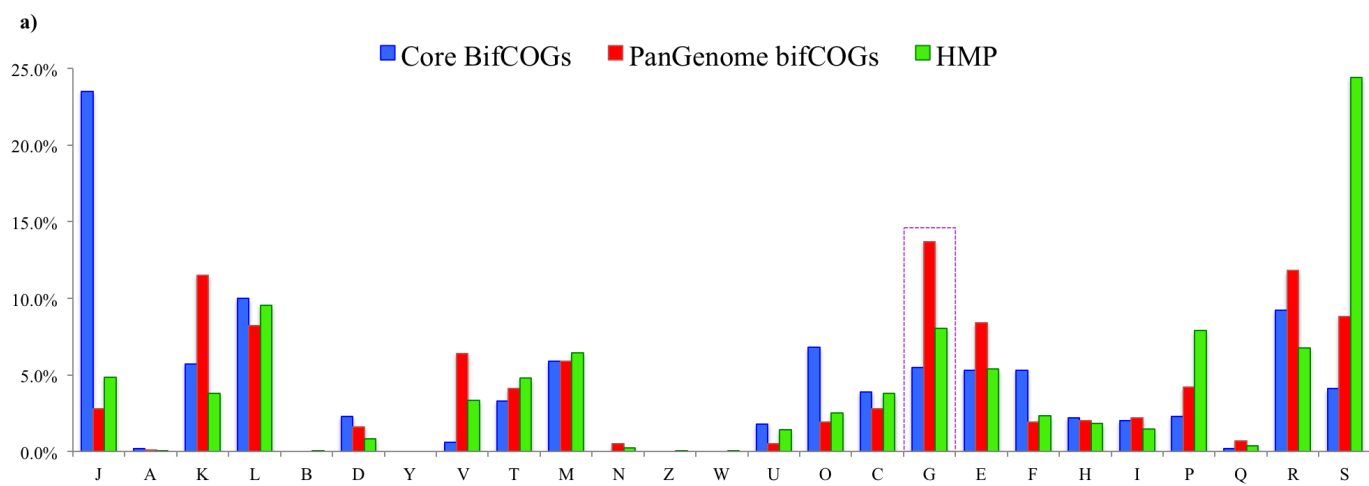


Fig. S1

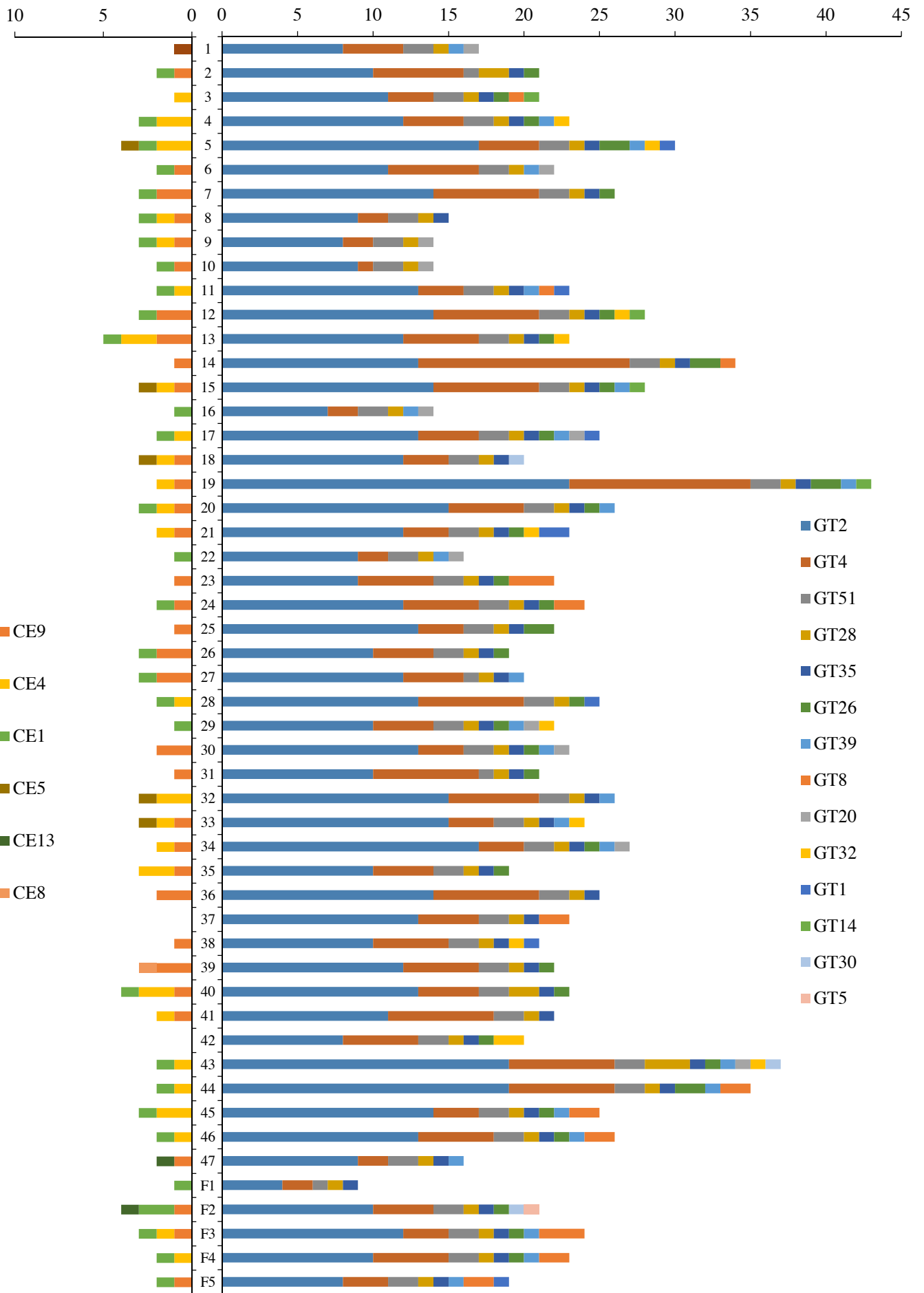


Fig. S3

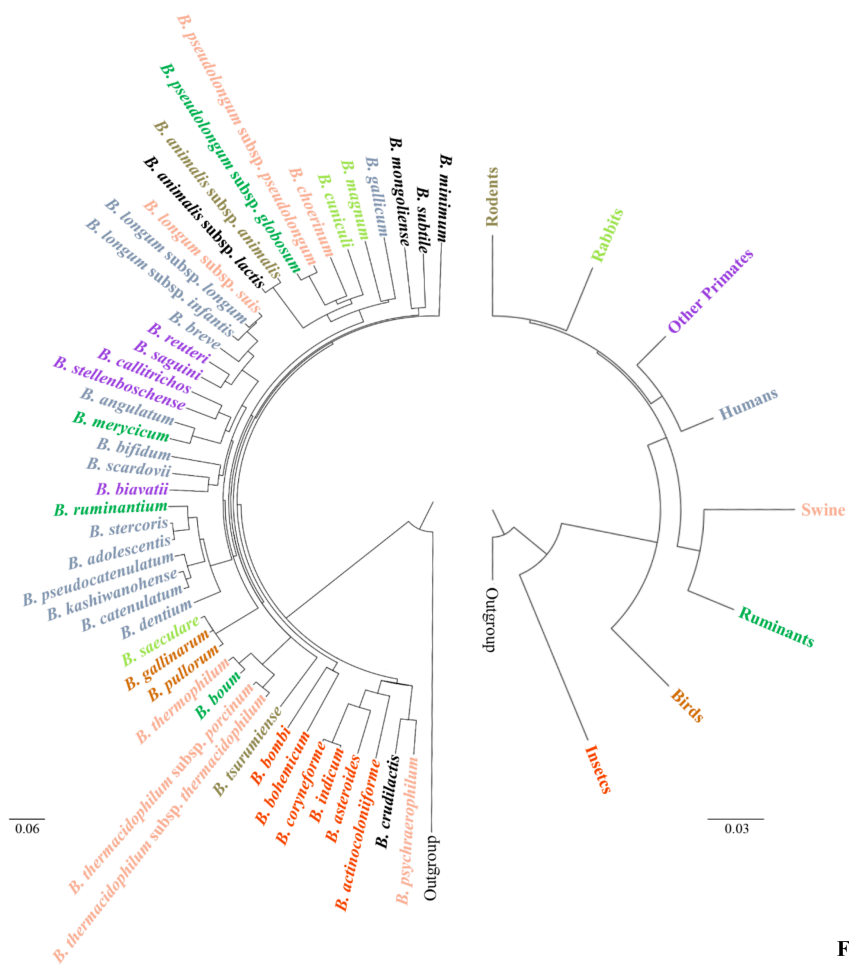


Fig.S4

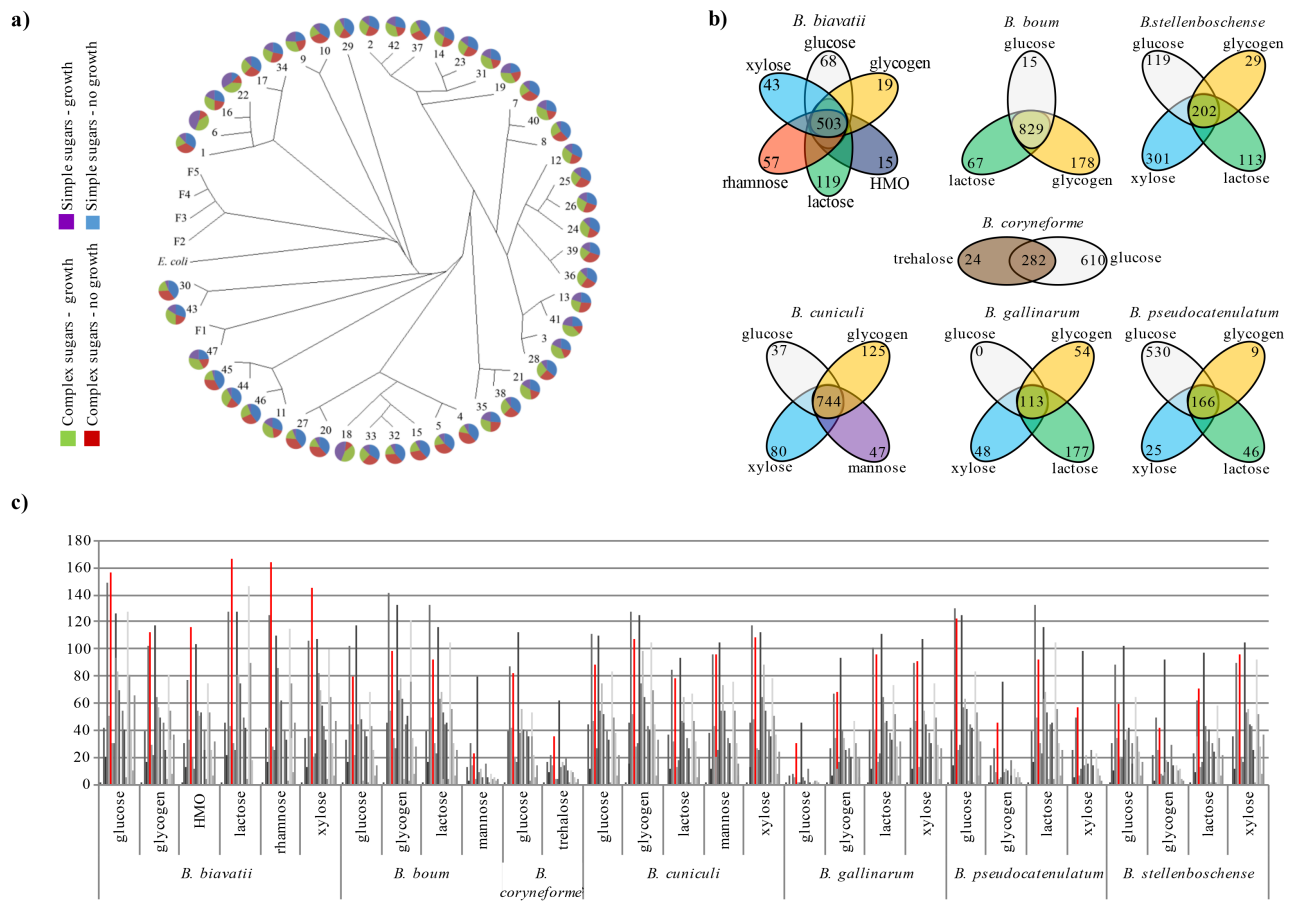


Fig. S5

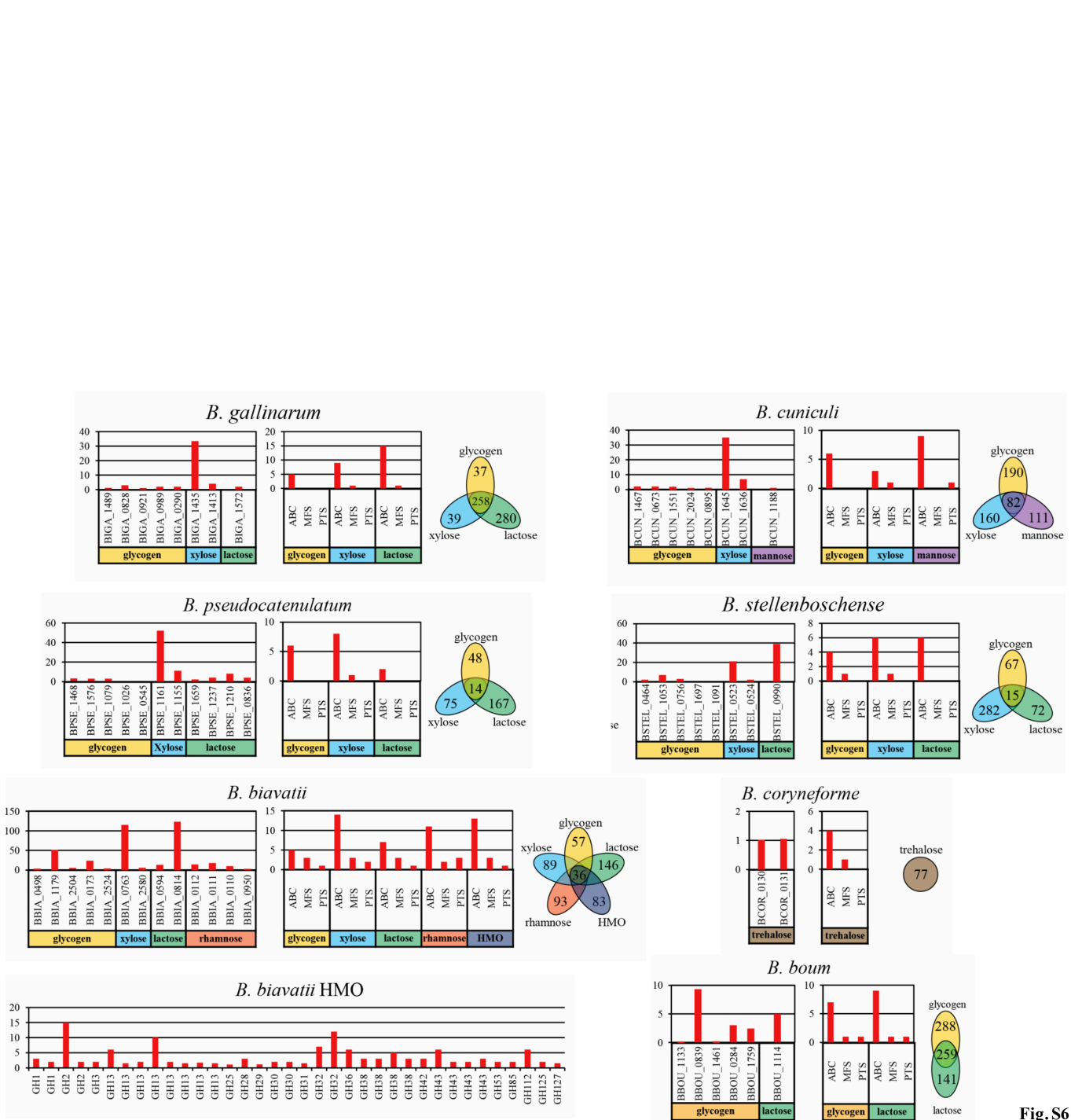


Fig. S6

Table S1. General features of the *Bifidobacterium* genomes.

Number	<i>Bifidobacterium</i> Strains	Genome Status	Genome Size	Isolation	Accession Number
1	<i>B. actinocolonii</i> forme DSM 22766	Draft (4)	1823388	Bumblebee digestive tract	JGYK00000000
2	<i>B. adolescentis</i> ATCC 15703	Complete	2089645	Intestine of adult	AP009256.1
3	<i>B. angulatum</i> LMG 11039	Draft (6)	2003806	Human faeces	JGYL00000000
4	<i>B. animalis</i> subsp. <i>animalis</i> LMG 10508	Draft (13)	1915007	Rat feces	JGYM00000000
5	<i>B. animalis</i> subsp. <i>lactis</i> DSM 10140	Complete	1938606	Fermented milk	CP001606.1
6	<i>B. asteroides</i> LMG 10735 (PRL2011)	Complete	2167304	Honeybee hindgut	CP003325.1
7	<i>B. biavatii</i> DSM 23969	Draft (56)	3252147	Feces of tamarin	JGYN00000000
8	<i>B. bifidum</i> LMG 11041	Draft (2)	2208468	Brest-feed Infant faeces	JGYO00000000
9	<i>B. bohemicum</i> DSM 22767	Draft (5)	2052470	Bumblebee digestive tract	JGYP00000000
10	<i>B. bombi</i> DSM 19703	Draft (4)	1895239	Bumblebee digestive tract	ATLK00000000
11	<i>B. boum</i> LMG 10736	Draft (18)	2171356	Bovine rumen	JGYQ00000000
12	<i>B. breve</i> LMG 13208	Draft (31)	2263780	Infant intestine	JGYR00000000
13	<i>B. callitrichos</i> DSM 23973	Draft (33)	2887313	Feces of common marmoset	JGYS00000000
14	<i>B. catenulatum</i> LMG 11043	Draft (11)	2082756	Adult intestine	JGYT00000000
15	<i>B. choerinum</i> LMG 10510	Draft (20)	2096123	Piglet faeces	JGYU00000000
16	<i>B. coryneforme</i> LMG 18911	Complete	1755151	Honeybee hindgut	CP007287
17	<i>B. crudilactis</i> LMG 23609	Draft (6)	2362816	Raw cow milk	JHAL00000000
18	<i>B. cuniculi</i> LMG 10738	Draft (41)	2531592	Rabbit faeces	JGYV00000000
19	<i>B. dentium</i> LMG 11045 (Bd1)	Complete	2636367	Oral cavity	CP001750.1
20	<i>B. gallicum</i> LMG 11596	Draft (12)	2004594	Adult intestine	JGYW00000000
21	<i>B. gallinarum</i> LMG 11586	Draft (10)	2160836	Chicken caecum	JGYX00000000
22	<i>B. indicum</i> LMG 11587	Complete	1734546	Insect	CP006018
23	<i>B. kashiwanohense</i> DSM 21854	Draft (30)	2307960	Infant faeces	JGYY00000000
24	<i>B. longum</i> subsp. <i>infantis</i> ATCC 15697	Complete	2832748	Intestine of infant	AP010889.1
25	<i>B. longum</i> subsp. <i>longum</i> LMG 13197	Draft (8)	2384703	Adult intestine	JGYZ00000000
26	<i>B. longum</i> subsp. <i>suis</i> LMG 21814	Draft (36)	2335832	Pig faeces	JGZA00000000
27	<i>B. magnum</i> LMG 11591	Draft (13)	1822476	Rabbit faeces	JGZB00000000
28	<i>B. merycicum</i> LMG 11341	Draft (16)	2280236	Bovine rumen	JGZC00000000
29	<i>B. minimum</i> LMG 11592	Draft (18)	1892860	Sewage	JGZD00000000
30	<i>B. mongoliense</i> DSM 21395	Draft (43)	2170490	Fermented mare's milk	JGZE00000000
31	<i>B. pseudocatenulatum</i> LMG 10505	Draft (10)	2283767	Infant faeces	JGZF00000000
32	<i>B. pseudolongum</i> subsp. <i>globosum</i> LMG 11569	Draft (26)	1935255	Bovine rumen	JGZG00000000
33	<i>B. pseudolongum</i> subsp. <i>pseudolongum</i> LMG 11571	Draft (11)	1898684	Swine faeces	JGZH00000000
34	<i>B. psychraerophilum</i> LMG 21775	Draft (11)	2615078	Pig caecum	JGZI00000000
35	<i>B. pullorum</i> LMG 21816	Draft (11)	2153559	Chicken faeces	JGZJ00000000
36	<i>B. reuteri</i> DSM 23975	Draft (28)	2847572	Feces of common marmoset	JGZK00000000
37	<i>B. ruminantium</i> LMG 21811	Draft (23)	2249807	Bovine rumen	JGZL00000000
38	<i>B. saeculare</i> LMG 14934	Draft (14)	2263283	Rabbit faeces	JGZM00000000
39	<i>B. saguini</i> DSM 23967	Draft (33)	2787036	Feces of tamarin	JGZN00000000
40	<i>B. scardovii</i> LMG 21589	Draft (34)	3141793	Blood	JGZO00000000
41	<i>B. stellenboschense</i> DSM 23968	Draft (40)	2812864	Feces of tamarin	JGZP00000000
42	<i>B. stercoris</i> DSM 24849	Draft (15)	2304613	Adult faeces	JGZQ00000000
43	<i>B. subtile</i> LMG 11597	Draft (27)	2790088	Sewage	JGZR00000000
44	<i>B. thermacidophilum</i> subsp. <i>porcinum</i> LMG 21689	Draft (3)	2079368	Piglet faeces	JGZS00000000
45	<i>B. thermacidophilum</i> subsp. <i>thermacidophilum</i> LMG 21395	Draft (8)	2233072	Anaerobic digester	JGZT00000000
46	<i>B. thermophilum</i> JCM 1207	Draft (12)	2099496	Swine faeces	JGZV00000000
47	<i>B. tsurumiense</i> JCM 13495	Draft (25)	2164426	Hamster dental plaque	JGZU00000000

Table S2. qPCR primers used in this study.

Primer	Sequence (5'-3')	Targeted ORF/gene	Organism
Bbia-fw	GGATATGTTGTTTCGTATGGGA	BBIA_0409	<i>B. biavatii</i> DSM 23969
Bbia-rv	GACTCGCTGTGTATGTGCTTC		
Bste-fw	ATACGGCAAGATCGTCAACAT	BSTEEL_1950	<i>B. stellenboschense</i> DSM 23968
Bste-rv	GATTGACCTTGTTGTAGGCTT		
Blosu-fw	ACACCTTCCAATCTACTGCAT	BLSS_1597	<i>B. longum</i> subsp. <i>suis</i> LMG 21814
Blosu-rv	CATCACTGTCTACGAAACCCA		
Btp-fw	ACACCACGTATAGTCTCGGC	BPORC_0092	<i>B. thermoacidophilum</i> subsp. <i>porcinum</i> LMG 21689
Btp-rv	GCTTTTGGTTTTCTCCCGGT		
Bcun-fw	GTGATGTACCTGGTAGAAGGAA	BCUN_0008	<i>B. cuniculi</i> LMG 10738
Bcun-rv	TGTGCCGTATGTGGTGAGATAT		
Bmag-fw	CAATGCGAACTATGCGGACA	BMAGN_1584	<i>B. magnum</i> LMG 11591
Bmag-rv	AAATCAGCGCGAGTCCATTC		

1 **Supplementary figures.**

2 **Figure S1.** COG functional annotation and Truly Unique Genes (TUG) of the *Bifidobacterium* genus.

3 Panel a shows the COG functional classification of the core BifCOGs in blue, pangenome BifCOGs
4 in red, and average abundance in samples from the Human Microbiome Project in green. Panel b
5 displays in red the TUG number in each bifidobacterial genome and in orange the number of TUGs
6 with unknown function, representing 54.1 % of the total TUG pool. The number of TUG with
7 predicted or unknown function is reported above each column in the graph. COG functional families
8 are abbreviated as follow: [J] Translation, ribosomal structure and biogenesis, [A] RNA processing
9 and modification, [K] Transcription, [L] Replication, recombination and repair, [B] Chromatin
10 structure and dynamics, [D] Cell cycle control, cell division, chromosome partitioning, [Y] Nuclear
11 structure, [V] Defense mechanisms, [T] Signal transduction mechanisms, [M] Cell
12 wall/membrane/envelope biogenesis, [N] Cell motility, [Z] Cytoskeleton, [W] Extracellular
13 structures, [U] Intracellular trafficking, secretion, and vesicular transport, [O] Posttranslational
14 modification, protein turnover, chaperones, [C] Energy production and conversion, [G] Carbohydrate
15 transport and metabolism, [E] Amino acid transport and metabolism, [F] Nucleotide transport and
16 metabolism, [H] Coenzyme transport and metabolism, [I] Lipid transport and metabolism, [P]
17 Inorganic ion transport and metabolism, [Q] Secondary metabolites biosynthesis, transport and
18 catabolism, [R] General function prediction only, [S] Function unknown.

19

20 **Figure S2.** Prediction of the glycosyl hydrolases (GH) and carbohydrate-binding modules (CBM) in
21 the *Bifidobacterium* genus. Panels a and b show, respectively, the GH and CBM families profile of
22 all analysed bifidobacterial species, based on the CAZy database. Each bifidobacterial genome
23 analysed is numbered according to the numbering of the species displayed in Supplementary Table
24 1.

25

26 **Figure S3.** Prediction of the glycosyl transferases (GT) and carbohydrate esterases (CE) in the
27 *Bifidobacterium* genus. Left and right bar plots show respectively the CE and GT families profile of
28 all the bifidobacterial species, based on the CAZy database. Each bifidobacterial genome analysed is
29 numbered according to the numbering of the species displayed in Supplementary Table 1. Additional
30 members of the *Bifidobacteriaceae* family (other than those belonging to the genus *Bifidobacterium*)
31 are as follows: F1 *Gardnerella vaginalis*, F2 *Metascardovia criceti*, F3 *Parascardovia denticolens*,
32 F4 *Scardovia inopinata* and F5 *Scardovia wiggsiae*.

33

34 **Figure S4.** Comparison between phylogenetic evolution of bifidobacterial species and their
35 eukaryotic hosts. The tree on the left depicts the evolutionary development of the 47 analyzed
36 members of the genus *Bifidobacterium*, while the tree on the right illustrates the phylogeny of their
37 eukaryotic hosts. Colors are used to correlate each bifidobacterial species to their respective host.

38

39 **Figure S5.** Whole-genus carbohydrate utilization survey and glycan transcriptomic analyses of
40 representative bifidobacterial species from the seven *Bifidobacterium* phylogenetic groups. Panel a
41 shows a phylogenetic core-supertree of the *Bifidobacteriaceae* family. Each terminal node is a cake
42 diagram describing the number of simple and complex carbohydrates that each tested bifidobacterial
43 strain is able to utilize of a total of 23 simple carbohydrates and 27 complex sugars. Each
44 bifidobacterial genome analysed is numbered according to the numbering of the species displayed in
45 Supplementary Table 1. Remaining members of the *Bifidobacteriaceae* family are named as follows:
46 F1 *Gardnerella vaginalis*, F2 *Metascardovia criceti*, F3 *Parascardovia denticolens*, F4 *Scardovia*
47 *inopinata* and F5 *Scardovia wiggsiae*. Panel b shows a Venn diagram representation of the observed
48 transcriptome of seven bifidobacterial taxa, chosen as representatives of the seven phylogenetic
49 groups, grown on a total of eight simple or complex carbohydrates. Panel c consists of a bar plot
50 representing the COG annotation of the observed transcriptomes. Highlighted in red is the
51 carbohydrate metabolism and transport (G) COG class.

52

53 **Figure S6.** Expression modulation of genes involved in carbohydrate transport and metabolism
54 possessed by representative bifidobacterial species from the seven *Bifidobacterium* phylogenetic
55 groups. Differential expression are reported comparing normalized read counts for the different
56 conditions versus growth on glucose. Venn diagrams depict the number of differentially transcribed
57 (>2-fold increased transcription) genes in the different growth conditions as well as actively
58 transcribed genes shared by all conditions. Bar plots represent fold transcriptional induction of genes
59 involved in degradation pathways and the number of transporter-encoding genes overexpressed (>2-
60 fold increased transcription). Glycogen degradation I is constituted by glycogen phosphorylase,
61 glycogen debranching protein, phosphoglucomutase, amylomaltase, and glucokinase, xylose
62 degradation I is constituted by xylose isomerase and xylulokinase, lactose degradation is constituted
63 by β -galactosidases, L-rhamnose degradation II is constituted by L-rhamnofuranose 1-
64 dehydrogenase, L-rhamnoate dehydratase, 2-keto-3-deoxy-L-rhamnoate aldolase and L-lactaldehyde
65 dehydrogenase, while trehalose degradation I is constituted by trehalose phosphatase and α,α -
66 phosphotrehalase.