REVIEW ARTICLE

The first 1000 cultured species of the human gastrointestinal microbiota

Mirjana Rajilić-Stojanović[1,2] & Willem M. de Vos[2,3]

[1]Department for Biotechnology and Biochemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia; [2]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands; and [3]Departments of Bacteriology and Immunology, and Veterinary Biosciences, University of Helsinki, Helsinki, Finland

Correspondence: Mirjana Rajilić-Stojanović, Department for Biotechnology and Biochemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia. Tel.: +381 11 337 0460; fax: +381 11 337 0387; e-mail: mrajilic@tmf.bg.ac.rs

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Abstract

The microorganisms that inhabit the human gastrointestinal tract comprise a complex ecosystem with functions that significantly contribute to our systemic metabolism and have an impact on health and disease. In line with its importance, the human gastrointestinal microbiota has been extensively studied. Despite the fact that a significant part of the intestinal microorganisms has not yet been cultured, presently over 1000 different microbial species that can reside in the human gastrointestinal tract have been identified. This review provides a systematic overview and detailed references of the total of 1057 intestinal species of *Eukarya* (92), *Archaea* (8) and *Bacteria* (957), based on the phylogenetic framework of their small subunit ribosomal RNA gene sequences. Moreover, it unifies knowledge about the prevalence, abundance, stability, physiology, genetics and the association with human health of these gastrointestinal microorganisms, which is currently scattered over a vast amount of literature published in the last 150 years. This detailed physiological and genetic information is expected to be instrumental in advancing our knowledge of the gastrointestinal microbiota. Moreover, it opens avenues for future comparative and functional metagenomic and other high-throughput approaches that need a systematic and physiological basis to have an impact.

Introduction – a historical perspective

Human beings, similar to other higher organisms, live in symbiosis with their coevolved microbiota (Bäckhed *et al.*, 2005). The majority of the human microorganisms reside in the gastrointestinal tract, where, besides contributing to the digestion, they perform various other functions that are essential for the human host. These functions include the production of vitamins, education of the immune system, communication with the intestinal cells, and modulation of the host's behavior (Bäckhed *et al.*, 2005; Cryan & Dinan, 2012; Rajilić-Stojanović, 2013). The first report of living creatures in the human gastrointestinal tract dates from 1681 when Antonie van Leeuwenhoek reported a variety of 'little animals' in his stool samples and identified what is now thought to be a *Giardia* spp. when suffering from diarrhea (Dobell, 1932). Almost two centuries passed before the first detailed descriptions of pure cultures of gastrointestinal microorganisms were reported, of which the earliest is most likely the description of the eukaryal intestinal parasite *Pentatrichomonas hominis* (at the time named *Trichomonas hominis*), by Casimir Davaine in 1854 (Hemmeter, 1902). Since *P. hominis*, similar to other intestinal Eukarya, has a very low prevalence, this discovery did not trigger further analysis of the gastrointestinal microbiota. However, intensive studies of the gastrointestinal microbiota followed the first cultivation of the intestinal bacterium, now known as *Escherichia coli*. From a historical perspective, this and several other events, here termed turning points, can be recognized as having impacted the discovery of the gastrointestinal microbiota constituents. These turning points are evident when the number of described gastrointestinal tract species is considered in view of time (Fig. 1).

The first turning point (Fig. 1) marks the first description of a gastrointestinal bacterium, which is the isolation of *Bacterium coli commune* (later renamed to *E. coli*), by

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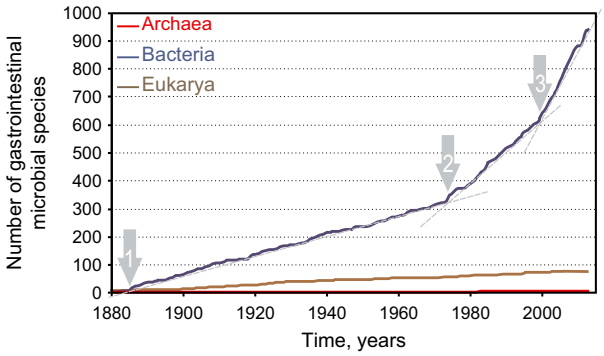

Fig. 1. Graphical representation of the cumulative number of cultured species from *Bacteria*, *Archaea* and *Eukarya* from the human gastrointestinal tract as a function of time. The arrows indicate the turning points of the gastrointestinal microbiota research: (1) Isolation of the first gastrointestinal bacterial species, (2) Introduction of strictly anaerobic techniques, and (3) Introduction of molecular techniques in the field of the gastrointestinal microbiota research.

the German pediatrician Teodor Escherich in 1885 (Shulman *et al.*, 2007). The studies that followed shortly thereafter led to the description of representatives of a number of the major gastrointestinal bacterial groups, including the genera *Bacteroides*, *Bifidobacterium,* and *Bacillus* as well as proteolytic cocci (Flügge, 1886; Veillon & Zuber, 1898; Moro, 1900; Tissier, 1900; Passini, 1905; Tissier, 1908; Distaso, 1911). During this period that lasted till the late sixties of the 20th century, *Bifidobacterium* and *Bacteroides* spp. were considered to be the dominant groups in the human gastrointestinal tract. Aerobes, referred as coliforms, streptococci and lactobacilli, were found as minor groups, while clostridia, staphylococci and aerobic spore-formers were reported as rare and not always detectable (Haenel, 1970). However, the vast majority of the gastrointestinal microorganisms are now known to be strict anaerobes, and this was for the first time shown in 1931 (Sanborn, 1931). Therefore, the early cultivation studies provided only a partial view of the gastrointestinal microbiota and it enabled isolation of only a minority (10–25%) of the gastrointestinal microorganisms (Finegold, 1969).

The improvements of anaerobic cultivation techniques by Hungate (1969) marked the second turning point in the gastrointestinal microbiota research, approximately 50 years ago (Fig. 1). In this second period of the gastrointestinal microbiota research that lasted from the early seventies till the molecular revolution in the beginning of this century (Fig. 1), it was recognized that the microbiota in the gastrointestinal tract is dominated by bacterial species that belong to the following genera: *Bacteroides, Clostridium, Eubacterium, Veillonella, Ruminococcus, Bifidobacterium, Fusobacterium, Lactobacillus, Peptostreptococcus,* and *Peptococcus* (Moore & Holdeman, 1974a). Using strict anaerobic techniques, it was reportedly possible to cultivate up to 88% of the total microscopic counts in fecal samples (Moore & Holdeman, 1974a). However, due to the enormous complexity of the gastrointestinal microbiota, many of the hundreds of isolates were not characterized beyond the genus level (Finegold *et al.*, 1974; Moore & Holdeman, 1974a; Benno *et al.*, 1986). Moreover, as processing of even a single sample yielded an enormous amount of different isolates, it was physically impossible to compare these all and make a full description based on the morphological, biochemical and physiological characteristics that could be determined at that time (Moore & Holdeman, 1974b). Hence, due to these technical limitations, the gastrointestinal microbiota remained only partially characterized.

Finally, the third turning point in the gastrointestinal microbiota research can be ascribed to the incorporation of molecular techniques about a dozen years ago (Fig. 1). These include global and culture-independent studies based on the sequence analysis of the small subunit ribosomal RNA (SSU rRNA) that had provided the molecular basis for microbial taxonomy that is currently used (Woese *et al.*, 1990). However, the complexity of the gastrointestinal tract microbial ecosystem hampers the rapid application of SSU rRNA-based methods as well as (meta)genomics (Zoetendal *et al.*, 2008). Hence, the first gastrointestinal tract study using SSU rRNA sequences dealt with a single adult sample (Wilson & Blitchington, 1996). Subsequent SSU rRNA-based studies in multiple adults demonstrated the individuality, temporal stability and site specificity of the intestinal microbiota with a diversity that was only partially grasped in cultivation-based studies (Zoetendal *et al.*, 1998; Suau *et al.*, 1999; Zoetendal *et al.*, 2001). These novel findings sparked a revival of the scientific interest in the gastrointestinal microbiota that was initially compared to a Renaissance (Tannock, 1999). However, the years that followed showed this to be more of a revolution that incorporated metagenome and whole-genome characterizations (Nelson *et al.*, 2010; Qin *et al.*, 2010; Brown *et al.*, 2013).

When integrated with cultivation-based studies, the analysis of the SSU rRNA gene sequences as phylogenetic markers enabled rapid identification of the new gastrointestinal isolates, and illustrated the need for the reclassification of many species. In addition, the SSU rRNA gene sequences enabled the detection of not yet cultured microorganisms and their phylogenetic positioning. Finally, the research field expanded to another dimension with the application of high-throughput approaches, including next-generation sequencing of the SSU rRNA gene sequences or the entire genomic material (Zoetendal *et al.*, 2008). The latter metagenomic analyses generated a

baseline of over 3 million, mainly bacterial, genes present in the human gastrointestinal tract (Qin *et al.*, 2010; Brown *et al.*, 2013) and demonstrated that the majority of the gastrointestinal microorganisms contain genomes that have not yet been characterized (Qin *et al.*, 2010; Le Chatelier *et al.*, 2013).

The present-day view of the gastrointestinal microbiota composition is quite different than prior to the molecular revolution. Most importantly, it is evident that still many of the gastrointestinal microorganisms have not yet been cultured and this in particular concerns phylogenetically distinct bacterial groups belonging to the *Firmicutes* phylum (Rajilić-Stojanović *et al.*, 2007). Furthermore, several of the bacterial groups that based on cultivation studies had been recognized as dominant gastrointestinal genera, have been reclassified and renamed. Most notably this concerns the *Bacteroides* spp. that have been reclassified into the genera *Alistipes, Prevotella, Paraprevotella, Parabacteroides,* and *Odoribacter.* Moreover, it is evident that different members of the *Bacteroidetes* phylum and not the *Bacteroides* genus *sensu stricto* are dominant in the human gastrointestinal tract. Furthermore, the abundance of the *Peptostreptococcus* spp. demonstrated in cultivation-based studies, could primarily be attributed to *Peptostreptococcus productus* (Holdeman *et al.*, 1976). However, SSU rRNA gene analysis has shown that this species does not belong to the genus *Peptostreptococcocus*, and the species was reclassified first as *Ruminococcus productus* (Ezaki *et al.*, 1994) and finally as *Blautia producta* (Liu *et al.*, 2008). Today, it is clear that *Blautia* spp., in contrast to *Peptostreptococcus* spp., form one of the most abundant groups in the human gastrointestinal tract. Many other so-called dominant genera are still in need for major reclassification, and the best example of this is the *Clostridium* genus, for which a detailed phylogenetic analysis led to a proposed grouping into 19 clusters (Collins *et al.*, 1994). Bacteria belonging to the *Clostridium* spp. are highly abundant in the adult gastrointestinal tract, and in particular, the members of the species that cluster within the *Clostridium* cluster IV (*C. leptum* group, which major constituent is the *Ruminococcacea* family) and the *Clostridium* cluster XIVa (*C. coccoides* group, which resembles the *Lachnospiraceae* family). Furthermore, the *Ruminococcus* genus is polyphyletic or paraphyletic and its members cluster within two families — the *Ruminococcaceae* and *Lachnospiraceae.* A recent metagenomic study reported that the abundance of *Ruminococcus* spp. is a driver of one of the proposed enterotype status of the microbiota (Arumugam *et al.*, 2011). However, as the present metagenomic analyses do not provide accurate phylogenetic information, it is unclear which of the two distinct groups of *Ruminococcus* spp., is the actual driver of this enterotype status. This example illustrates the need for a systematic and detailed presentation of the microbiota analysis in a phylogenetic framework.

The extensive period of the studying of the gastrointestinal microbiota, its complexity and its variation between individuals have generated a massive amount of information, which is scattered in the literature. To unify the knowledge of the gastrointestinal microbiota that has accumulated since its discovery, we have performed a search of the publications covering more than a century (Fig. 1). We found references that link the human gastrointestinal microbiota with a total of 1057 intestinal species belonging to the *Eukarya* (92), *Archaea* (8) and *Bacteria* (957; Fig. 2, Supporting information, Tables S1–S3). These species were analyzed in ARB software-based database of the SSU rRNA sequences (Pruesse *et al.*, 2007). The phylogenetic trees presented here were extracted from the reference phylogenetic tree of the SILVA database (Yarza *et al.*, 2008). From this phylogenetic and literature analysis, it is clear that bacteria that cluster within the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria,* are the most diverse and abundant microorganisms in the adult gastrointestinal tract (Fig. 2). The gastrointestinal microbiota also contains members of the less diverse, although in some cases still abundant, bacterial phyla, including the *Verrucomicrobia*, *Lentisphaerae*, *Synergistetes, Planctomycetes, Tenericutes* and the *Deinococcus-Thermus* group. In addition to these established phylogenetic groups, the SSU rRNA gene sequences of not yet cultured bacteria that cluster within the TM7 candidate phylum, *Melainabacteria* and *Gemmatimonacetes,* can be detected in the human gastrointestinal tract (Fig. 2). Several archaeal species that cluster within two phyla have been detected in the human gastrointestinal tract. The *Euryarchaeota* include the methanogens that are relatively abundant. Among the *Eukarya* there are organisms that are highly adapted to the human gastrointestinal tract, such as some *Candida* spp., while many other eukaryote species can be present at a low abundance and may be passengers. Altogether, our present analysis confirms that the human gastrointestinal microbiota is composed of representatives of all three domains of life — *Bacteria*, *Archaea,* and *Eukarya*.

The gastrointestinal microbiota research is very dynamic, and in the last decade, 239 novel gastrointestinal tract species have been detected or described, confirming the earlier notion that the majority of the gastrointestinal microorganisms are cultivable but not yet cultured. While traditional cultivation media and strategies are efficient in obtaining novel species within the *Actinobacteria*, *Bacteroidetes*, *Firmicutes,* and *Proteobacteria* phyla, for the detection of the gastrointestinal representatives of the phyla *Verrucomicrobia* and *Lentisphaerae,* the development of specific media and culturing approaches

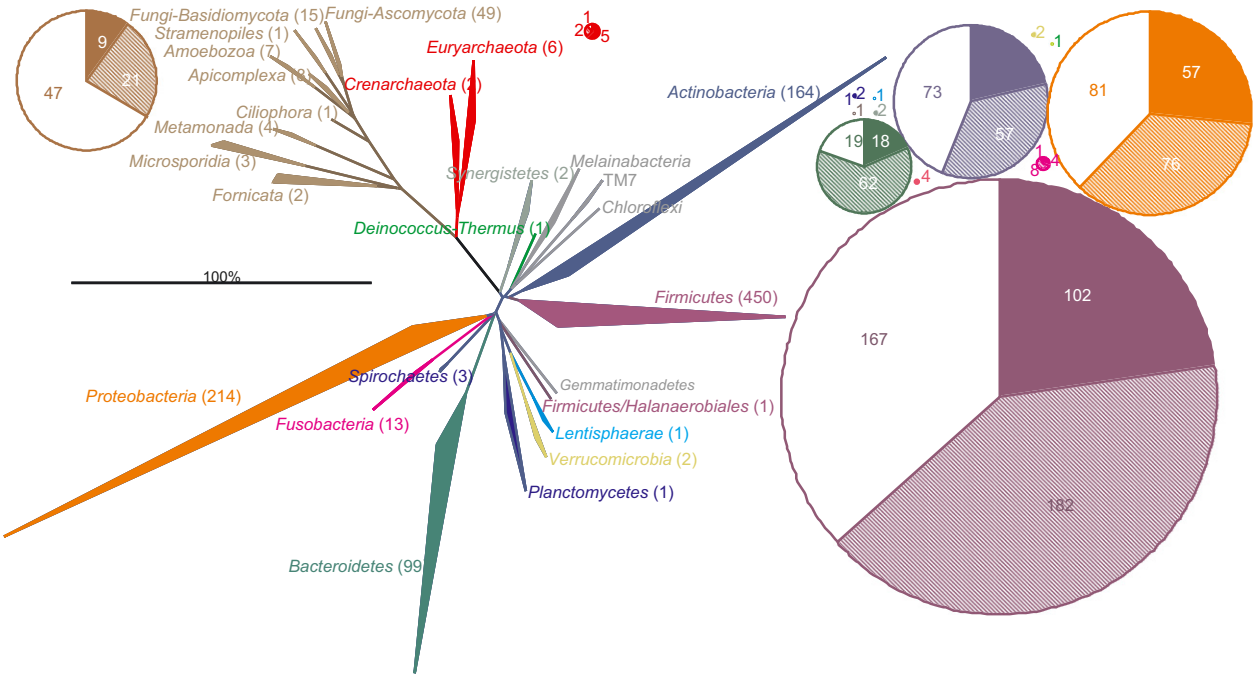

Fig. 2. Phylogenetic tree of the human gastrointestinal microbiota. The numbers in parentheses indicate the number of cultured species given per phylum. The pie charts illustrate distribution between the number of species with full genome sequence genome (full sectors), the number of species with partial genome sequence (semi-full sectors) and number of species without any genome sequence (empty sectors) given for *Archaea*, *Eukarya* and per phylum for *Bacteria*. The color code of pies corresponds to the color code of the phylogenetic tree.

was needed (Zoetendal *et al.*, 2003; Derrien *et al.*, 2004). This suggests that for the cultivation of the gastrointestinal microorganisms belonging to phyla that lack any cultured representatives from the human intestine (e.g., TM7 candidate phylum or the *Oscillospira* genus), alternative and creative cultivation approaches should be developed and applied. Some new and promising developments include the use of high-throughput solid phase growth (Ingham *et al.*, 2007), advanced culturing approaches using gnotobiotic mice (Goodman *et al.*, 2011), or gel microdroplet culturing (Fitzsimons *et al.*, 2013). The use of high-throughput culturing systems that employ a large set of growth media coupled to genomic characterization has proven to be very fruitful (Lagier *et al.*, 2012a; Dubourg *et al.*, 2013; Hamad *et al.*, 2013; Pfleiderer *et al.*, 2013). This recent attention for culturing the gastrointestinal microorganisms reflects the perceived need for detailed physiological, ecological and genetic studies. While a variety of functional metagenomics approaches have been described and applied, it is the integration with culturing approaches that is needed to further advance the understanding of the function of the intestinal ecosystem in health and disease. The power of this combination has recently been illustrated with the example of the abundant mucus-utilizing bacterium, *Akkermansia muciniphila* as a paradigm (Belzer & de Vos, 2012). Currently, the complete genome of at least one strain of 225 gastrointestinal species has been fully sequenced, assembled, and published, while many other genomic sequencing projects are ongoing (Fig. 2, Tables S1–S3). The physiological and genetic characteristics of these currently recognized gastrointestinal species and their association with particular functions of the ecosystem or diseases are systematized in this review that aims to provide the basis for future comparative and functional metagenomic and other high-throughput approaches applied on the gastrointestinal microbiota.

Actinobacteria

Actinobacteria are common and abundant in the human gastrointestinal tract. They are also known as gram-positive bacteria with a high G + C content in their DNA. As they are particularly difficult to lyse and their SSU RNA needs specific PCR primers to be amplified (Satokari *et al.*, 2001), this group of bacteria is often underrepresented in molecular surveys of the gastrointestinal microbiota (notably in one of the first global studies of the infants' microbiota; Palmer *et al.*, 2007). Members of the orders *Bifidobacteriales* (in particular *Bifidobacterium* spp.) and *Coriobacteriales* (mainly *Collinsella* spp.) are highly prevalent already since early life, while members of the

order *Actinobacteriales* are human-associated bacteria that are subabundant and only scarcely detected in the gastrointestinal tract.

Actinobacteria-Bifidobacteriales

Bifidobacterium spp. form a dominant fraction of the human gastrointestinal microbiota, particularly in infants (Benno *et al.*, 1984). Bifidobacteria are present in the abundance ranging between 10^8 and 10^{10} cells g^{-1} of intestinal content (Finegold *et al.*, 1974; Moore & Holdeman, 1974a; Tannock, 1995). The majority of *Bifidobacterium* spp. have been recovered exclusively from human or animal gastrointestinal samples, and for two species (*B. minimum* and *B. subtile*) that were isolated from sewage (Scardovi & Trovatelli, 1974), an intestinal origin can be suspected, showing the high adaption of this genus to the gastrointestinal tract. Phylogenetically, *Bifidobacterium* spp. form a homogenous group, with 20 cultured species linked to the human gastrointestinal tract (Fig. 3). The first *Bifidobacterium* spp. was recovered from infant feces in 1900 by Henri Tissier, as a part of his PhD thesis work (Tissier, 1900). It was named *Bacillus bifidus-communis.* Already in 1924, this bacterium was renamed to *Bifidobacterium bifidum*, but *Bifidobacterium* was not recognized as an independent genus until 1974 (Biavati *et al.*, 2000). The members of the *Bifidobacterium* genus are nonmotile, anaerobic or microaerophilic bacteria that produce acetate and lactate as major fermentation products from sugars. The degradation of sugars by these bacteria is

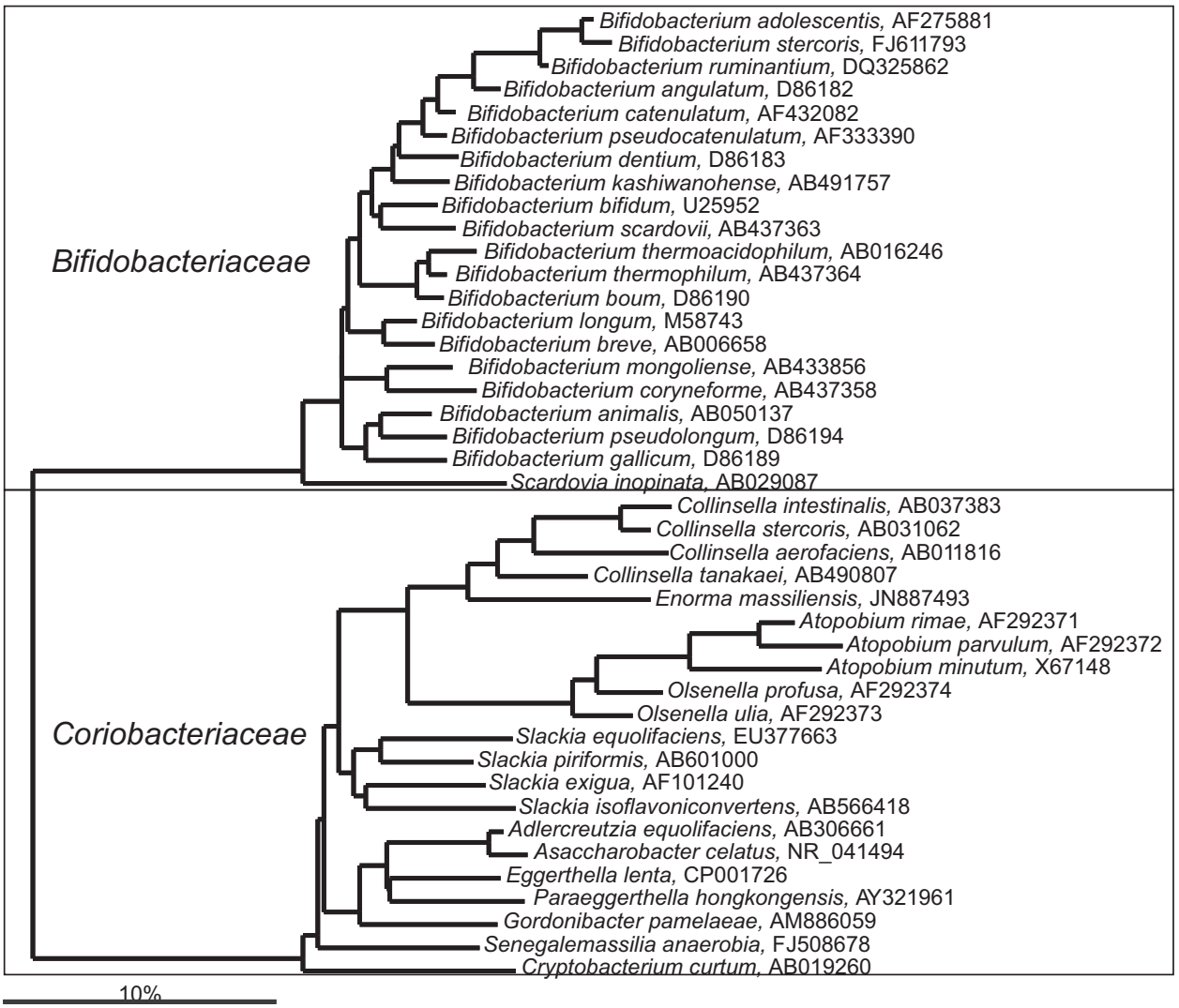

Fig. 3. Phylogenetic tree of the human gastrointestinal species that belong to the orders of the *Bifidobacteriales and Coriobacteriales*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

performed through a phosphoketolase pathway, also known as the Bifido shunt. *Bifidobacterium* spp. degrade monosaccharides, galacto-, manno-, and fructo-oligosaccharides, while some strains are able to ferment complex carbohydrates such as starch, arabinogalactan, arabic gum, and gastric mucin (Crociani *et al.*, 1994). As mother milk's contains nondigestible oligosaccharides that can be degraded by *Bifidobacterium* spp. (Marcobal *et al.*, 2011), this bacterial group is strongly stimulated in breast-fed infants resulting in the dominance of *Bifidobacterium* spp. in the gastrointestinal microbiota before weaning. The *Bifidobacterium* spp. are assumed to have a beneficial effect on health (Mitsuoka, 1990) and several members of the *Bifidobacterium* genus are commercially applied as probiotics. The most relevant observation is that these bacteria have decreased abundance in relation to a number of diseases including vitamin K deficiency (Benno *et al.*, 1985), atopic diseases (Kalliomaki *et al.*, 2001), irritable bowel syndrome (Kerckhoffs *et al.*, 2009; Rajilić-Stojanović *et al.*, 2011), and autism (Wang *et al.*, 2011a). Moreover, *Bifidobacterium* spp. represent a very stable component of the gastrointestinal microbiota of each person, the composition of which hardly changes throughout years (Rajilić-Stojanović *et al.*, 2013b). Remarkably, a recent study of the microbiota of the Hazda tribe from Tanzania suggested that these adult hunter-gatherers do not carry any *Bifidobacterium* spp., which was explained by absence of dietary components such as meat and dairy that could support growth of these bacteria (Schnorr *et al.*, 2014).

Actinobacteria-Coriobacteriales

Coriobacteriales species constitute a frequently detected group of the gastrointestinal microbiota composed of representatives of 12 different genera (Fig. 3). *Collinsella* is the most dominant among other members of the order, and a representative of these rod-shaped, nonmotile obligate anaerobes was for the first time detected in human feces in 1935 (Eggerth, 1935). Both cultivation- and molecular-based studies show that *Collinsella aerofaciens* is a prevalent and an abundant gastrointestinal microorganism (Moore & Holdeman, 1974a; Benno *et al.*, 1986; Kageyama *et al.*, 2000). Four different types of *Collinsella aerofaciens* were initially recognized and later reclassified into distinct species (Kageyama & Benno, 2000). *Collinsella* spp. can ferment a wide range of different carbohydrates including complex sugars, such as starch but also glycogen to produce hydrogen gas, ethanol, formate, and lactate (Eggerth, 1935; Kageyama *et al.*, 1999a). Experiments with an *in vitro* model of the human colon showed that *Collinsella* spp. along with *Bifidobacterium* spp. are the major lactose utilizers in the human gastrointestinal microbiota (Kovatcheva-Datchary, 2010). Moreover, *Collinsella* spp. are capable of deconjugation of bile acids and their abundance shows significant positive correlation with plasma cholesterol levels (Lahti *et al.*, 2013).

Eggerthella are assacharolytic bacteria that produce acids only from glucose, but not from other sugars. The first representative of this bacterial group was isolated in 1935 by Arnold Eggerth (Eggerth, 1935). These bacteria produce formate and lactate. Until now, only *Eggerthella lenta* and the still not fully characterized *Eggerthella* sp. YY7918 are associated with the human gastrointestinal tract. *Eggerthella lenta* has been implied in producing anti-tumor substances that stimulate natural killer cells (Hatta, 1995), while *Eggerthella* sp. YY7918 has been reported to produce s-equol (Yokoyama & Suzuki, 2008), which has anticarcinogenic properties (Yuan *et al.*, 2007).

Slackia spp. are asaccharolytic bacteria with the common feature of converting dietary isoflavones. These isoflavones have been proposed to prevent hormone-dependent diseases, while their conversion by gastrointestinal bacteria impacts their biological effectiveness. Among the bacterial products, s-equol appears to be the most relevant to human physiology (Yuan *et al.*, 2007). At least two *Slackia* spp. are capable of equol production from isoflavones (Matthies *et al.*, 2009; Jin *et al.*, 2010), while *Adlercreutzia equolifaciens* that also belongs to the *Coriobacteriales* order, is another gastrointestinal species capable to produce equol (Maruo *et al.*, 2008). The ability to produce s-equol is more abundant among microbiota of Asian than the Caucasian subjects (Song *et al.*, 2006) and can be explained by the adaptation of the microbiota to the higher availability of isoflavones — particularly those derived from soy beans.

Atopobium species are anaerobic bacteria that cluster within the *Actionobacteria* phylum, and, in contrast to the rest of the phylum, contain DNA with a low G + C content. The main product of their metabolism is lactate, which is in line with the previous classification of these bacteria within the *Lactobacillus* and *Streptococcus* genera (Collins & Wallbanks, 1992). Based on the literature data, it can be concluded that *Atopobium* spp. are among the earliest colonizers of the human intestinal tract as they are reported to be present in gastrointestinal contents of 6-week-old infants (Fallani *et al.*, 2011). However, the data on *Atopobium* quantification are based on the application of a FISH probe for the *Atopobium* cluster, which in addition to *Atopobium*, hybridizes to species that belong to the *Coriobacterium, Eggerthella* and *Collinsella* genera (Harmsen *et al.*, 2000). Therefore, it is not clear if the *Atopobium* or the other targeted genera are colonizing the gastrointestinal tract of infants. Bacteria belonging to the *Atopobium* cluster are significantly associated with the major products of protein fermentation, suggesting that

these bacteria are responsible for protein degradation in the gastrointestinal tract (Shen *et al.*, 2010; Thompson-Chagoyan *et al.*, 2011).

Actinobacteria-Actinomycetales

The *Actinobacteria* of the human gastrointestinal tract include diverse members of the order *Actinomycetales* (Figs 4 and 5 — for clarity the phylogenetic tree of this numerous order was split into two parts). These bacteria are rarely detected in human gastrointestinal samples, but this is most likely due to their low abundance in the gastrointestinal tract that is in the range from 10^2 to 10^3 cells g^{-1} of feces (Hoyles *et al.*, 2012). Their low abundance can explain the fact that many representatives of this group were detected only in studies that specifically targeted this group of bacteria (Hoyles *et al.*, 2013), or in studies that targeted low abundant bacteria within the gastrointestinal microbiota (Lagier *et al.*, 2012a; Dubourg *et al.*, 2013). Various different *Actinomycetales* species, of which many are still uncultured, were identified in a molecular study of these specific subcommunity within the gastrointestinal microbiota of healthy humans of different ages, showing a high prevalence of these bacteria (Hoyles *et al.*, 2013). The most diverse and the frequently detected *Actinomycetales* of the human gastrointestinal tract include *Propionibacterium* spp. and *Corynebacterium* spp. (Fig. 4). These bacteria typically colonize the human skin and are found in high abundance in infants that are born using Caesarean section

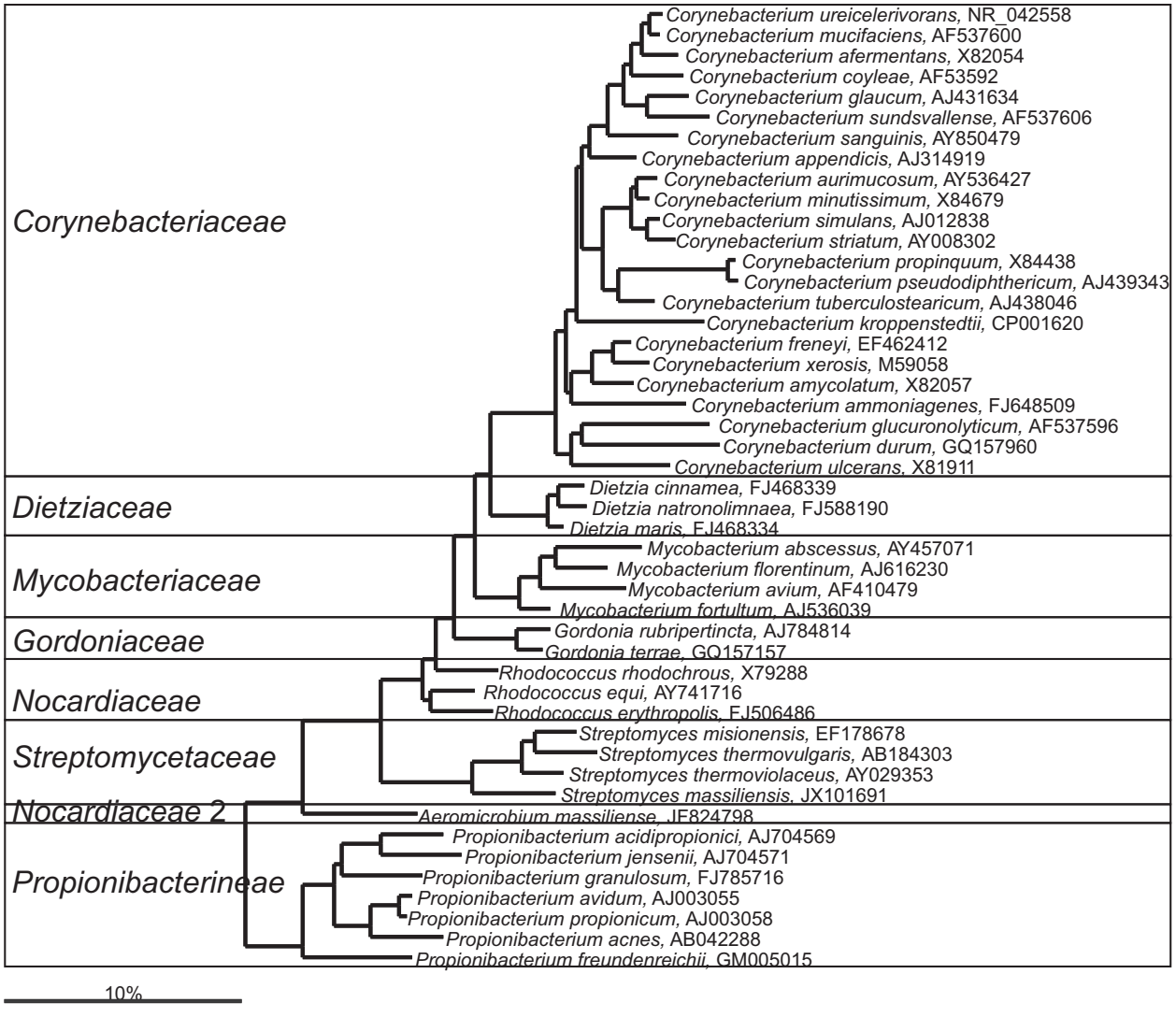

Fig. 4. Phylogenetic tree of a fraction of the human gastrointestinal species that belong to the order of the *Actinomycetales*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. For the other human gastrointestinal species that cluster within the *Actinomycetales* see Fig. 5.

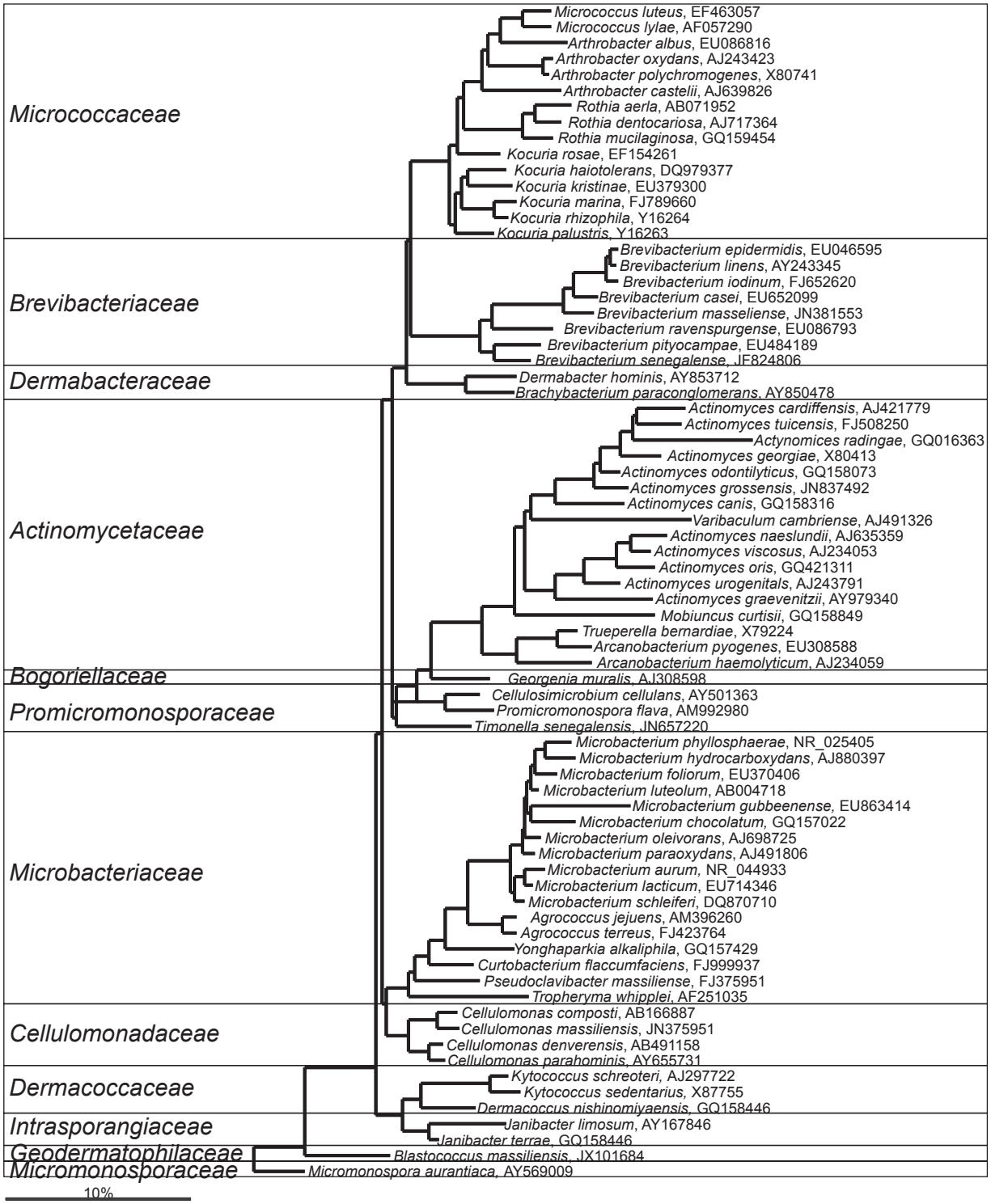

Fig. 5. Phylogenetic tree of a fraction of the human gastrointestinal species that belong to the order of the *Actinomycetales.* GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. For the other human gastrointestinal species that cluster within the *Actinomycetales* see Fig. 4.

(Dominguez-Bello *et al.*, 2010). In adults, *Corynebacterium* spp. are more frequently detected in the samples of the upper gastrointestinal tract (Justesen *et al.*, 1984). Cultivation studies have indicated that *Propionibacterium* spp. are the major proteolytic bacteria in the human intestine (Macfarlane *et al.*, 1986). *Propionibacterium* spp. are applied as probiotics as they are major vitamin B_{12} producers and produce propionate from lactate. *In vitro* experiments showed that the metabolic products of two gastrointestinal *Propionibacterium* spp. can induce apoptosis of colorectal carcinoma cells (Jan *et al.*, 2002).

Bacteria belonging to the *Rhodococcus* genus are rarely detected in the human gastrointestinal microbiota but have been found in an extreme abundance (up to 68%) in mucosal biopsies of ulcerative colitis patients (Lepage *et al.*, 2011). *Mycobacterium* spp., notably *Mycobacterium avium*, have also been implied in ulcerative colitis and the expression of intestinal cells in inflammatory bowel disease patients appears to have similarities to *Mycobacterium* infection (Sibartie *et al.*, 2010).

The gastrointestinal *Actinomycetales* also include *Rothia* spp. that are frequently detected in the upper gastrointestinal tract (Ou *et al.*, 2009), but rarely in fecal samples. These species contribute to the degradation of gluten (Zamakhchari *et al.*, 2011) and their abundance and activity might be relevant for celiac disease and other conditions related to gluten digestion. Another species of this order — *Actinomyces graevenitzii* — which is detected in an increased abundance in the small intestine of celiac disease patients, might be a relevant risk factor for the development of this disease (Ou *et al.*, 2009). Various other *Actinomyces* spp. can be detected in low abundance in the fecal samples of healthy humans (Hoyles *et al.*, 2012; Hoyles *et al.*, 2013), and while the role of these bacteria in the gastrointestinal tract is still to be determined, it is noteworthy that an *Actinomyces* spp. was detected as colonizer of the infant gastrointestinal tract using sensitive molecular methods already in the first days of life (Favier *et al.*, 2002).

Micrococcus spp. are relatively prevalent (present in 20% of the analyzed subjects) in the samples of the upper gastrointestinal tract in patients predisposed to the development of the small intestinal bacterial overgrowth syndrome (Bouhnik *et al.*, 1999). Although representatives of this genus can be detected in the fecal samples (Finegold *et al.*, 1974), these bacteria typically inhabit human skin. Similar applies to six *Kocuria* spp., which are human skin and oropharynx mucosa commensals (Savini *et al.*, 2010), although two recent studies have reported presence of *Kocuria* spp. in gastrointestinal samples (Lagier *et al.*, 2012a; Fitzsimons *et al.*, 2013).

The other members of the *Actinomycetales* order include the representative species of the following genera: *Brevibacterium, Cellulomonas,* and *Microbacterium*. These genera are typically associated with other ecosystems, namely the skin (*Brevibacterium*), and soil (*Cellulomonas* and *Microbacterium*). Nevertheless, most of these bacteria are already recognized as relevant for human health, as many of these species can cause infections of different tissues, particularly in immuno-suppressed patients (Funke *et al.*, 1997). It has been suggested that gastrointestinal tract represents the natural niche of these bacteria (Funke *et al.*, 1997).

Bacteroidetes

The Gram-negative bacteria that belong to the phylum *Bacteroidetes* are common, abundant and diverse within the human gastrointestinal tract. The first *Bacteroides* species — *Bacteroides fragilis* — was isolated in 1898 as a human pathogen linked to appendicitis among other clinical cases (Veillon & Zuber, 1898). Although some *Bacteroides* spp. are still considered to be opportunistic pathogens, several decades of research have testified that many *Bacteroidetes* species are highly adjusted to the gastrointestinal tract, where they live in high abundance (up to 10^{11} cells g^{-1} of intestinal material; Eggerth & Gagnon, 1933; Moore & Holdeman, 1974a; Benno *et al.*, 1986). Hence, they perform metabolic conversions that are essential for the host, often related to the degradation of proteins or complex sugar polymers. The colonization of the gastrointestinal tract with the *Bacteroidetes* is promoted already in infants, as mother milk's nondigestible oligosaccharides support the growth of both *Bacteroides* and *Bifidobacterium* spp. (Marcobal *et al.*, 2011). Furthermore, animal model experiments have shown that the colonization of the normal gastrointestinal tract, as illustrated by experiments with pure cultures of *Bacteroides* spp., is a result of the recognition and selection by the immune system of the host (Rakoff-Nahoum *et al.*, 2004), mediated through the toll-like receptors (Round *et al.*, 2011; Lopez-Siles *et al.*, 2012) and other specific host-microorganism interactions (Hooper *et al.*, 2012).

For a long time, it was thought that the majority of Gram-negative gastrointestinal tract bacteria belonged to the *Bacteroides* genus, but in recent years many earlier designed *Bacteroides* spp. were assigned to other genera within the *Bacteroidetes* phylum. Currently, only four gastrointestinal *Bacteroides* spp. form deep branches in the phylogenetic tree (Fig. 6), suggesting that these bacteria (*B. ureolyticus, B. galacturonicus, B. pectinophilus,* and *B. coagulans*) still should be reclassified to other phylogenetic groups. A similar situation applies to *Anaerorhabdus furcosa*, which is still classified as a member of the *Bacteroidaceae* family, but based on its SSU rRNA gene sequence clusters within the *Firmicutes* phylum. The majority of the gastrointestinal *Bacteroidetes* spp. belongs

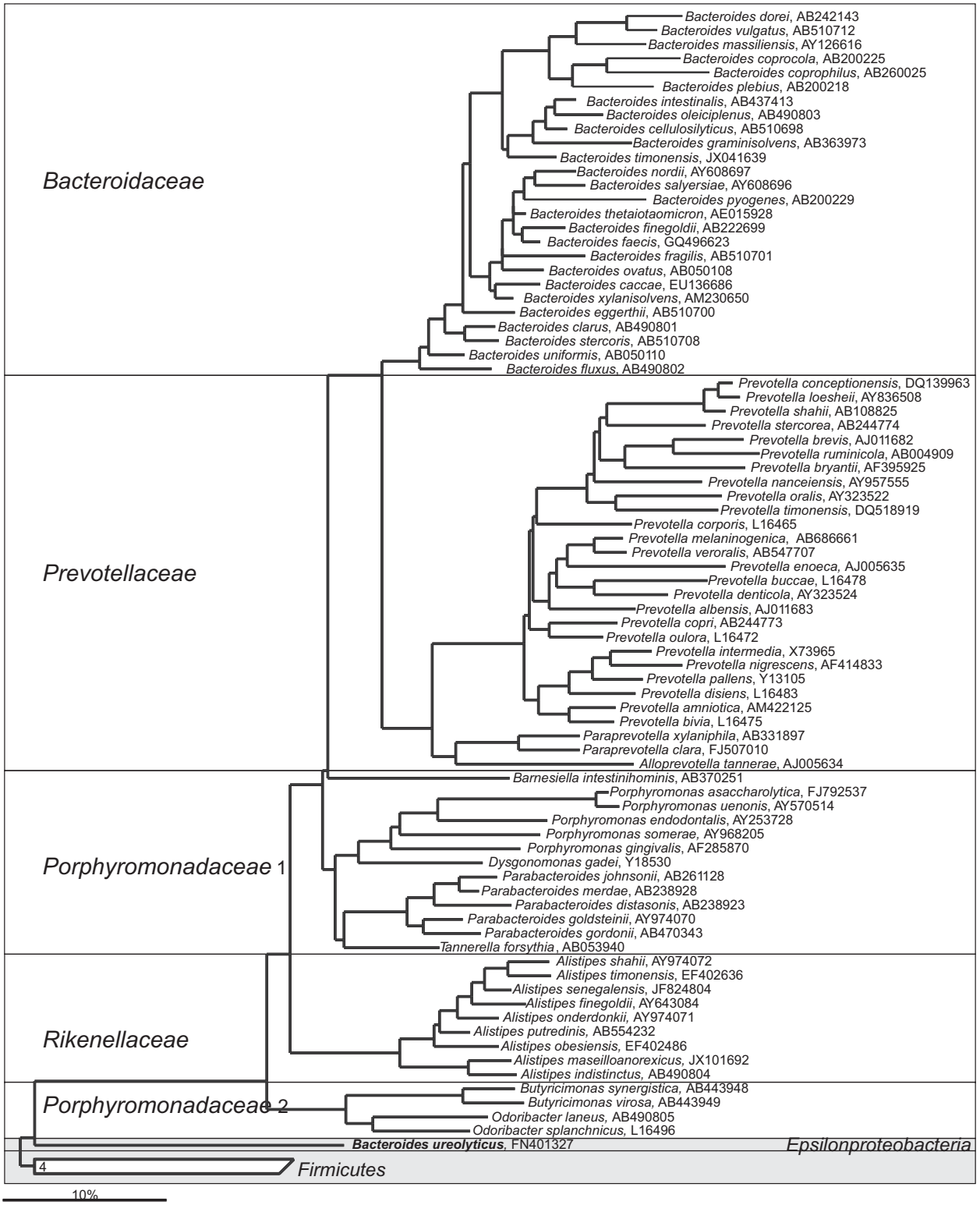

Fig. 6. Phylogenetic tree of the human gastrointestinal species that belong to the class of the *Bacteroidia*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. Deeply rooted *Bacteroides* spp., which based on the SSU rRNA gene sequence cluster within distant phylogenetic groups are depicted in the gray area.

to the following bacterial families: *Bacteroidaceae*, *Prevotellaceae*, *Rikenellaceae*, and *Porphyromonadaceae* (Fig. 6). These bacterial species share the common feature that they produce succinic acid, acetic acid, and in some cases propionic acid, as the major end-products. Species belonging to the genera *Alistipes, Bacteroides, Parabacteroides, Prevotella, Paraprevotella, Alloprevotella, Barnesiella,* and *Tannerella* are saccharolytic, while species belonging to *Odoribacter* and *Porphyromonas* are predominantly asaccharolytic. Some *Bacteroides* spp. and *Prevotella* spp. can degrade complex plant polysaccharides such as starch, cellulose, xylans, and pectins (Wu *et al.*, 1992; Morotomi *et al.*, 2009; Sakamoto & Ohkuma, 2012). The *Bacteroidetes* species play also an important role in protein metabolism, as some species have proteolytic activity, assigned to the proteases that are linked to the cell wall (Macfarlane *et al.*, 1986; Macfarlane *et al.*, 1988), while some *Bacteroides* spp. have a potential to utilize urea as a nitrogen source (Yatsunenko *et al.*, 2012). Other important functions of *Bacteroides* spp. include the deconjugation of bile acids (Narushima *et al.*, 2006) and growth on mucus (Leitch *et al.*, 2007). The *Bacteroidetes* contribute to the recently proposed classification of the gastrointestinal microbiota into enterotypes (Arumugam *et al.*, 2011). The importance of the *Bacteroidetes* is further illustrated by the fact that this group is the most stable component of the gastrointestinal microbiota over time in healthy adults (Rajilić-Stojanović *et al.*, 2013b). Anecdotally, a unique case report described the microbiota of a critically ill patient that harbored no *Bacteroidetes* — this patient passed away soon after sampling (Dubourg *et al.*, 2013).

Because of their broad metabolic potential, the role of the *Bacteroidetes* in the gastrointestinal microbiota is complex: while the reduced abundance of the *Bacteroidetes* in some cases is associated with obesity (Ley, 2010) and irritable bowel syndrome (Rajilić-Stojanović *et al.*, 2011), this bacterial group appears to be enriched in patients suffering from type 1 and type 2 diabetes (Larsen *et al.*, 2010). Moreover, *Bacteroides* spp. in contrast to *Prevotella* spp. were recently found to be enriched in the metagenomes of subjects with low gene richness that were associated with adiposity, insulin resistance and dyslipidaemia as well as an inflammatory phenotype (Le Chatelier *et al.*, 2013).

Bacteroidetes species that belong to classes *Flavobacteriales* and *Sphingobacteriales* are only occasionally detected in the gastrointestinal tract (Fig. 7, Table S1). With an exception of *Capnocytophaga* spp. and *Sphingobacterium* spp. that can be detected in the human oral cavity, the other bacteria of this group are typically associated with other ecosystems (primarily soil). There is no data about the role of these bacteria in the gastrointestinal microbiota, but it is noteworthy that several of these bacteria were detected only in the SSU rRNA gene clone libraries of the microbiota of inflammatory bowel disease patients (Frank *et al.*, 2007).

Firmicutes

Firmicutes are the most diverse and abundant group of the gastrointestinal microbiota, making up over half and in many cases around 80% of the gastrointestinal microbiota of healthy adults. The gastrointestinal *Firmicutes* are distributed over four classes: *Bacilli, Clostridia, Erysipelotrichi,* and *Negativicutes*. Traditionally, this group is considered to include Gram-positive bacteria with a low GC

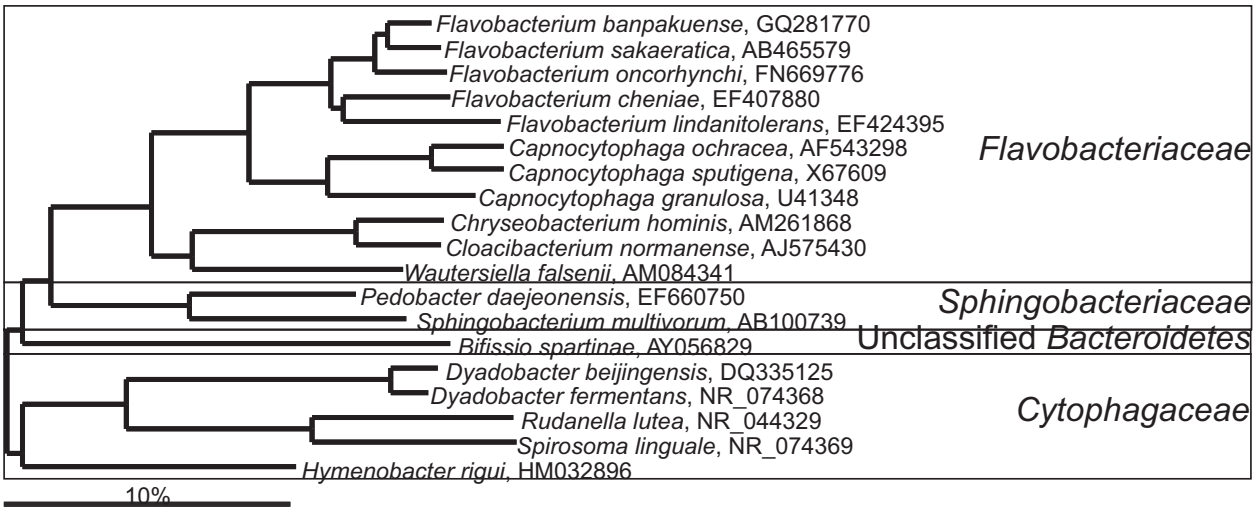

Fig. 7. Phylogenetic tree of the human gastrointestinal species that belong to the classes of the *Cytophagia* and *Sphingobacteria*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

content in their DNA, although recent studies have shown that Gram-positive staining is not a feature of many *Firmicutes*. This can be illustrated with *Faecalibacterium prausnitzii*, which is a Gram-negative-staining bacterium, previously classified within the *Fusobacteria* phylum (Duncan *et al.*, 2002a), novel gastrointestinal isolates such as *Christensenella minuta* (Morotomi *et al.*, 2012), but also typical Gram-negative bacteria such as members of the *Veillonellacea* family (Marchandin *et al.*, 2010). While the vast majority of the *Firmicutes* are indeed low GC content bacteria, this also, is not a common feature of the phylum as seen in an example of *Anaerofustis stercorihominis*, which DNA has a content of the GC of around 70% (Finegold *et al.*, 2004). Most of the *Firmicutes*, notably the *Clostridium* spp. and *Bacillus* spp., are spore-formers and this property confers special survival value in and beyond the gastrointestinal tract.

The most abundant gastrointestinal microorganisms are members of the class *Clostridia* and within this class the families *Ruminococcaceae* and *Lachnospiraceae* (Tap *et al.*, 2009; Jalanka-Tuovinen *et al.*, 2011). Another diverse group of the *Firmicutes* is the class *Bacilli* that includes the genera of *Lactobacillus*, *Enterococcus,* and *Streptococcus,* which are dominant in the upper part of the gastrointestinal tract. In line with its enormous diversity, the *Firmicutes* in the gastrointestinal tract perform a number of different functions that stretch from health promoting of some probiotic *Lactobacillus* spp. to pathogenic properties of *Clostridium difficile*. The vast majority of the currently uncultured gastrointestinal inhabitants belong to the phylum *Firmicutes* (Rajilić-Stojanović *et al.*, 2007), which illustrates that future research is expected to dramatically expand our knowledge about the functional contribution of this group to the ecosystem and the host.

Bacilli

The first representative of the *Bacilli* class retrieved from the gastrointestinal tract was a member of *Lactobacillales* order and was isolated in 1900 — *Bacillus acidophilus* (Moro, 1900). The description of this species is vague, based on the currently accepted standards, and as the original strain was lost, it is not clear if this species is *Lactobacillus acidophilus* or one of the other five species derived from the so-called *L. acidophilus* group (Mitsuoka, 1992). Lactobacilli comprise a group of gastrointestinal inhabitants that has received particular scientific attention (Tannock, 2004), mainly because of the health claims proposed by Metchnikoff (1908) in the beginning of the nineteenth century and their later application as probiotics. Although highly important for the health, Lactobacilli are rarely detected as markers of the gastrointestinal microbiota dysbiosis, but such reports exist and include a reduced abundance in patients suffering from inflammatory bowel disease (Keighley *et al.*, 1978; Ott *et al.*, 2004), type 1 diabetes (Murri *et al.*, 2013). This might be related to the fact that lactobacilli are only a minor fraction of the fecal microbiota where they can reach counts of up to 10^8 cells g^{-1} (Simon & Gorbach, 1984), and most of the analysis of the gastrointestinal microbiota is based on the use of stool samples. In the small intestine *Lactobacillus* spp. represent one of the predominant groups obtained by culturing (Reuter, 2001). However, while molecular studies could confirm their presence in the upper intestinal tract, these also showed that the Lactobacilli are quite variable and not as abundant as other gastrointestinal genera at that location, such as *Streptococcus* and *Veillonella* (Booijink, 2009; Booijink *et al.*, 2010). This may explain why *Lactobacillus* spp. should be part of the diet, as consumed probiotic strains of *Lactobacillus* spp. have a beneficial effect on human health and specific induction of gene expression has been observed in duodenal biopsies after exposure of *Lactobacillus plantarum* (van Baarlen *et al.*, 2009). Specific media, developed already in the 1950s (Rogosa *et al.*, 1951), enabled the isolation of numerous *Lactobacillus* spp. Nevertheless, new *Lactobacillus* spp. from human gastrointestinal tract are still being reported (Roos *et al.*, 2005; Oki *et al.*, 2012), indicating that even the 38 known gastrointestinal *Lactobacillus* sp. (Fig. 8) are not covering the group's full diversity. Several previously misclassified *Lactobacillus* spp. have now been reclassified into novel genera, including *Weissella, Atopobium*, *Eggerthia,* and *Kandleria* (Collins & Wallbanks, 1992; Bjorkroth *et al.*, 2002; Salvetti *et al.*, 2011). Currently, only *Lactobacillus rogosae* is strongly outgrouping from the remaining *Lactobacillus* spp., although even after exclusion of the strongly outgrouping species, the species show a large degree of the SSU rRNA gene variation and form several groups in the phylogenetic tree (Fig. 8) As *Lactobacillus* spp. produce lactic acid as the major fermentation production that can be accompanied with ethanol and carbon dioxide in some species and under some conditions, traditionally *Lactobacillus* spp. are classified into three groups: obligately homofermentative, facultatively homofermentative, and obligately heterofermentative. However, the phylogenetic position of the species does not seem to be related to their fermentation profile.

In addition to *Lactobacillus* spp., other related, lactic acid bacteria can be detected in the gastrointestinal tract. Members of the genera *Leuconostoc* and *Weissella* used to be considered as occasional and possibly transient members of the gastrointestinal microbiota. However, a recent study showed that *Leuconostoc* spp. and *Weissella* spp. are abundant (representing up to 24% of total microbial community) and widely distributed in colonic mucosa

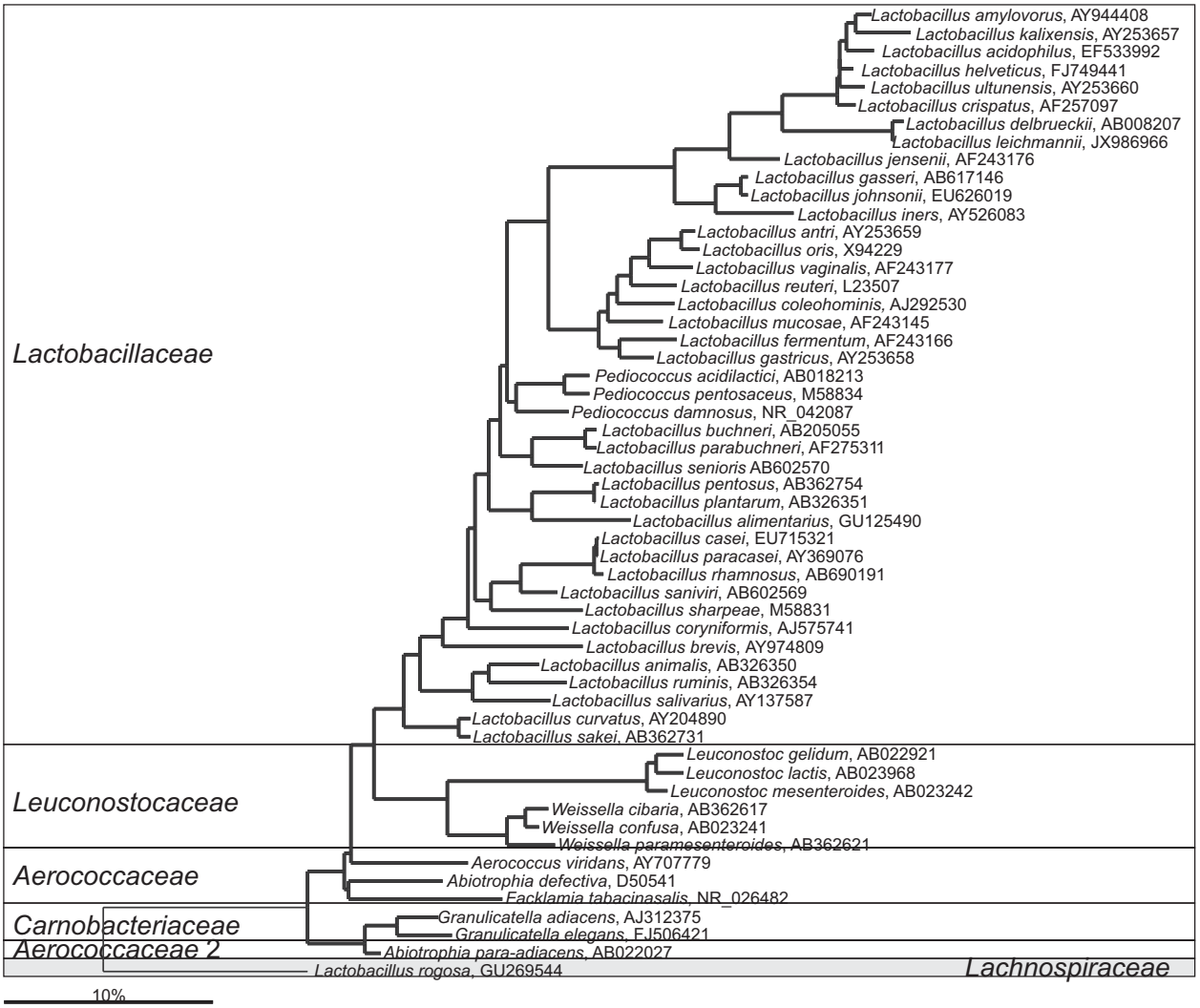

Fig. 8. Phylogenetic tree of a fraction of the human gastrointestinal species that belong to the order of the *Lactobacillales*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. For the other gastrointestinal species that belong to the order of the *Lactobacillales* see Fig. 9.

after bowel cleansing (Hong *et al.*, 2011). Moreover, *Leuconostoc* was identified as the most abundant bacterial genus, representing almost a quarter of the total microbial community in a group of meconium samples of newborns, which persisted in the gastrointestinal tract until 7 months of age (Gosalbes *et al.*, 2013). In the same study, *Weissella* spp. were also detected as the earliest colonizers of the gastrointestinal tract of some newborns. These bacteria utilize simple sugars and their presence in the lower parts of the gastrointestinal tract is dependent on the activity of other gastrointestinal microorganisms that have the ability to degrade complex sugars, resistant to human digestive enzymes.

Other relevant gastrointestinal bacteria belonging to the *Lactobacillales* order include members of the genera *Streptococcus* and *Enterococcus*. These two genera have only recently been separated, although the presence of the subgroup within the genus *Streptococcus* was noticed as late as in the 1930s (Sherman, 1938). They are one of the dominant bacterial fractions in the upper part of the small intestine (Simon & Gorbach, 1986; Reuter, 2001). Forty-six species of these two genera are known to be gastrointestinal inhabitants (Fig. 9). In addition, *Streptococcus pleomorphus*, which also can be part of the gastrointestinal microbiota, forms a deep branch in the SSU rRNA gene sequence–based phylogenetic tree, suggesting that this species should be reclassified into another genus within the *Erysipelotrichaceae* family. The ample presence of the *Enterococcus* and *Streptococcus* spp. can be explained by the fact that the species are oxygen

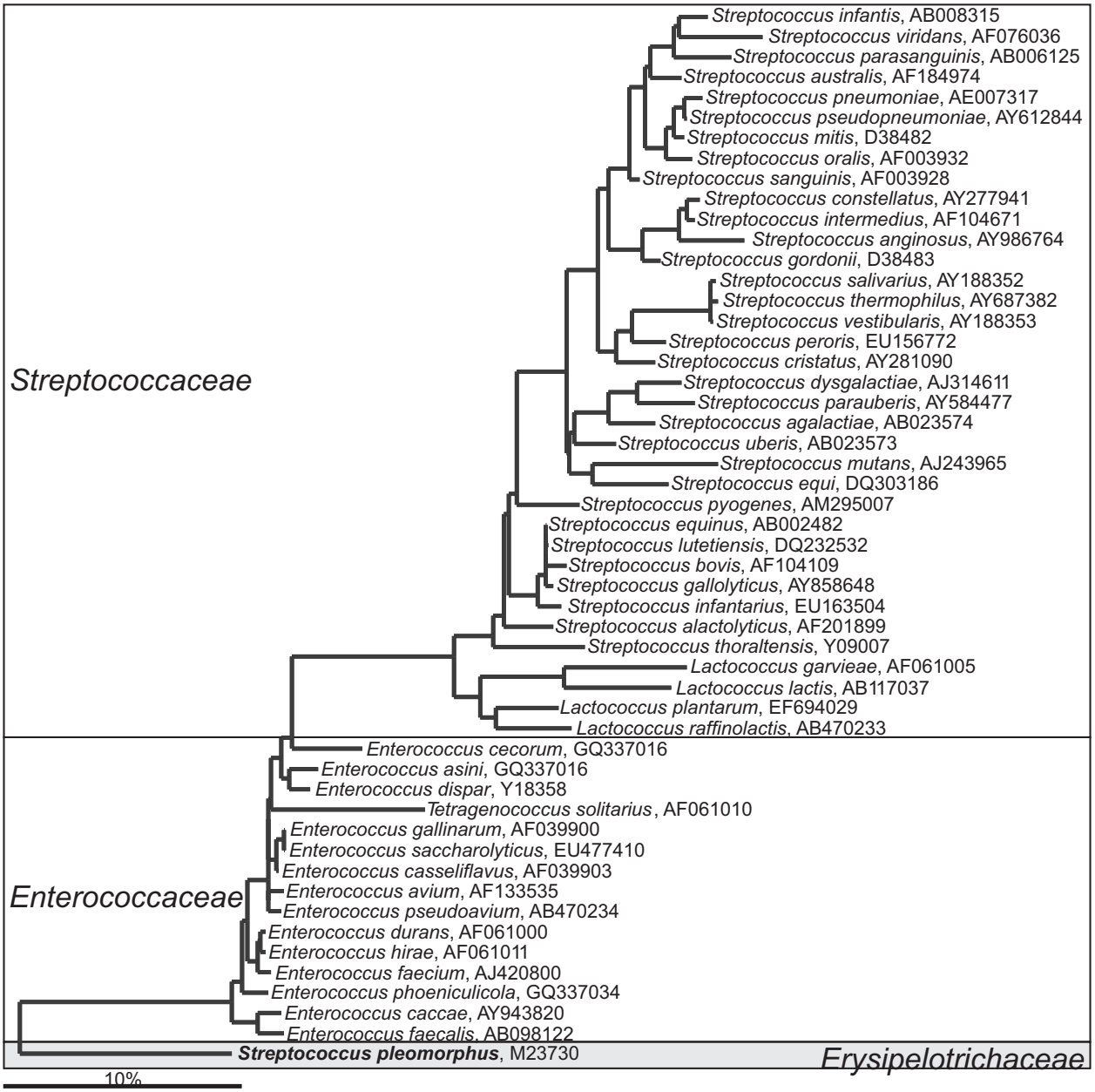

Fig. 9. Phylogenetic tree of the human gastrointestinal species that belong to the families of *Streptococcaceae* and *Enterococcaceae*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. For the other gastrointestinal species that belong to the order of the *Lactobacillales* see Fig. 8.

tolerant and easily cultivable. The oldest isolate of the group, *Enterococcus faecalis*, was for the first time plated in 1899 from a case of endocarditis (MacCallum & Hastings, 1899), and only 7 years later it was recovered from intestinal samples (Andrewes & Horder, 1906). Both *Streptococcus* and *Enterococcus* spp. are among the first established species in the infant's gastrointestinal tract that can be detected already in the first day of life (Solís *et al.*, 2010; Gosalbes *et al.*, 2013). Although this early presence would suggest an important role in the ecosystem, the data on the role of *Streptococcus* and *Enterococcus* spp. in human health are conflicting. *Enterococcus* spp. are widely recognized as opportunistic pathogens, although these species are common, and can even exhibit probiotic properties (Ó Cuív *et al.*, 2013). The abundance of a *Streptococcus* species is decreased in mucosal biopsies in Crohn's disease patients (Li *et al.*, 2012), while *Streptococcus* and *Enterococcus* phylotypes are found to be

increased in fecal samples of colorectal cancer patients (Wang *et al.*, 2012). However, in the adult gastrointestinal tract, *Streptococcus* spp. are particularly abundant in the upper part of the gastrointestinal tract, where they are active in the process of simple sugar fermentation into lactate (Zoetendal *et al.*, 2012). Moreover, they may form a tropic chain with the equally abundant *Veillonella* spp. that convert the produced lactate into propionate (Zoetendal *et al.*, 2012).

Various members of the *Bacillales* order can be low-level constituents of the human gastrointestinal microbiota (Figs 10 and 11 — for clarity the phylogenetic tree of this numerous order was split into two parts). Among them, a large number of *Staphylococcus* spp., which typically are associated with the human skin, can be detected in the human gastrointestinal tract (Fig. 11). These bacteria are one of the earliest colonizers of the gastrointestinal tract, particularly in infants that were delivered by cesarean section (Dominguez-Bello *et al.*, 2010). The predominant early colonization with *Staphylococcus* spp. is, however, coupled with several health risks, as it induces strong stimulation of the immune system, which can be a trigger for the development of asthma and rhinitis in later childhood (Johansson *et al.*, 2012). Furthermore, predominant colonization of the gastrointestinal tract of premature infants with *Staphylococcus* spp. is associated with fatal sepsis (Madan *et al.*, 2012). An increased abundance of bacteria belonging the *Staphylococcus* genus, both in

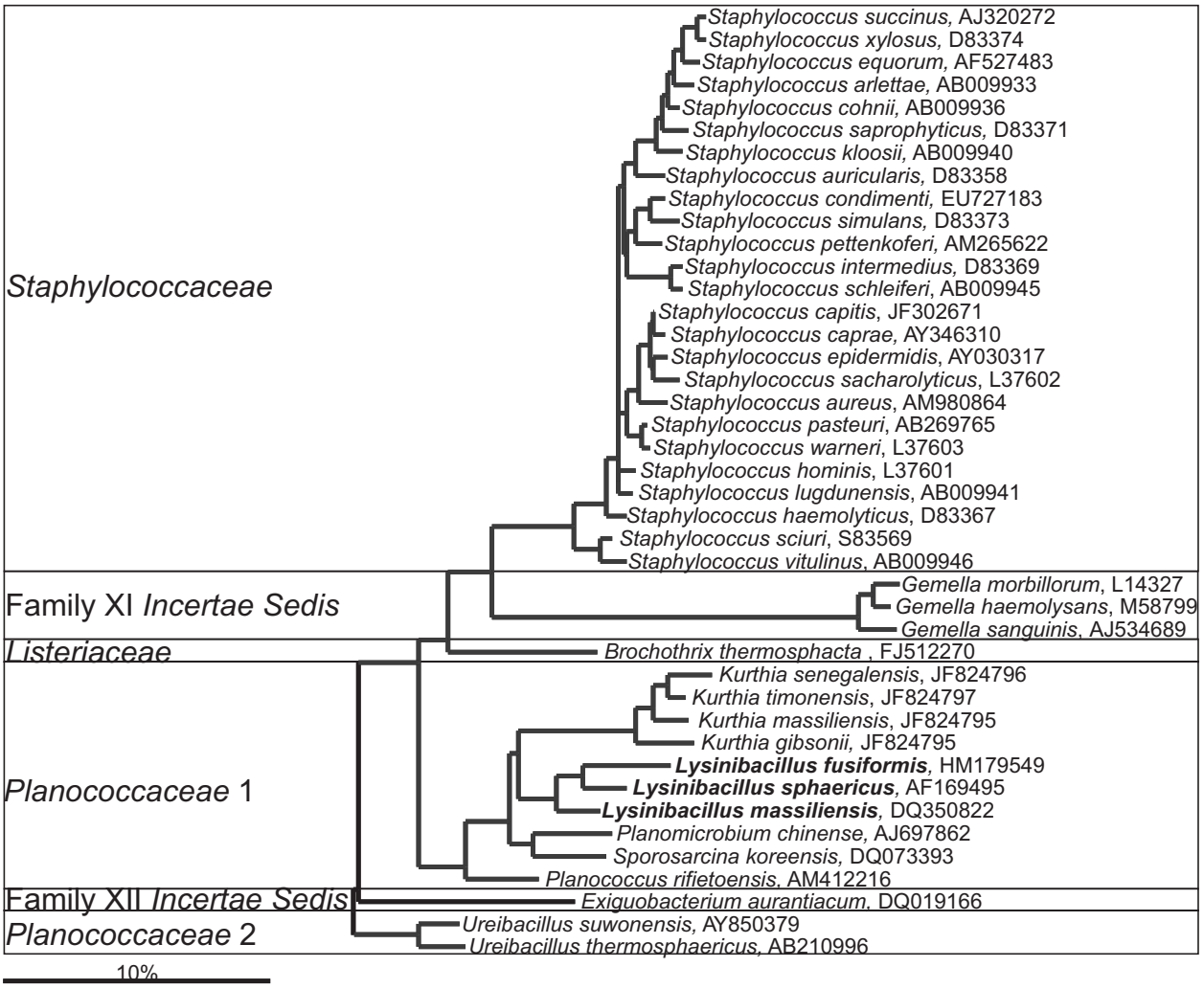

Fig. 10. Phylogenetic tree of a fraction of the human gastrointestinal species that belong to the order of the *Bacillales*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification. For the other gastrointestinal species that belong to the order of the *Bacillales* see Fig. 11.

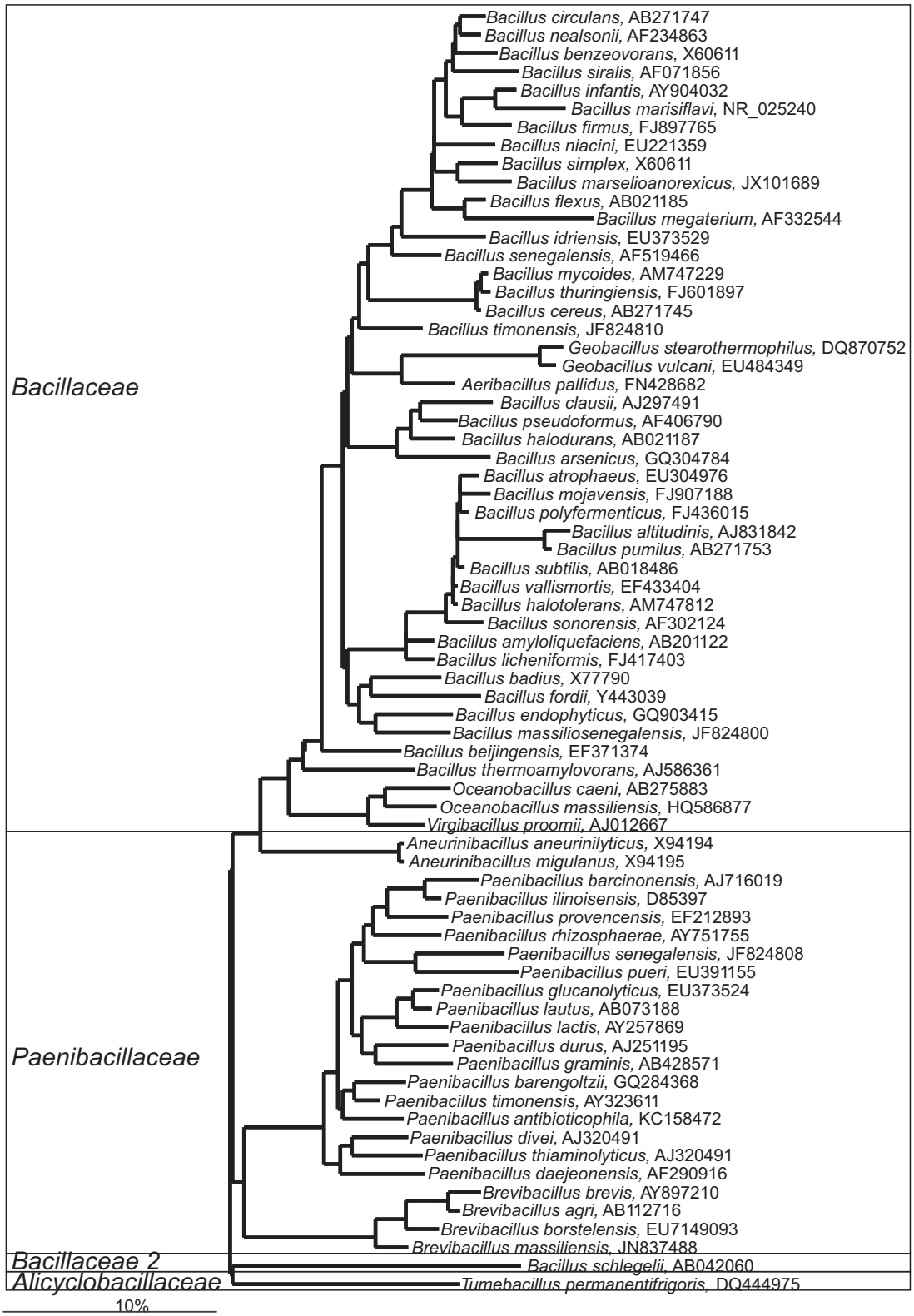

Fig. 11. Phylogenetic tree of a fraction of the human gastrointestinal species that belong to the order of the *Bacillales*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. For the other gastrointestinal species that belong to the order of the *Bacillales* order see Fig. 10.

the upper and lower gastrointestinal tract, is associated with celiac disease, although the abundance of these bacteria can be depleted with the withdrawal of gluten from the diet of patients (Collado *et al.*, 2009).

Gemella spp. are abundant in the upper gastrointestinal tract and specially the proximal small intestine (Ou *et al.*, 2009). While their function in the gastrointestinal tract has not been determined, these species can include pathogenic strain that translocate to other organs. For instance, *Gemella* spp. have been described as likely causing agents of endocarditis, particularly in patients that suffer from gastrointestinal disorders (such as colon cancer; Lopez-Dupla *et al.*, 1996).

Numerous members of the *Bacillus* and *Paenibacillus* genera have been detected in the samples of the human gastrointestinal tract. The first representatives of this group of bacteria were isolated in 1919 by Marjorie Batchelor, who reported *Bacillus cereus* as the most prevalent member of the aerobic sporogenic bacteria in infant feces (Batchelor, 1919). Members of the *Bacillus* genus were often reported in the older cultivation-based studies, but the vast majority of the species of this genus and related genera were reported only recently (Hoyles *et al.*, 2012; Lagier *et al.*, 2012a; Zoetendal *et al.*, 2012). One of these studies was designed for the targeted cultivation of *Bacillus* and related species from human samples (Hoyles *et al.*, 2012). It has been shown that *Bacillus* spp. could be retrieved from all analyzed samples, although these bacteria have very low abundance of 10^2–10^4 cells mL^{-1} of intestinal content. Many of the *Bacillus* spp. isolated in this study exhibited notable antimicrobial activity. This feature is in line with the use of several *Bacillus* spp. as potent probiotics with immunomodulatory potential (Duc *et al.*, 2004). Little is known about the function of these bacteria in the ecosystem, but it is noteworthy that two independent studies have shown that members of the *Bacillales* order, more specifically *Aneurinibacillus* spp., have an increased abundance in feces of irritable bowel syndrome patients (Krogius-Kurikka *et al.*, 2009; Rajilić-Stojanović *et al.*, 2011), while a significantly higher abundance of *Bacillus subtilis* was found in the feces of bottle-fed than breast-fed babies (Benno *et al.*, 1984).

Clostridia

The class *Clostridia* clusters bacteria that are dominant and frequently detected in the lower gastrointestinal tract that are distributed within the families: *Clostridiaceae*, *Christensenellaceae*, *Eubacteriaceae*, *Lachnospiraceae, Peptostreptococcaceae, Ruminococcacea* as well as bacteria with an unclear taxonomic status that are classified within *Clostridiales Incertae Sedis* families XI and XIII (Garrity *et al.*, 2005). Members of the Clostridia class are heterogeneous and many of its members were initially assigned to *Clostridium* genus and subsequently reclassified into novel genera. The *Clostridium sensu stricto* — the real *Clostridium* spp. — are grouped around the type species *Clostridium butyricum* and belong to the *Clostridium* cluster I within the *Clostridiaceae* family (Fig. 12; Stackebrandt *et al.*, 1999). In addition to *Clostridium* spp., the *Clostridiaceae* officially groups *Sarcina* spp., *Butyricicoccus pullicaecorum* and *Lactonifactor longoviformis*, *Anoxynatronum sibiricum* while the latter three, based on their SSU rRNA gene sequence, should be assigned to various other *Clostridiales* families (Figs 13, 14 and 16). The first human gastrointestinal *Clostridium* isolate, *C. perfringens*, was recovered in 1905 (Passini, 1905). The same species, previously known as *Bacillus aerogenes capsulatus* and *Clostridium welchii*, was earlier isolated from a case of endocarditis (Welch & Nuttall, 1892). Both isolation sites fit the nowadays known properties of *C. perfringens*, which is a commensal gastrointestinal bacterium that can cause bacteraemia (Petit *et al.*, 1999). Up to now, 72 *Clostridium* spp. have been detected in the human gastrointestinal samples, of which 30 belong to the *Clostridium sensu stricto* (Fig. 12). The other *Clostridium* spp. belong to different families within the *Firmicutes* phylum, while *Clostridium rectum* belongs to the *Fusobacteria* phylum. Members of the *Clostridium sensu stricto* are generally perceived as pathogenic, although cultivation-based studies show that *C. perfringens* and other real clostridia can be found in densities of up to 10^{10} cells g^{-1} intestinal content of healthy individuals (Finegold *et al.*, 1974), and up 10^7 cells g^{-1} intestinal content of healthy infants (Mevissen-Verhage *et al.*, 1987). Still, the presence of these bacteria, notably as seen for *C. perfringens* in elderly Irish subjects, is interpreted as an indicator of a less healthy microbiota (Lakshminarayanan *et al.*, 2013).

The most abundant and diverse gastrointestinal family is the *Lachnospiraceae*. This family groups 24 different genera, most of which can be detected in the human gastrointestinal tract. In addition, a number of species that are officially classified into the genera *Clostridium, Eubacterium,* and *Ruminococcus,* cluster within the *Lachnospiraceae* based on their SSU rRNA gene sequence (Fig. 13). Members of the *Lachnospiraceae* are also among the first to be established in the gastrointestinal tract. A recent study showed that *Ruminococcus gnavus* is an exclusive representative of this family in 2-months old breast-fed infants, while infants fed with cow-milk based formula have a more diverse Lachnospiraceae community (Tannock *et al.*, 2013). Analysis of the microbiota of children and adults showed that this group of bacteria is predominant in both young children and in adults, which indicates the early establishment of these bacteria (Ringel-Kulka *et al.*, 2013).

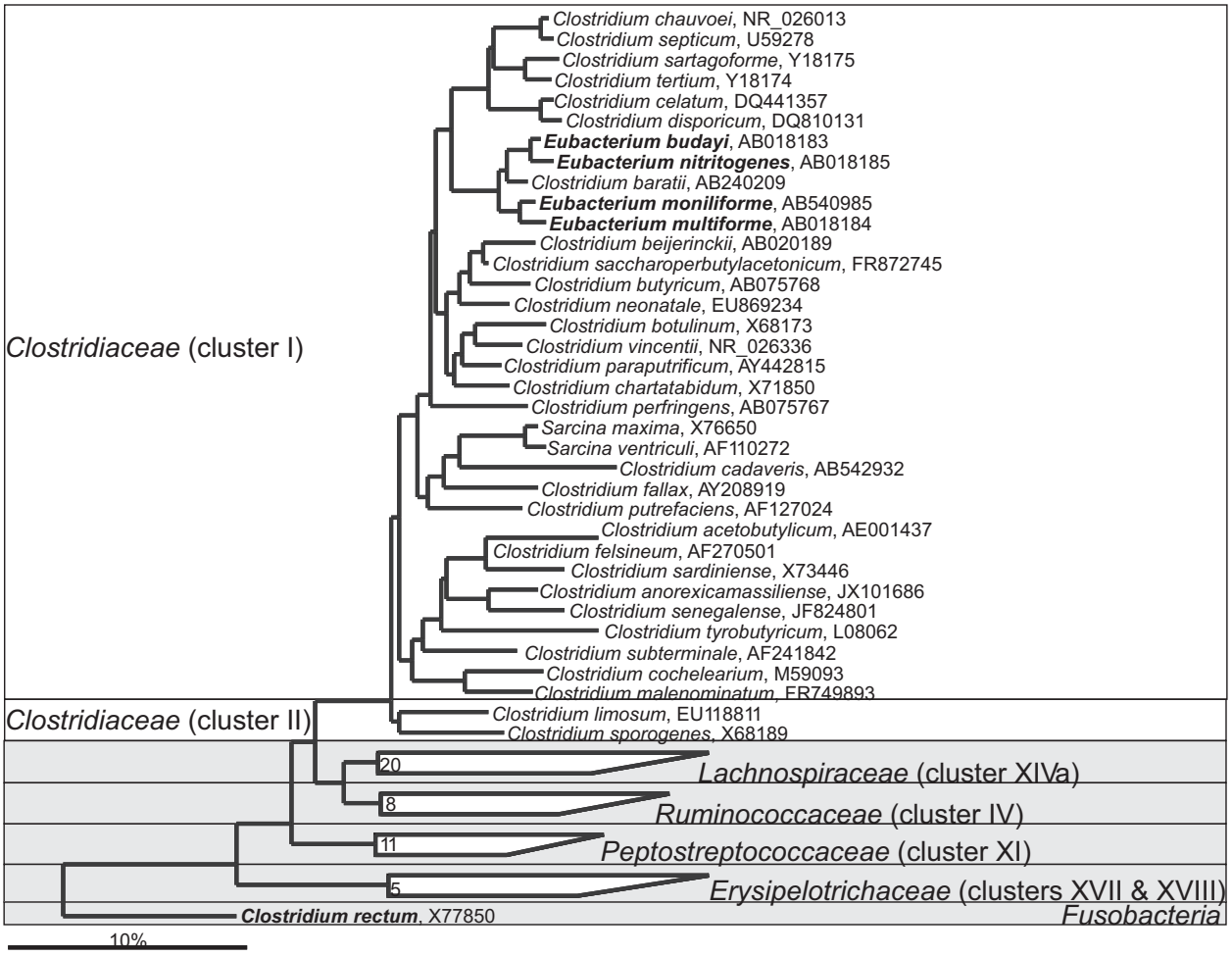

Fig. 12. Phylogenetic tree the human gastrointestinal species that belong to the family of *Clostridiaceae*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification. Deeply rooted *Clostridium* spp., which based on the SSU rRNA gene sequence cluster within distant phylogenetic groups, are depicted in the gray area.

Several members of the *Lachnospiraceae* family are butyrate producers including *Anaerostipes* spp., *Butyrivibrio* spp., *Coprococcus* spp., *Roseburia* spp., *Eubacterium rectale*- and *Eubacterium hallii*-related species. Butyrate can be used as an energy source by the gut epithelial cells, and it has anticarcinogenic and anti-inflammatory properties (Hamer *et al.*, 2008). Furthermore, a recent study shows that butyrate produced by intestinal microorganisms has beneficial effects on glucose and energy homeostasis (De Vadder *et al.*, 2014). The decrease in the relative abundance of the butyrate-producing *Lachnospiraceae* in the gastrointestinal microbiota is associated with compromised health status of subjects suffering from colorectal cancer (Wang *et al.*, 2012), ulcerative colitis (Rajilić-Stojanović *et al.*, 2013a), type 1 (Murri *et al.*, 2013) and type 2 diabetes (Qin *et al.*, 2012). This bacterial group seems to be stimulated by an omnivore diet, since it is present in lower abundance in vegetarians (Kabeerdoss *et al.*, 2012). This is an intriguing but not yet explained finding, as it could be expected that vegetarian, plant-based diets, which are rich in fibers, would favor butyrate production in the colon and promote health.

The gastrointestinal *Lachnospiraceae* include *Dorea* spp., which are the major gas producers in the gastrointestinal tract and its end-products of glucose fermentation include both hydrogen and carbon dioxide (Taras *et al.*, 2002). *Dorea* spp. were found in an increased in abundance in both pediatric and adult irritable bowel syndrome patients (Rajilić-Stojanović *et al.*, 2011; Saulnier *et al.*, 2011), which probably could explain the symptom of bloating, experienced by the majority of these patients.

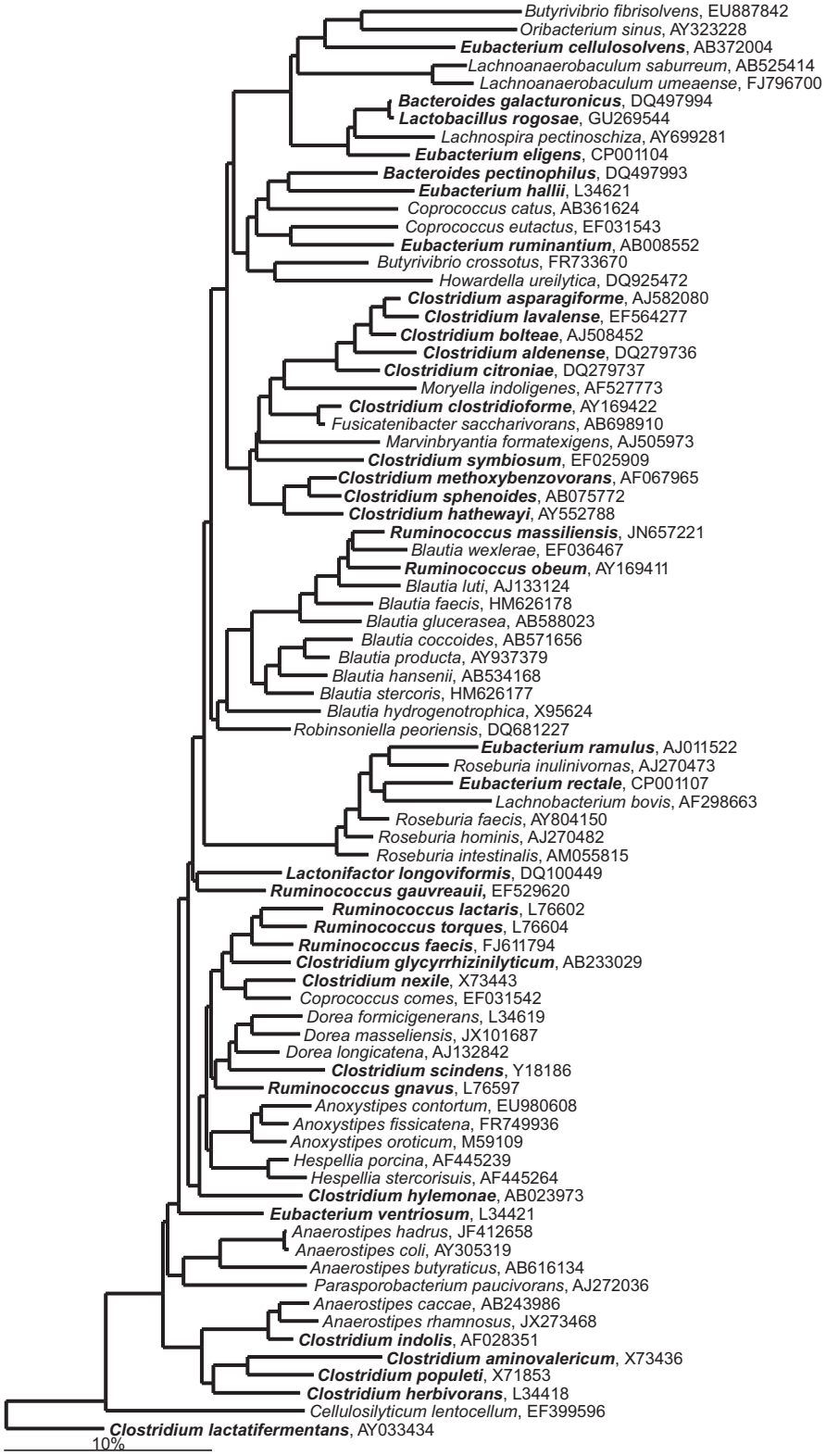

Fig. 13. Phylogenetic tree the human gastrointestinal species that belong to the family of the *Lachnospiraceae*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification.

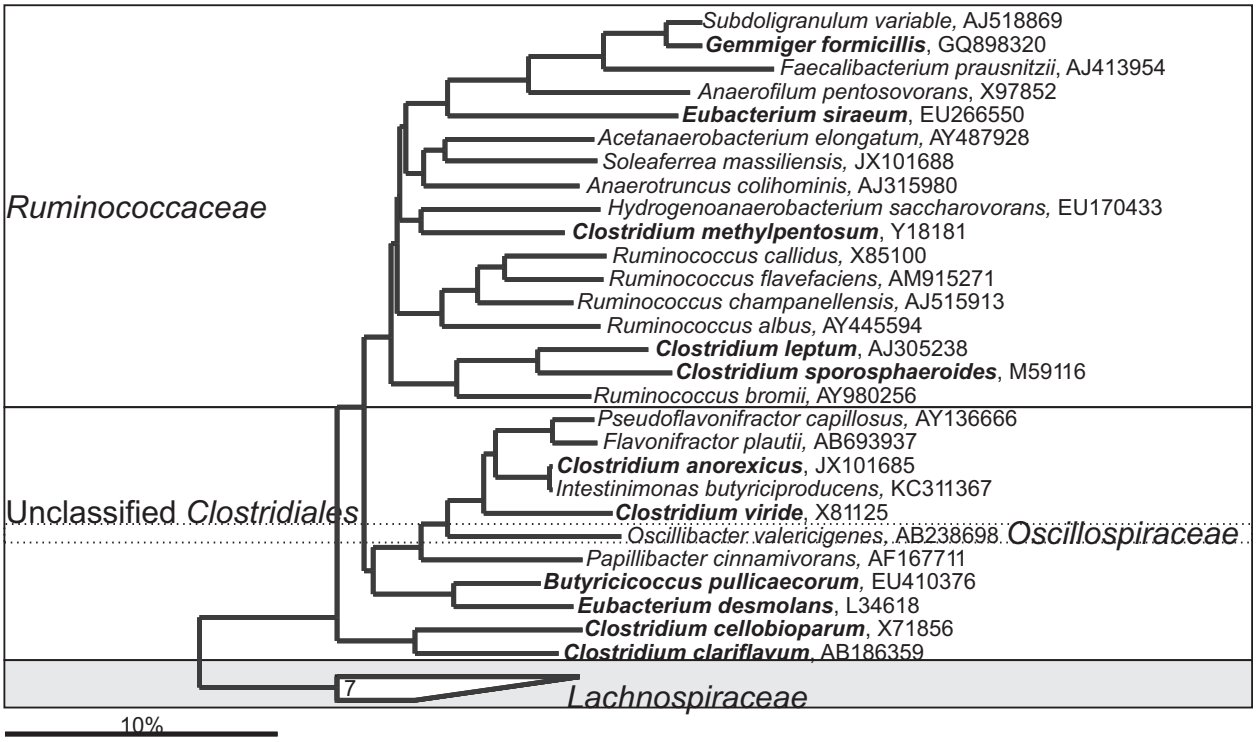

Fig. 14. Phylogenetic tree the human gastrointestinal species that belong to the *Clostridium* cluster IV, most of which belong to the family of the *Ruminococcaceae*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification. Deeply rooted *Ruminococcus* spp., which based on the SSU rRNA gene sequence cluster within distant phylogenetic groups, are depicted in the gray area.

Blautia is a recently described bacterial genus that groups several abundant gastrointestinal bacteria that were previously assigned to the *Ruminococcus* genus – notably those related to *Ruminococcus obeum* (Fig. 14). The common feature of *Blautia* spp. is the utilization of hydrogen and carbon dioxide to form acetate (Bernalier *et al.*, 1996). *Blautia* spp. are among the most abundant members of the entire gastrointestinal tract and can encompass between 2.5% and 16% of the total microbiota (Zoetendal *et al.*, 2002). This abundant bacterial group is significantly depleted in elderly subjects (Hayashi *et al.*, 2003; Biagi *et al.*, 2010) and in mucosal samples of colorectal cancer patients (Chen *et al.*, 2012). In contrast, increased levels of *Blautia* spp. are observed in irritable bowel syndrome patients (Rajilić-Stojanović *et al.*, 2011), but this could reflect the adaption of the ecosystem to the larger amount of gasses produced by *Dorea* spp., which can be utilized by *Blautia* spp.

An interesting group within the *Lachnospiraceae* family is the misclassified *Ruminococcus* spp., including *R. gnavus, R. torques, R. lactaris,* and *R. faecis*. These bacteria are abundant in the gastrointestinal tract (Holdeman & Moore, 1974), and apparently associated with a number of important metabolic functions. *R. torques* and other currently uncultured species related to *R. torques*, are among the most potent mucus utilizes that enable mucus degradation by secretion of several different extracellular glycosidases (Hoskins *et al.*, 1985). Furthermore, the abundance of these bacteria is strongly associated with the level of triglycerides in blood serum (Lahti *et al.*, 2013). Several studies of the microbiota of irritable bowel syndrome patients and controls have shown that organisms related to these misclassified *Ruminococcus* spp. are significantly elevated in patients (Kassinen *et al.*, 2007; Rajilić-Stojanović *et al.*, 2011; Saulnier *et al.*, 2011). Moreover, the abundance of these bacteria is positively correlated with irritable bowel syndrome symptoms (Malinen *et al.*, 2010) and significantly reduced by probiotics consumption that reduces these symptoms (Lyra *et al.*, 2010).

The *Ruminococcaceae* family is another relevant group of gastrointestinal bacteria within the *Clostridiales* order. It includes the true *Ruminococcus* spp. — members of the *Ruminococcus sensu stricto* namely *R. albus, R. bromii, R. callidus, R. champanellensis,* and *R. flavefaciens*. Several other frequently detected gastrointestinal genera that are

recognized members of the *Clostridium* cluster IV (*Clostridium leptum* group) are either members or are closely related to this family (Fig. 14). The true *Ruminococcus* spp. are an abundant fraction of the human gastrointestinal microbiota that can reach densities of up to 10^{10} cells g^{-1} of intestinal content (Finegold *et al.*, 1977). Being strictly anaerobic cellulolytic cocci, *Ruminococcus* spp. were isolated from human gastrointestinal samples only after the improvement of the anaerobic techniques and media for studying rumen anaerobes (Hungate, 1947). The first human gastrointestinal *Ruminococcus* spp. reported is *Ruminococcus bromii* isolated in 1972 (Moore *et al.*, 1972). Similar to the other gastrointestinal bacteria, the initially defined *Ruminococcus* spp. are a heterogeneous group, which based on the SSU rRNA gene sequence clusters within the *Ruminococcaceae* and *Lachnospiraceae* families. Recently, five gastrointestinal *Ruminococcus* spp. were reclassified into *Blautia* genus, leaving seven others to be reclassified (Liu *et al.*, 2008; Figs 9 and 10). Bacteria that belong to the *Ruminococcus sensu stricto* degrade complex sugars to produce acetate as the major fermentation product. Both *in vitro* and *in vivo* studies have shown that *R. bromii* is the major degrader of the resistant starch in the human gastrointestinal tract (Kovatcheva-Datchary *et al.*, 2009; Walker *et al.*, 2011). Application of the resistant starch in the diet has a wide range of health-promoting effects, suggesting the importance of the metabolic activity of *R. bromii* for the wellbeing of the host (Higgins & Brown, 2013). The importance of the members of the *Ruminococcus sensu stricto* for the intestinal health is indicated by their reduced abundance in feces of Crohn's disease (Kang *et al.*, 2010) and ulcerative colitis patients (Rajilić-Stojanović *et al.*, 2013a).

Among the *Ruminococcaceae* family, *Faecalibacterium prausnitzii* (previously known as *Fusobacterium prausnitzii*) is the most prevalent and abundant gastrointestinal microorganism (Holdeman *et al.*, 1976). *Faecalibacterium prausnitzii* can utilize glucose, fructose, and fructo-oligosacharides, as well as complex molecules such as pectin and *N*-acetylglucosamine to produce butyrate, formate and lactate (Duncan *et al.*, 2002a; Lopez-Siles *et al.*, 2012). It is one of the major butyrate producers in the gastrointestinal tract, which is a relevant feature because of the health-promoting properties of butyrate. The reduced abundance of this bacterium is detected in association with Crohn's disease (Sokol *et al.*, 2006; Kang *et al.*, 2010) and with colon cancer (Chen *et al.*, 2012). This bacterium is important for the gastrointestinal microbiota homeostasis as it has found to show anti-inflammatory properties in mice (Sokol *et al.*, 2008) and is associated with a range of metabolic processes in the human mucosa (Lepage *et al.*, 2011). Health-promoting properties are also exhibited by *B. pullicaecorum,* another species with the SSU rRNA gene sequence that is related to the *Ruminococcaceae* family. This bacterium is significantly reduced in inflammatory bowel disease patients, while its oral administration strengthens the epithelial barrier function in animal models by increasing the trans-epithelial resistance (Eeckhaut *et al.*, 2013).

The first *Eubacterium* spp. from a human gastrointestinal sample was isolated already in 1908 when Henri Tissier plated *Bacillus ventriousus*, later renamed into *Eubacterium ventriosum* (Tissier, 1908). The genus *Eubacterium* was for a long time recognized as one of the most abundant genera of the human gastrointestinal microbiota, with densities of up to 10^{10} cells g^{-1} of intestinal content (Moore & Holdeman, 1974a). However, *Eubacterium*, similar to *Clostridium*, is a genus that is very vaguely described. Defined as anaerobic, rod-shaped, Gram-positive bacteria that do not form endospores, *Eubacterium* genus includes a consortium of distantly related species. Some *Eubacterium* spp. have been reclassified into novel genera within two bacterial phyla — *Actinobacteria* and *Firmicutes* — of which six genera (*Dorea*, *Collinsella*, *Eggerthella*, *Flavonifractor*, *Mogibacterium* and *Pseuodramibacter*) can be members of the gastrointestinal microbiota (Willems & Collins, 1996; Kageyama *et al.*, 1999a, b; Nakazawa *et al.*, 2000; Taras *et al.*, 2002; Carlier *et al.*, 2010). Further reclassification of the genus can be expected, as only four gastrointestinal *Eubacterium* spp. belong to the *Eubacterium sensu stricto* (Fig. 15). In a recent study of the gastrointestinal microbiota of centenarians, *Eubacterium* spp. (notably those related to *Eubacterium limosum*) were reported as signature bacteria of the long life, being 10-fold increased in centenarians (Biagi *et al.*, 2010). It is known that *E. limosum* has the ability to transform dietary phytoestrogens into forms that might have a positive impact on health (Clavel *et al.*, 2006; Possemiers & Verstraete, 2009). Furthermore, *E. limosum* is selectively stimulated by prebiotics that improve the symptoms of inflammatory bowel disease patients (Kanauchi *et al.*, 2005).

Mogibacterium is a genus established by reclassification of the intestinal bacterium – *Eubacterium timidum. Mogibacterium* spp. are enriched in mucosa-associated microbiota in colon cancer patients but not much is known about this group of bacteria belonging to *Clostridium* Family XIII *Incertae Sedis* (Chen *et al.*, 2012).

Prior to the molecular revolution of the gastrointestinal microbiota research (Fig. 1), *Peptococcus* spp. and *Peptostreptococcus* spp. were considered as the dominant and abundant in the human gastrointestinal tract (Holdeman *et al.*, 1976). However, the latter research has shown that these two genera are heterogeneous and led to the major reclassification resulting in definition of novel genera that include *Anaerococcus*, *Blautia, Finegoldia*, *Parvimonas,* and *Peptoniphilus* (Murdoch & Shah, 1999; Ezaki *et al.*, 2001;

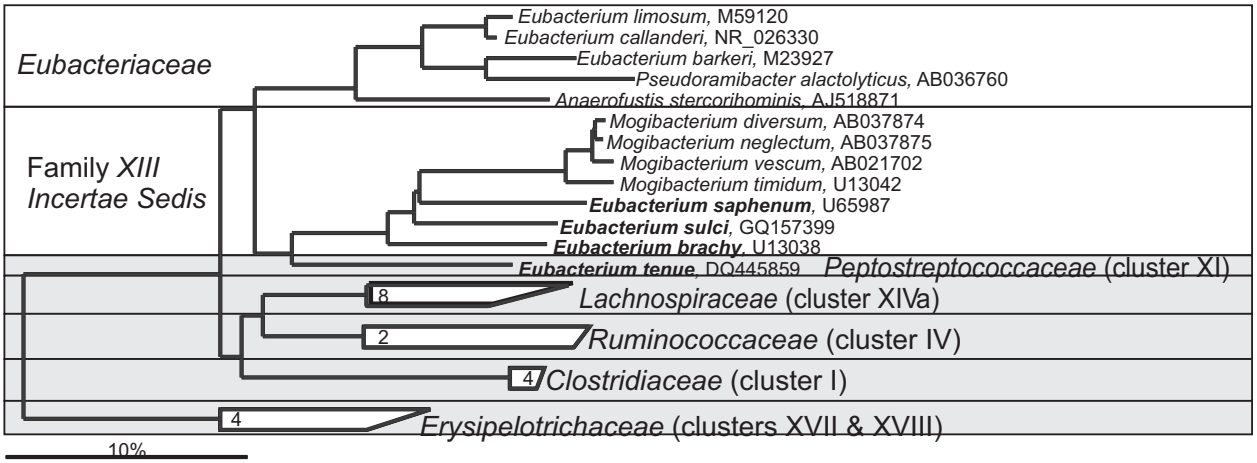

Fig. 15. Phylogenetic tree the human gastrointestinal species that belong to the family of the *Eubacteriaceae* and *Clostridiales* Family XIII *Incertae Sedis*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification. Deeply rooted *Eubacterium* spp., which based on the SSU rRNA gene sequence cluster within distant phylogenetic groups are depicted in the gray area.

Tindall & Euzeby, 2006; Liu *et al.*, 2008). All these genera, with exception of *Blautia* that is a genus in the *Lachnospiraceae* family, belong to the *Clostridiales* Family XI *Incertae Sedis* (Fig. 16). Among this group of bacteria, *Peptoniphilus asaccharolyticus* is the most frequently detected and also was the first to be cultured and described (Distaso, 1911). Currently, only two true *Peptostreptococcus* spp. have been recognized as gastrointestinal inhabitants, although a number of other species that are officially classified within different genera (predominantly *Clostridium*) belong to the *Peptostreptococcaceae* family according to their SSU rRNA gene sequence (Fig. 16). Members of the *Peptostreptococcaceae* family are, in principle, associated with compromised health, and the most convincing example of this is *Clostridium difficile*. Although *C. difficile* can be present in low numbers in healthy subjects without exhibiting pathogenic properties (Ozaki *et al.*, 2004), many strains are toxin producing and are well-established pathogens that cause severe diarrhea. Furthermore, recent studies have shown that *Peptostreptococcus* spp. have an increased abundance in association with ulcerative colitis (Rajilić-Stojanović *et al.*, 2013a) and colorectal cancer (Chen *et al.*, 2012; Wang *et al.*, 2012). Based on the SSU rRNA gene sequence, the members of the *Peptococcaceae* family form two paraphyletic groups within the *Firmicutes* phylum, of which the group that contains the two human gastrointestinal bacteria is closely related to *Negativicutes* class and is discussed in the following section.

In addition to the already mentioned species, five other gastrointestinal bacteria that officially belong to the *Clostridia* class, form distinct branches in the phylogenetic tree (Fig. 17). Two of these gastrointestinal bacteria, namely *Catabacter hongkongensis* and *C. minuta*, form a separate cluster within the *Clostridiales* order of the *Clostridia* class and are the only cultured representatives of a phylogenetic group that was previously detected only in various molecular studies and was in a previous review designated as uncultured *Clostridiales* II (Rajilić-Stojanović *et al.*, 2007). These two species are officially assigned to two different families (*Catabacteriaceae* and *Christensenellaceae*), although based on the SSU rRNA gene sequence similarity (96.5%), they should be grouped in the same family, and, most likely, in the same genus. *Catabacter hongkongensis* was isolated in 2007 from a blood sample, although the intestinal origin of the bacterium was suspected (Lau *et al.*, 2007). This bacterium was later isolated from patients with acute appendicitis, but also from other tissues where it was a causative agent of fatal bacteremia (Lau *et al.*, 2012). *Christensenella minuta* is an intestinal isolate, described in 2012 (Morotomi *et al.*, 2012). Not much is known about the role of this group of strictly anaerobic bacteria in the human gastrointestinal tract, but it is noteworthy that bacteria that belong to the *Christensenella/Catabacter* group were reported to be dramatically (20-fold) depleted in fecal samples of ulcerative patients relative to controls (Rajilić-Stojanović *et al.*, 2013a) and significantly (fivefold) depleted in fecal samples of patients with postinfectious irritable bowel syndrome (Jalanka-Tuovinen *et al.*, 2013).

Two members of the *Peptococcaceae* family were reported as members of the human gastrointestinal

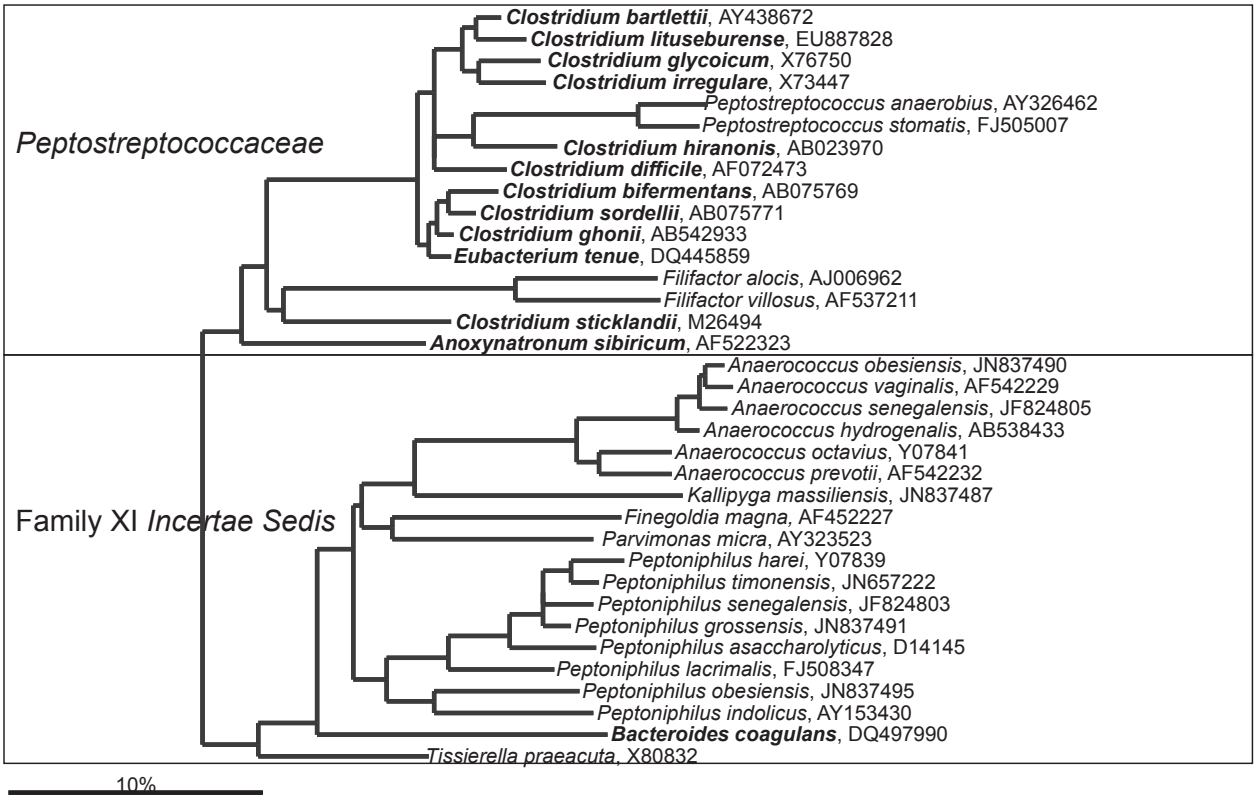

Fig. 16. Phylogenetic tree the human gastrointestinal species that belong to families of the *Peptostreptococcaceae* and *Clostridiales* Family XI *Incertae Sedis*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification.

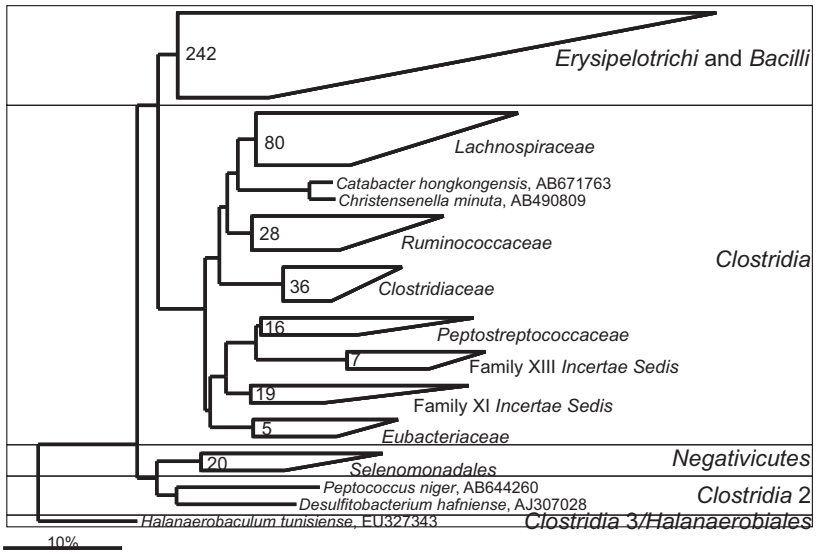

Fig. 17. Partially opened phylogenetic tree the human gastrointestinal species that belong to the *Firmicutes* phylum on which five species that cluster within the three families with low diversity are indicated.

microbiota (Fig. 17). These bacteria are officially classified within the *Clostridia* class and *Clostridiales* order, although based on the SSU rRNA gene sequence they are closely related to the members of the *Selenomonadales* order within the *Negativicutes* class (Fig. 18). Based on their SSU rRNA gene sequence, gastrointestinal members of the *Peptococcaceae* family will be reclassified either the *Negativicutes* class or into another novel class, different

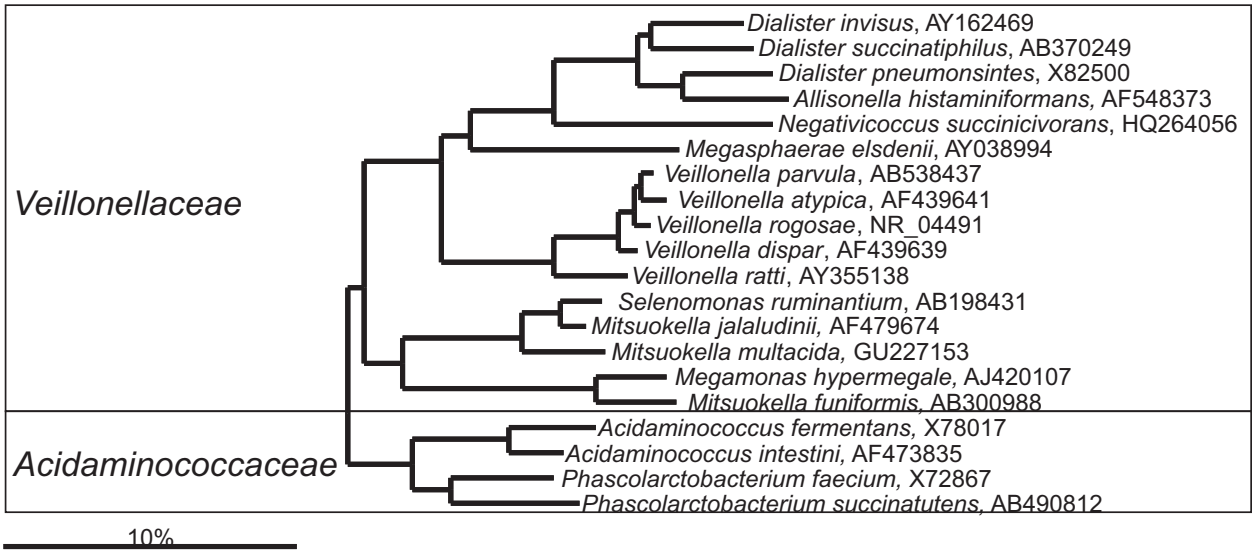

Fig. 18. Phylogenetic tree the human gastrointestinal species that belong to the order of the *Negativicutes*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

from *Clostridia sensu stricto*. *Peptococcus niger* is the only representative of the genus. This strictly anaerobic bacterium has been isolated from various body sites, while the strain isolated from feces showed an ability to desulfate and perform other chemical transformation of steroid molecules (Van Eldere *et al.*, 1987). This feature of *P. niger* makes it an important player in enterophatic circulation of various steroid molecules, primarily steroid hormones, which has a major impact on human metabolism. Another member of the *Peptococcaceae* family, *Desulfitobacterium hafniense*, has been reported only once as an inhabitant of the human gastrointestinal tract (van de Pas *et al.*, 2001). In contrast to other members of the genus, the human *D. hafniense* is not able to use chloroethenes or chlorophenol as terminal electron acceptors (Smidt & de Vos, 2004). This bacterium is capable of using sulfite as terminal electron acceptor and hence produces hydrogen sulfide. Hydrogen sulfide is also produced by *P. niger* (Wilkins *et al.*, 1975), and this property that may be detrimental to health as described below for the *Deltaproteobacteria*.

Finally, *Halanaerobaculum tunisiense* has been identified by pyrosequencing of the V6 variable region of the SSU rRNA gene in a recent study that compared efficiency of culturomics and pyrosequencing approach for studying the gastrointestinal microbiota diversity (Lagier *et al.*, 2012a). *Halanaerobaculum tunisiense* is a recently described anaerobic bacterium that was isolated from hypersaline lake in Tunisia (Hedi *et al.*, 2009). Given the conditions that the isolated strain of this bacterium requires for its growth (the minimal required NaCl concentration of 14%), it is not likely that it represents a member of the gastrointestinal microbiota, although this should be confirmed in further studies.

Negativicutes

The *Negativicutes* include bacteria that were previously assigned to the *Clostridium* cluster IX (Collins *et al.*, 1994; Marchandin *et al.*, 2010), distributed within the following genera: *Acidamoinococcus, Dialister, Megamonas*, *Megasphaera, Phascolarctobacterium* and *Veillonella* (Fig. 18). Bacteria of this group used to be classified within the order *Clostridiales,* although based on their SSU rRNA gene sequence, they are distant from other *Clostridiales*. Therefore, following the description of a novel gastrointestinal inhabitant — *Negativicoccus succinicivorans*, the novel class of *Negativicutes* and novel order of *Selenomonadales* were introduced to accommodate the Gram-negative staining bacteria within the *Firmicutes* phylum (Marchandin *et al.*, 2010). The first record of this bacterial group dates from 1898 when Veillon and Zuber isolated *Staphylococcus parvulus*, which was later reclassified as *Veillonella parvula,* from infected appendix tissue (Veillon & Zuber, 1898). The *Negativicutes* are typically isolated from the oral cavity or the proximal small bowel (Rogosa, 1965; Simon & Gorbach, 1986), but representative species from this group can be detected in high abundances even in the lower intestinal tract. In that line *V. parvula*, can reach densities of up to 10^{11} cells g^{-1} of feces (Finegold *et al.*, 1977), while molecular quantification of *Phascolarctobacterium* spp.

showed that these bacteria represent more than 2% of the total fecal microbiota in some subjects (Paliy *et al.*, 2009). Members of the *Negativicutes* are assacharolytic and utilize end=products of sugar metabolisms of other gastrointestinal bacteria (such as lactate or succinate) to produce propionate, forming an important trophic chain. Propionate is a beneficial product of the gastrointestinal microbiota as it has anti-inflammatory potential, is utilized by adipose tissue and the liver, plays a role in the satiety sensation, influences glucose and energy homeostasis, and improves insulin sensitivity (Vipperla & O'Keefe, 2012; De Vadder et al., 2014). In the upper gastrointestinal tract, *Veillonella* spp. are an indispensable component of the gastrointestinal microbiota (van den Bogert *et al.*, 2011) where they form a trophic chain with the lactate and acetate-producing *Streptococcus* spp. (Zoetendal *et al.*, 2012). Currently, there is no evidence about the role of *Veillonella* spp. in human health, although several studies have shown an increased abundance of *Veillonella* spp. in fecal samples of irritable bowel patients (Malinen *et al.*, 2005; Tana *et al.*, 2010; Saulnier *et al.*, 2011), which could indicate an increased transit of the ileal microbiota to the lower part of the gastrointestinal tract.

Erysipelotrichi

The *Erysipelotrichi* constitute a class of bacteria within the *Firmicutes* phylum that was introduced into bacterial systematics in 2009, to accommodate members of earlier established family *Erysipelotrichaceae* (Ludwig *et al.*, 2009). The majority of the human gastrointestinal bacteria that based on their SSU rRNA gene sequence cluster within the *Erysipelotrichi* class are still officially classified within other groups of the *Firmicutes* (Fig. 19). This indicates that a major revision of this group can be expected in the future. There are several studies that link *Erysipelotrichi* with compromised health. An increased abundance of *Erysipelotrichi* in patients suffering from colon cancer was reported (Chen *et al.*, 2012). Animal model experiments have shown that members of this group are increased on high fat, and western type diets (Turnbaugh *et al.*, 2009; Fleissner *et al.*, 2010), while their increased abundance is associated with obesity (Turnbaugh *et al.*, 2006). Furthermore, it has been shown that an increased abundance of *Erysipelotrichaceae* correlates with choline deficiency-induced fatty liver disease (Spencer *et al.*, 2011), which causes multiple organ dysfunctions. Choline is an important component of our diet, and recently, it was found that choline and phospatidylcholine are converted by the intestinal microbiota to trimethylamine, which is further metabolized to proatherogenic trimethylamine-N-oxide, linking diet and microbiota to cardiovascular disease (Wang *et al.*, 2011b; Koeth *et al.*, 2013).

Tenericutes

Tenericutes is a recently introduced phylum that accommodates the *Mollicutes* class, which was previously positioned within the *Firmicutes* phylum. The assignment of the *Mollicutes* to the novel phylum was supported by the unique properties of these bacteria, in particular the lack of rigid cell walls (Ludwig *et al.*, 2009), although based

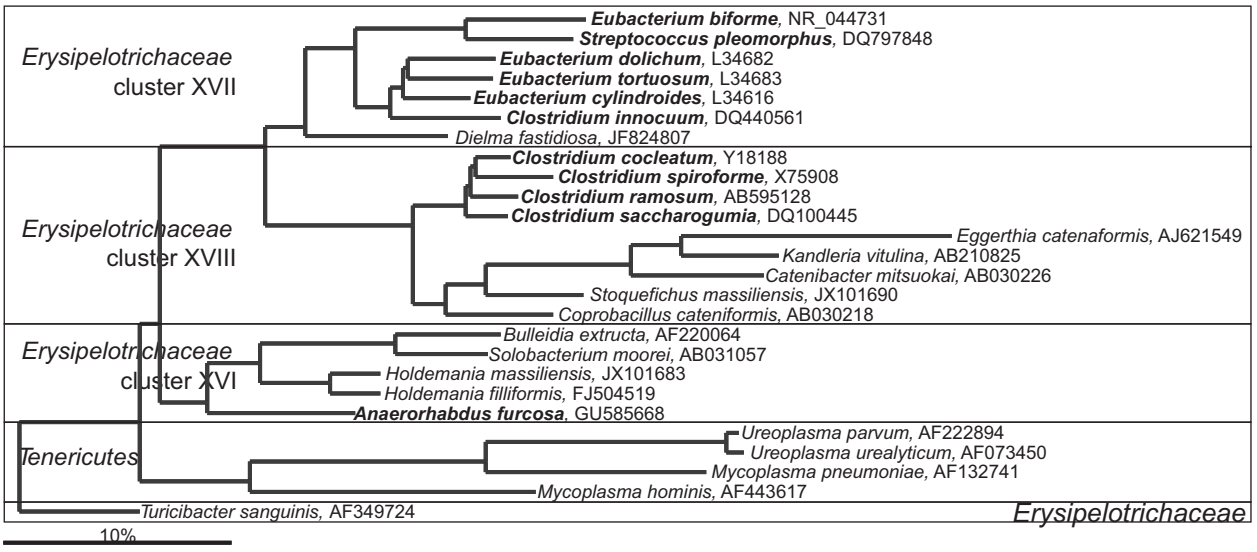

Fig. 19. Phylogenetic tree the human gastrointestinal species that belong to the order of the *Erysipelotrichi.* GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species, while the family is divided over *Clostridium* clusters (Collins *et al.*, 1994). The species indicated in bold are based on the SSU rRNA gene sequence clustering within the *Erysipelotrichi* in contrast to their official classification.

on the SSU rRNA gene sequence, they are interrelated with the members of the *Erysipelotrichi* class of the *Firmicutes* (Fig. 19). The majority of the gastrointestinal *Tenericutes* are currently uncultured species that are frequently detected in the molecular surveys. Their detection even in the studies of the limited depth suggests a relatively high relative abundance of these bacteria (see for instance, Suau *et al.*, 1999). Concerning their function in the gastrointestinal tract, a recent study has shown a significant positive correlation between the abundance of the *Tenericutes* and the levels of trimethylamine-N-oxide, which is a metabolite that is believed to accelerate atherosclerosis (Koeth *et al.*, 2013).

It has been suggested that members of the *Tenericutes* are involved in inflammatory bowel disease, as they have the ability to adhere to and to fuse with epithelial and immune system cells, which could explain intracellular epithelial structures in the Crohn's disease patients' biopsies detected by electron microscopy (Roediger & Macfarlane, 2002). A molecular survey has revealed that a cultured representative of this group — the pathogenic *Mycoplasma pneumoniae* — can be detected in mucosal biopsies of both inflammatory bowel disease patients and healthy controls alike, although Crohn's disease patients had a significantly higher abundance of this bacterium than ulcerative colitis patients and healthy subjects (Chen *et al.*, 2001). The first report of the *Mycoplasma* in the human gastrointestinal tract dates from 1973 (Bhat *et al.*, 1973), while specific searches for *Ureaplasma urealyticum* and *Mycoplasma hominis* revealed the presence of these two species in anal swaps of more than half of the analyzed subjects (Munday *et al.*, 1981).

Fusobacteria

Fusobacteria are another phylum of the frequently detected gastrointestinal bacteria, the majority of which belong to the genus *Fusobacterium* (Fig. 20). These bacteria are pointed, nonsporulating, Gram-negative, anaerobic bacilli (Knorr, 1922). The first record of a *Fusobacterium* spp. originates from 1886 when *Bacillus fusiforme* (now known as *Fusobacterium necrophorum*) was reported as a pathogen related to appendicitis (Flügge, 1886). Although *Fusobacterium* spp. can be isolated from gastrointestinal samples of healthy humans in densities of up to 10^{10} cells g^{-1} of feces (Benno *et al.*, 1989), this group of bacteria seems to be relevant for intestinal inflammation. Recent studies have shown that the majority of cases of acute appendicitis are associated with a local infection of *Fusobacterium* spp. (Swidsinski *et al.*, 2011), while the increased abundance of *Fusobacterium* spp. is associated with ulcerative colitis (Rajilić-Stojanović *et al.*, 2013a) and colorectal cancer (Castellarin *et al.*, 2012; Kostic *et al.*, 2012). The human gastrointestinal tract-associated *Fusobacteria* also include representatives of the *Leptotrichia* genus and the misclassified *C. rectum* (Fig. 20).

Proteobacteria

Bacteria belonging to the phylum *Proteobacteria* are commonly detected in the gastrointestinal samples and this group of the true Gram-negative bacteria is particularly diverse, although not very abundant – typically all *Proteobacteria* account about 1% of the total microbiota (Holdeman *et al.*, 1976). Members of five different classes of *Proteobacteria*, namely the *Alpha-*, *Beta-*, *Gamma-*, *Delta-* and *Epsilonproteobacteria* can be part of the gastrointestinal microbiota, and among them *Enterobacteriaceae* within *Gammaproteobacteria* are the most abundant and the prevalent group.

Alphaproteobacteria

Gemmiger formicilis was the first and the only bacterium from the *Alphaproteobacteria* class that was associated with the human gastrointestinal tract, throughout the

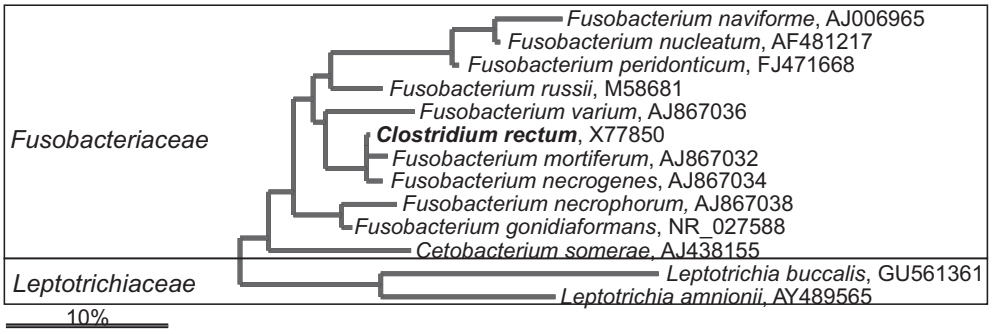

Fig. 20. Phylogenetic tree the human gastrointestinal species that belong to the phylum of the *Fusobacteria*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold are based on the SSU rRNA gene sequence clustering within the families that are designated on the figure in contrast to their official classification.

20th century (Holdeman *et al.*, 1976; Benno *et al.*, 1986; Moore & Moore, 1995; Macfarlane *et al.*, 2004). Ironically, the recent SSU rRNA gene sequence analysis of *G. formicilis* indicated that this species has been misclassified and belongs to *Firmicutes* (*Clostridia*/*Clostridiales*/*Ruminococcaceae*) and has almost an identical SSU rRNA gene sequence as *Subdoligranulum variabile* (98.3% sequence similarity). Nevertheless, other representatives of the true *Alphaproteobacteria* seem to be a part of the normal gastrointestinal microbiota. In a recent study, four alphaproteobacterial species were cultivated from gastrointestinal samples (Lagier *et al.*, 2012a). Twenty-four other members of this subdivision were detected by retrieving SSU rRNA gene sequences identical to previously cultured bacteria from gastrointestinal samples (Fig. 21, Table S1). *AlphapProteobacteria* seem to be characteristic for the upper part of the gastrointestinal tract as sequences of these bacteria were detected only in studies where samples from the upper intestine were included (Eckburg *et al.*, 2005; Wang *et al.*, 2005), or when the microbiota of patients with ileal pouch was analyzed (McLaughlin *et al.*, 2010). There are no data that correlate *Alphaproteobacteria* with any specific function in the gastrointestinal tract or any disease. However, some genera are known to perform specific metabolic transformations — for example, *Methylobacterium* spp. include bacteria that can oxidize methylamine or methanol to generate energy. Although the metabolism of these compounds has not been studied in the gastrointestinal tract, and the methylotrophic community is not typically associated with the human body, a recent study has demonstrated that methanotrophs are ubiquitous in the human oral microbiota (Hung *et al.*, 2011). Similarly, it can be anticipated that they are present in the upper gastrointestinal tract, where SSU rRNA gene sequences of these bacteria were detected, and where oxygen needed for their metabolic activity is present. *Sphingomonas* spp. include metabolically versatile aerobic bacteria that can be found in different environments. Studies of these and most other *Alphaproteobacteria* focus on outbreaks of infections involving these bacteria in immuno-suppressed patients. However, animal models studies have shown that *Sphingomonas* spp. are important for the development of the immune system of the host, since these species can stimulate the maturation of invariant natural killer T cells (Wingender *et al.*, 2012).

Betaproteobacteria

The first bacterium from the *Betaproteobacteria* class — *Alcaligenes faecalis* — was isolated from a human fecal sample in 1896 (Petruschky, 1896). This asaccharolytic rod that can utilize urea, a range of amino-acids, and can produce nitric oxide (NO; Denault *et al.*, 1953) has fre-

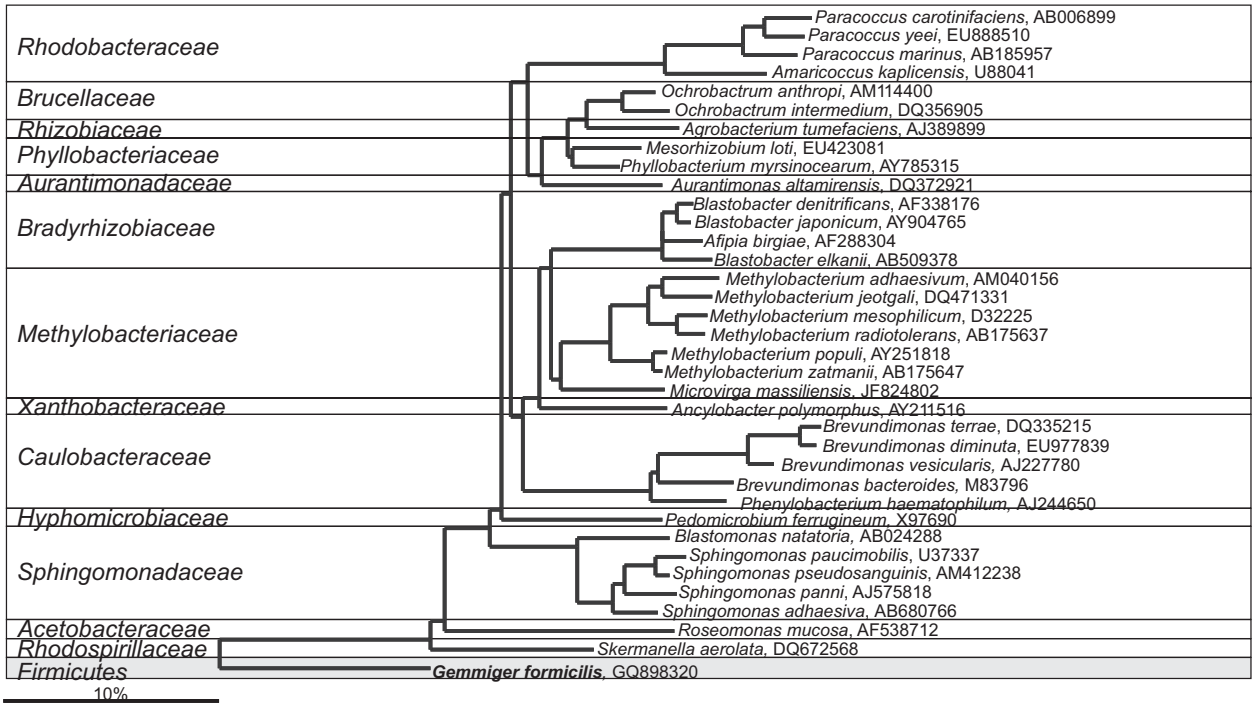

Fig. 21. Phylogenetic tree the human gastrointestinal species that belong to the class of the *Alphaproteobacteria* class. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

quently been detected in later studies of the gastrointestinal microbiota. A number of other bacteria within this class have been detected in recent years, showing that *Betaproteobacteria* are diverse and ubiquitous members of the gastrointestinal microbiota (Fig. 22). Among them, *Sutterella* and *Parrasutterella* are the most frequently encountered and the abundant gastrointestinal microorganisms (Nagai *et al.*, 2009; Mukhopadhya *et al.*, 2011). These bacteria are typical for the gastrointestinal microbiota ecosystem, assacharolytic and inactive in classical microbiological tests. Their abundance is increased in autistic children (Williams *et al.*, 2012) and in patients suffering from type 2 diabetes (Larsen *et al.*, 2010). Hence, it is of interest to define which metabolic transformations are catalyzed by these bacteria, as they might be particularly relevant for understanding the global impact of the gastrointestinal microbiota on human health. *Alcaligenes faecalis* and related bacteria are common gastrointestinal tract inhabitants, notably of the ileum. *Alcaligenes* spp. may be opportunists that inhabit Peyers' patches and signal to the immune system as shown in model animals (Obata *et al.*, 2010). Moreover, *Alcaligenes* spp. produce NO, which is an important biological regulator (Culotta & Koshland, 1992; Anderson *et al.*, 1993).

Oxalobacter formigenes is another relevant intestinal inhabitant. It has been isolated in 1985 and described as a unique intestinal bacterium that degrades exclusively oxalate and can reach densities of up to 10^7 cells g^{-1} of feces (Allison *et al.*, 1985). Due to its metabolic activity, *O. formigenes* regulates oxalate concentrations in feces and urine, and indirectly influences the formation of kidney stones (Duncan *et al.*, 2002b). This has led to its application in probiotic formulations. Recently, it has been shown that some strains of another intestinal inhabitant *Ancylobacter polymorphus* (belonging to the *Alphaproteobacteria*) can utilize oxalate (Lang *et al.*, 2008), suggesting that there are alternative pathways for oxalate removal from the gastrointestinal tract.

Neisseria spp. are inhabiting mucosal surfaces of the genital, the respiratory and the upper gastrointestinal tract. *Neisseria gonorhhoea* and *Neisseria meningitis* are the most studied as they are important pathogens causing gonorrhoea and meningitis. However, the majority of *Neisseria* spp. are nonpathogenic and their presence in the gastrointestinal tract samples was detected in the early cultivation-based studies (Gray & Shiner, 1967; Bhat *et al.*, 1980). The presence of specific *Neisseria* spp. in the gastrointestinal tract was only recently reported in a high-throughput culturing study (Lagier *et al.*, 2012a) and emerged from SSU rRNA gene sequencing-based studies (Fig. 22, Table S1). Nonpathogenic *Neisseria* spp. do not catabolize many carbohydrates, while some species are even asaccharolytic, but they can reduce nitrate. It is known that *Neisseria* spp. are able to grow on amino acids and can use sulfur directly from sulfate (McDonald & Johnson, 1975), but their function in the gastrointestinal tract has not been exploited.

The human gastrointestinal *Betaproteobacteria* also include a number of other species within various genera,

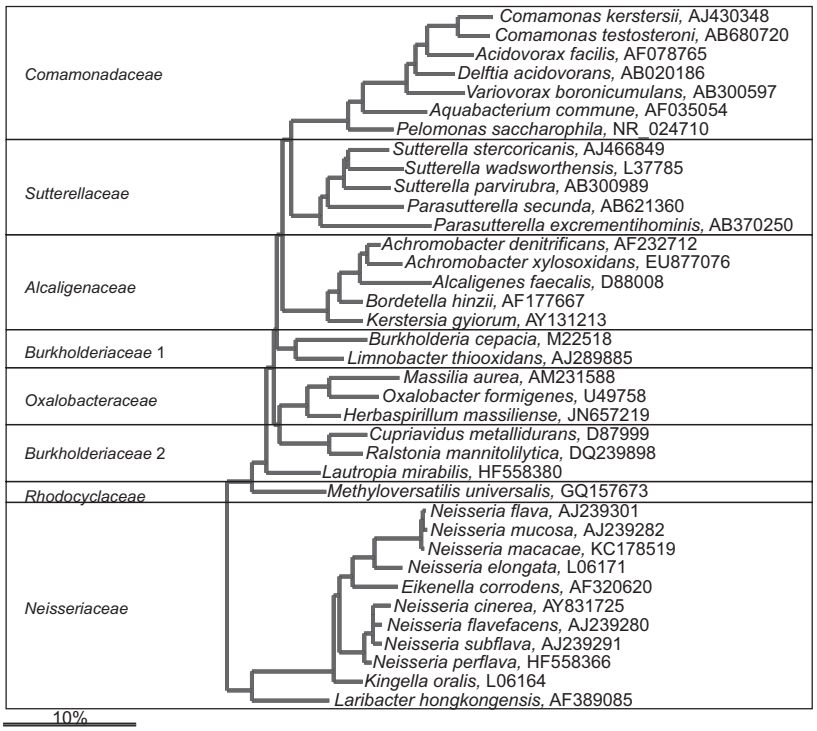

Fig. 22. Phylogenetic tree the human gastrointestinal species that belong to the class of the *Betaproteobacteria*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

which are occasionally reported as members of the gastrointestinal microbiota (Fig. 22). Among them, *Acidovorax* spp. were found to be ubiquitously present in the colonic mucosa (Hong *et al.*, 2011), *Burkholderia* spp. were found in an increased abundance in hepatic encephalopathy and were linked to poor cognition and inflammation (Bajaj *et al.*, 2012), while *Variovorax* spp. seem to be particularly abundant in the upper gastrointestinal tract (van den Bogert *et al.*, 2011). There is no available information about association of other species with specific gastrointestinal sites, diseases or functions of the gastrointestinal microbiota.

Gammaproteobacteria

Within the class *Gammaproteobacteria*, nine different families distributed within six different orders have been detected in the human gastrointestinal samples. *Escherichia coli* was the first bacterial isolate of the *Gammaproteobacteria* characterized from human gastrointestinal samples in 1885 (Shulman *et al.*, 2007). *Escherichia coli* belongs to the *Enterobacteriaceae* family, which is the most diverse, prevalent and abundant of all gastrointestinal *Proteobacteria* (Fig. 23). Most of *Enterobacteriaceae* members are associated with diarrhea (Thielman & Guerrant, 2004), although representatives of this family are not necessarily causing any symptoms and are actually one of the first to be found in the newborn gastrointestinal tract (Favier *et al.*, 2002). The abundance of this bacterial group increases with age, but it remains subdominant and in elderly subjects represents about 1% of the total gastrointestinal microbiota (Hopkins *et al.*, 2001). *Escherichia coli* is the most prevalent representative of this family that is often the most abundant facultative anaerobe in the gastrointestinal samples. The different strains of *E. coli* can exhibit different properties, varying from probiotic (Kruis *et al.*, 2004) to pathogenic causing diarrhea or infections on other sites (Ron, 2006). The majority of other *Enterobacteriaceae* spp. are infrequently isolated from gastrointestinal samples (Bucher & von Graevenitz, 1982; Müller, 1986). In that line, a study of dedicated isolation of the *Morganella-Proteus-Providencia* group from feces of almost 3000 healthy subjects and patients suffering from enteric diseases, showed that species of this group are subabundant and have joined prevalence between 10% and 20%, depending on the health status (Müller, 1986). Still, the *Enterobacteriaceae* is one of the most comprehensively described gastrointestinal families, which can be explained by its development as a paradigm for genetic studies and its clinical relevance (Grimont *et al.*, 1981; Hickman-Brenner *et al.*, 1984; Farmer *et al.*, 1985; Hickman-Brenner *et al.*, 1985a, b). A specific case is represented by the so-called adherent-invasive *E. coli* strains that have been implied in various forms of inflammation in the gastrointestinal tract of human and animal models (Negroni *et al.*, 2012; Chassaing *et al.*, 2014). Phylogenetically, the *Enterobacteriaceae* is a diverse group and while some genera form separate clusters (e.g., *Yersinia*), species of other genera are mixed up in the SSU rRNA phylogenetic tree (Fig. 23). The absence of genus-specific SSU rRNA gene sequences for these genera, could explain a recent the finding that sequences assigned to *E. coli, Salmonella enterica, Citrobacter koseri,* and *Enterobacter cancerogenus* appear together (Lozupone *et al.*, 2012).

In addition to the *Enterobacteriaceae*, representatives of eight other bacterial families with the *Gammaproteobacteria* class can be detected in the human gastrointestinal tract (Fig. 24). Among these, members of the *Moraxellaceae* are relatively frequently detected using both cultivation-based and molecular studies. Within this family, *Acinetobacter* spp. are the most diverse and are frequently detected in infants (Chang *et al.*, 2011; Pandey *et al.*, 2012), with an increased abundance in infants that develop allergy (Nakayama *et al.*, 2011). A recent study has indicated that members of the *Gammaproteobacteria* and in particular *Haemophilus* spp. are elevated in irritable bowel syndrome pediatric patients (Saulnier *et al.*, 2011). Members of the same phylogenetic group were found to correlate with irritable bowel syndrome symptom score in an independent study (Rajilić-Stojanović *et al.*, 2011). *Haemophilus* spp. are relatively frequently detected in the upper parts of the gastrointestinal tract of healthy humans of different ages (Justesen *et al.*, 1984; Ou *et al.*, 2009), but also can be detected inflamed and stool specimens from children with diarrhea, with relatively low prevalence (Mégraud *et al.*, 1988).

Aeromonas spp. are medically significant as these species are implicated in the development of the gastroenteritis and diarrhea. The role of these bacteria in human health has been a subject of a long-lasting debate resulting in a conclusion that at least four gastrointestinal species (*A. caviae, A. hydrophila, A. jandaei,* and *A. veronii*), are pathogenic (Janda & Abbott, 1998). The presence of these species in the gastrointestinal tract is not necessarily inducing any symptoms, although *in vitro* experiments have indicated that they are cytotoxic and induce lesions in the intestinal mucosa (Pitarangsi *et al.*, 1982).

The *Succinivibrionaceae* family groups strictly anaerobic bacteria that ferment carbohydrates to produce succinate and acetate. Representatives of three genera of this family can be detected in the human gastrointestinal tract: *Anaerobiospirillum* spp. are motile, spiral bacteria that are implicated in the development of diarrhea (Malnick, 1997); while *Succinatimonas* and *Succinivibrio* representatives are subdominant bacteria that were isolated from healthy humans (Table S1).

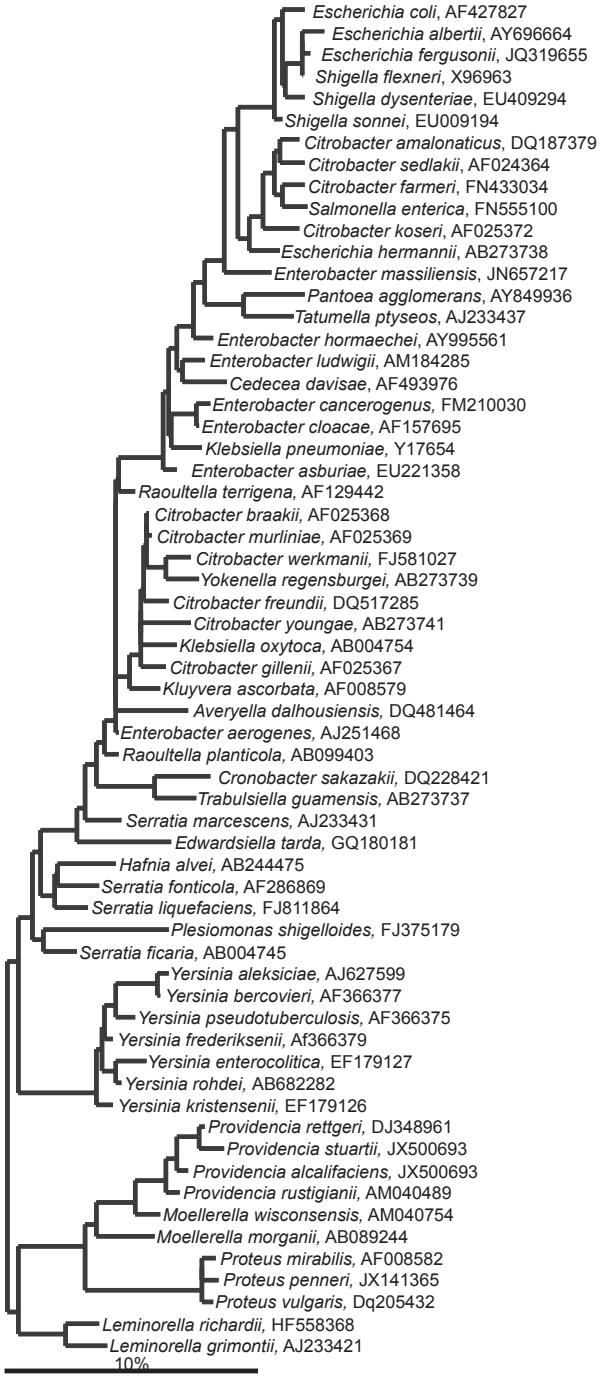

Fig. 23. Phylogenetic tree the human gastrointestinal species that belong to the family of the *Enterobacteriaceae*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

Members of the *Vibrionaceae* are pathogens that cause acute, self-limiting gastroenteritis (Hou *et al.*, 2011). The natural habitat of *Vibrio* spp. is the aquatic ecosystem, and hence they are only rarely detected in the human gastrointestinal samples — notably after infection that induces diarrhea.

Eight different *Pseudomonas* spp. can be detected in the human gastrointestinal tract. Among them, *Pseudomonas aeruginosa* is the most prevalent and was frequently reported as a member of the fecal microbiota of healthy humans (Finegold *et al.*, 1974; Benno *et al.*, 1986). Although a member of the normal gastrointestinal microbiota, *Pseudomonas aeruginosa* can act as an opportunistic pathogen in critically ill or immuno-suppressed patients and cause sepsis as it can interact with our immune system (Wu *et al.*, 2005) and can disrupt the intestinal epithelial barrier (Zaborina *et al.*, 2006). *Pseudomonas fluorescens* is a less prevalent member of the genus that has been implicated in the development of Crohn's disease (Wei *et al.*, 2002). A recent study showed that *Pseudomonas* spp., among other *Proteobacteria*, have increased abundance in infants with colic (de Weerth *et al.*, 2013).

Until today, only two bacteria from *Xanthomonadaceae* have been isolated from gastrointestinal samples: *Stenotrophomonas maltophilia* from a stool and an ileal sample derived from atypical clinical cases (Tamura *et al.*, 1988; Apisarnthanarak *et al.*, 2003), and *Lysobacter soli* in a recent high-throughput cultivation of the normal gastrointestinal microbiota (Lagier *et al.*, 2012a). In addition, four other related bacterial species were detected based on the SSU rRNA gene sequence. These bacteria were previously isolated from different ecosystems and include *Nevskia ramose* and *Rhodanobacter ginsenosidimutans* from soil, *Pseudoxanthomonas mexicana* from sludge and urine, and *Silanimonas lenta* from a hot spring. Based on their low prevalence in the gastrointestinal tract, it is most likely that *Xanthomonadaceae* are transient members of the gastrointestinal microbiota.

Deltaproteobacteria

Sulfate-reducing bacteria that cluster within the δ class of the phylum *Proteobacteria* inhabit the human gastrointestinal tract where they utilize sulfate that can be diet derived or released from mucins. Human gastrointestinal tract-associated sulfate-reducing bacteria include the acetate-utilizing *Desulfobacter* spp., the lactate-, and H_2-utilizing *Desulfovibrio* spp., and the propionate-utilizing *Desulfobulbus* spp. (Gibson *et al.*, 1988). This group of related bacteria has been subject of numerous studies because the end-product of their metabolism — hydrogen sulfide — is a highly toxic compound that inhibits butyrate oxidation within the colonocytes (Attene-Ramos *et al.*, 2006). Hydrogen sulfide overproduction in the gastrointestinal tract has been linked to ulcerative colitis and colon cancer. Although some cultivation, studies showed an association between the presence of sulfate-reducing

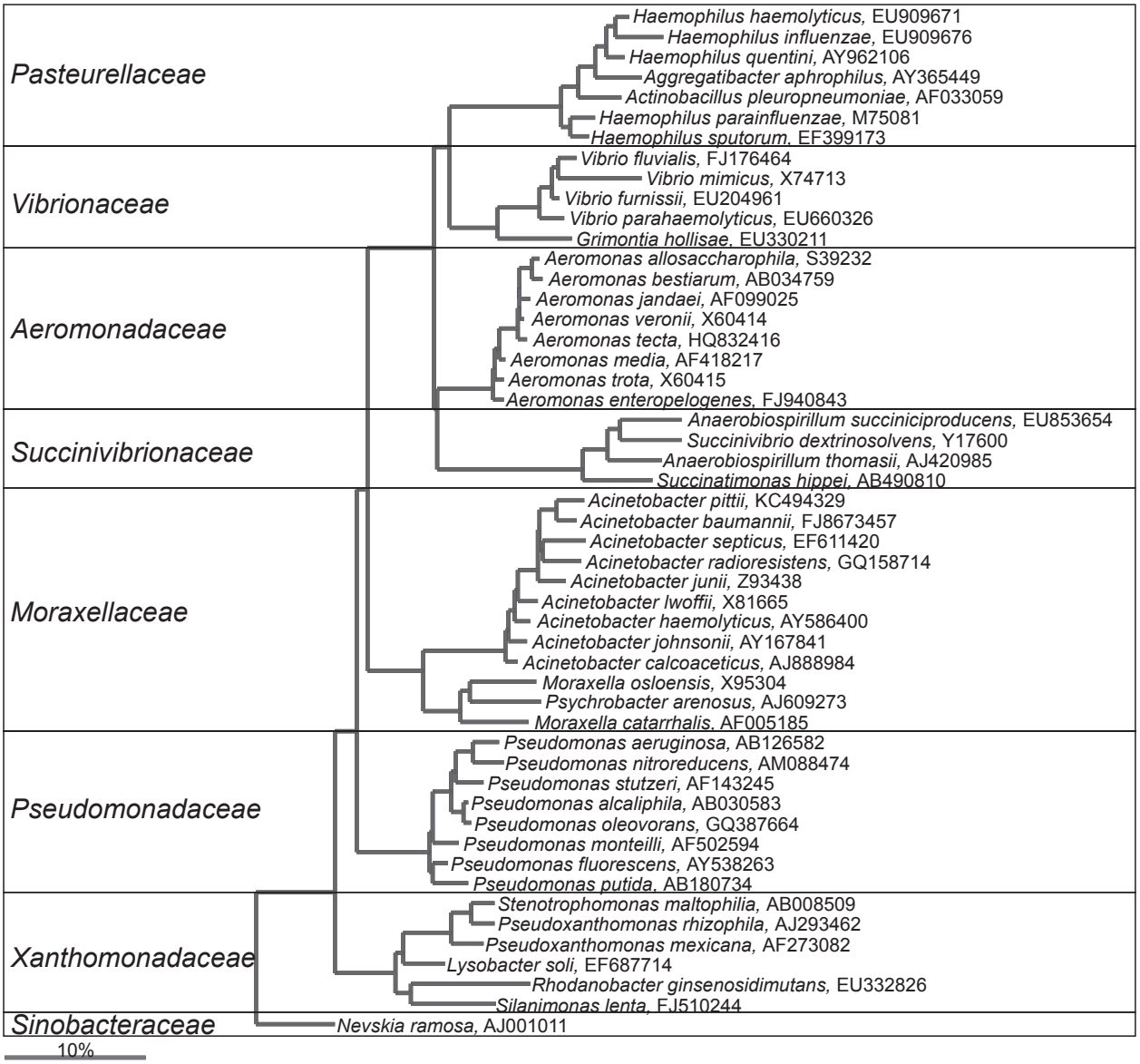

Fig. 24. Phylogenetic tree the human gastrointestinal species that belong to the class of the *Gammaproteobacteria* without *Enterobacteriaceae* family. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated.

bacteria and ulcerative colitis, the overproduction of hydrogen sulfide has a stronger correlation with dietary protein (Magee *et al.*, 2000) than with dietary sulfate (Deplancke *et al.*, 2003), challenging the hypothesis that the metabolic activity of sulfate-reducing bacteria is involved in compromised health, at least in healthy subjects. Sulfate-reducing bacteria are normally present in low abundance, of approximately 10^6–10^7 cells g^{-1} (Fite *et al.*, 2004), and although at least three different genera of this group of bacteria can be found in the human gastrointestinal tract, only *Desulfovibrio* spp. are characterized below the genus level (Fig. 25). In addition, *Bilophila wadsworthia* is another member of *Deltaproteobacteria* that is present in approximately half of the studied humans (Baron *et al.*, 1992; Baron, 1997). *Bilophila wadsworthia* is capable of utilizing taurine, which is released by deconjugation of bile salts or present in the diet, and also generates hydrogen sulfide as the major end-product. Its involvement in promoting colitis via taurine metabolism in mice has recently been established (Devkota *et al.*, 2012).

Epsilonproteobacteria

The class of *Epsilonproteobacteria* is represented by two main genera in the human gastrointestinal tract: *Campylo-*

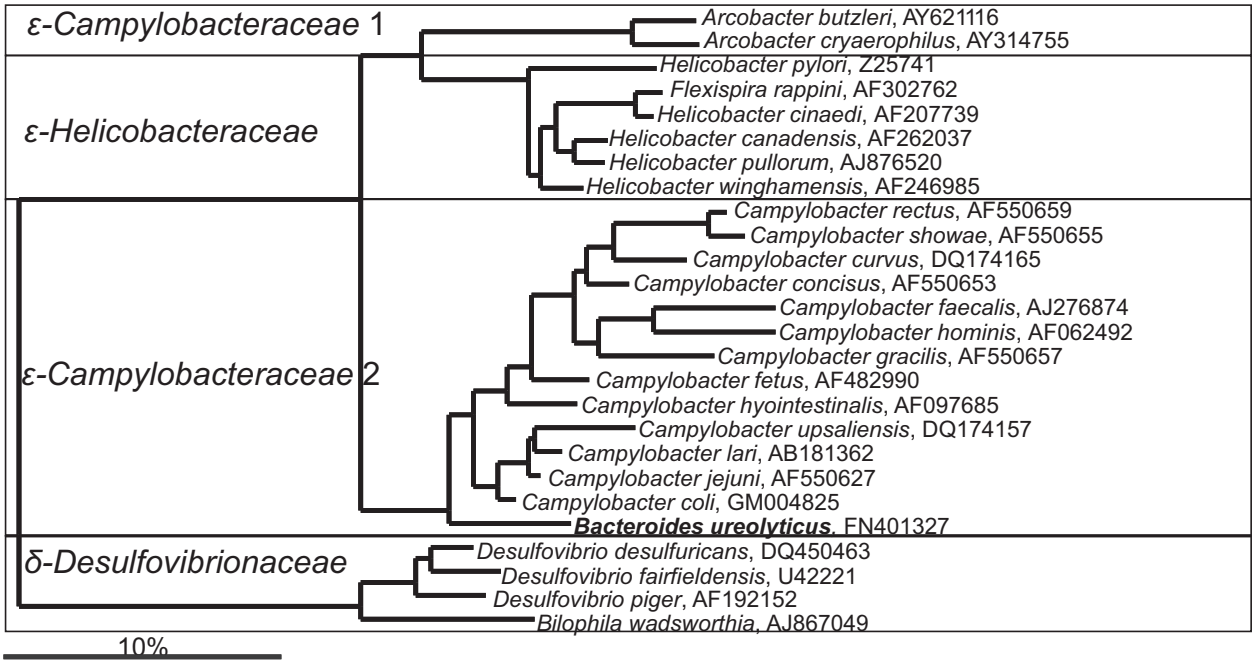

Fig. 25. Phylogenetic tree the human gastrointestinal species that belong to the classes of the *Deltaproteobacteria* and *Epsilonproteobacteria*. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the family names are indicated. The species indicated in bold is based on the SSU rRNA gene sequence clustering within the indicated family in contrast to its official classification.

bacter and *Helicobacter*. *Campylobacter* is a genus that groups diverse isolates from mucosal surfaces of gastrointestinal, oral and urogenital tract. The first report of *Campylobacter* isolation from the human gastrointestinal tract dates from 1946 (Levy, 1946). *Campylobacter* spp. were originally described as members of genus *Vibrio*, and reclassified into an independent genus in 1973 (Veron & Chatelain, 1973). Species of this genus are principally considered to be pathogenic organisms involved in diarrheal illness. However, *Campylobacter hominis* has been isolated from a gastrointestinal sample of a healthy subject (Lawson *et al.*, 2001), while *C. concisus* is a clinical isolate that also has been recovered from healthy individual (Engberg *et al.*, 2000). Finally, a recent study of the microaerophilic fecal microbiota of children revealed diverse and prevalent colonization of *Campylobacter* spp. of gastrointestinal tract of both healthy and children suffering from inflammatory bowel diseases (Hansen *et al.*, 2013). The presence of *Campylobacter* spp. in the gastrointestinal tract can cause watery or bloody diarrhea, whereas it also can be associated with intestinal diseases such as ulcerative colitis (Rajilić-Stojanović *et al.*, 2013a). However, *Campylobacter* spp. can also remain asymptomatic, suggesting that at least some species of this genus are commercial members of the gastrointestinal microbiota.

The *Arcobacter* genus was introduced to accommodate an independent cluster identified based on the SSU rRNA gene sequences, of species that were previously classified within the *Campylobacter* genus (Vandamme *et al.*, 1991). These bacteria are also associated with diarrheal disease, although the prevalence of their isolation, even from clinical samples is very low (Engberg *et al.*, 2000).

The genus *Helicobacter* has been derived from *Campylobacter* after reclassification of the latter (Goodwin *et al.*, 1989; Vandamme *et al.*, 1991). *Helicobacter* spp. are spiral-shaped bacteria that were detected in human gastric mucosa as late as in 1906 (Krienitz, 1906). They received exceptional attention once *Helicobacter pylori* was discovered to induce the gastric and duodenal ulcers (for recent review see, Fock *et al.*, 2013). *Helicobacter* spp. are mainly located in the stomach but can be detected in the other gastrointestinal samples of healthy individuals but only when highly sensitive techniques are applied, suggesting that they may lyse and disappear in transit from the stomach (MacKay *et al.*, 2003; Ceelen *et al.*, 2005). Hence, their prevalence is low in samples from the lower gastrointestinal tract (Hansen *et al.*, 2013).

Lentisphaerae

Victivallis vadensis is the only species within the phylum of *Lentisphaerae* that has been isolated from the gastrointestinal tract (Fig. 26). This species was isolated in 2003, as a bacterium that was able to grow in basal liquid

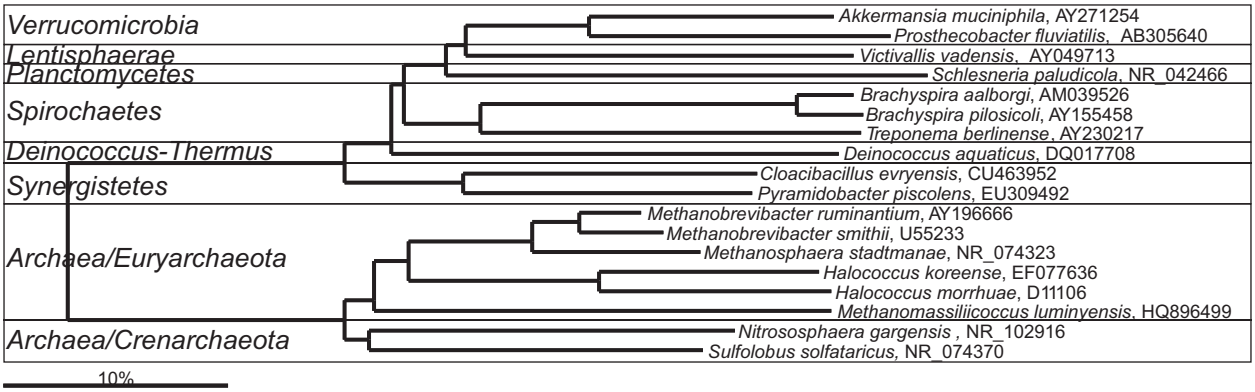

Fig. 26. Phylogenetic tree the human gastrointestinal species that belong to the different bacterial phyla with limited diversity, and two archaeal phyla. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the phylum names are indicated.

medium containing cellobiose as the sole carbon source, but not on the same medium solidified with agar (Zoetendal *et al.*, 2003). Further studies have confirmed its presence in the gastrointestinal tract of humans (Claesson *et al.*, 2012), indicating the adaptation of this species (and most likely the entire bacterial group) to the gastrointestinal tract conditions. The genome of this bacterium has been sequenced (van Passel *et al.*, 2011), revealing a host of functions, but its activity in the gastrointestinal tract and its impact on the human host remains to be determined.

Spirochetes

The first report of the *Spirochetes* in the human gastrointestinal samples dates from 1923 when a cultivation-based study reported the 28% prevalence of these bacteria in fecal samples of healthy individuals (Parr, 1923). The *Spirochetes* are established pathogens in veterinary medicine and their pathogenicity in humans has been debated for a long period of time. Two species of the phylum *Spirochetes* can be detected in the human gastrointestinal samples: *Brachyspira pilosicoli* and *Brachyspira aalborgi* (Fig. 26). Their presence in the gastrointestinal tract is termed intestinal spirochetosis, which in clinical cases is associated with abdominal pain and diarrhea. However, a retrospective review of cases diagnosed as intestinal spirochetosis revealed that the presence of *Spirochetes* in the gastrointestinal biopsies is often asymptomatic and may not have pathological significance (Carr *et al.*, 2010). The question of implication of the *Spirochetes* in clinical cases might be a question of their density in the gut. These bacteria typically colonize intestinal mucosa where they attach and penetrate short distances into the surface epithelial cells (Harland & Lee, 1967). In clinical cases of spirochetosis they form a dense biofilm that covers the entire colonic surface, as indicated by scanning electron microscopy images (Gad *et al.*, 1977). Another factor might be relevant to the currently undefined role of the *Spirochetes* in human health. A recent molecular study showed that in addition to the two cultured species, another, currently uncultured *Brachyspira* spp. is more frequent than the other two *Brachyspira* spp. in the human colonic biopsies. Although this study confirmed the absence of a correlation between these species and physical complaints, it appeared that *B. pilosicoli* is associated with intestinal inflammation (Westerman *et al.*, 2012).

Another relevant group of the gastrointestinal Spirochetes are formed by the *Treponema* spp. Members of the *Treponema* genus were detected in molecular-based studies of the gut microbiota of five geographically separate rural African and Native American tribes (De Filippo *et al.*, 2010; Tito *et al.*, 2012; Yatsunenko *et al.*, 2012; Ou *et al.*, 2013; Schnorr *et al.*, 2014). Only in one study, a representative of this group was identified at species level. A sequence 99% similar to *Treponema berlinense* was detected when analyzing the microbiota coprolite (fossilized feces) taken from archaeological site in Mexico (Tito *et al.*, 2012). *Treponema* spp. are in principle considered to be pathogenic in industrial societies (Giacani & Lukehart, 2014), but its reproducible detection in the gastrointestinal microbiota of isolated rural communities suggest alternative symbiotic roles played by these bacteria. These might include degradation of fiber rich foods and enhancement of anti-inflammatory capability, as suggested by de Fillipo and coauthors, who were the first to detected *Treponema* spp. in healthy human gut (De Filippo *et al.*, 2010).

Synergistetes

Synergistetes is a recently recognized bacterial phylum that is typically subdominant in the ecosystems where it resides, and its members can be present in abundance of about

0.01% in human fecal samples (Horz *et al.*, 2006). The first attempt to detect this group of bacteria in the human gastrointestinal tract yielded a sole SSU rRNA gene sequence that is identical to that of a later on cultured *Cloacibacillus evryensis* (Ganesan *et al.*, 2008). *Cloacibacillus* spp. are amino acid degrading bacteria that use sulfate as terminal electron acceptor and that are capable to use mucin as sole carbon source (Looft *et al.*, 2013). Their presence in the human gastrointestinal microbiota was confirmed by several molecular studies. Furthermore, a recent high-throughput culturomics study retrieved another gastrointestinal *Synergistetes* bacterium — *Pyramidobacter piscolens* (Lagier *et al.*, 2012a). *Pyramidobacter piscolens* was described in 2009, as an asaccharolytic, anaerobic oral isolate capable of hydrogen sulfide production.

Although a minor group, the gastrointestinal *Synergistetes* might be relevant for the human health, as indicated by their increased abundance in mucosal samples associated with colorectal cancer (Chen *et al.*, 2012). Mucin degradation coupled with hydrogen sulfide produced by these bacteria might be relevant for the colorectal cancer etiology, since the produced metabolite increases mucosal apoptosis, goblet cell depletion, and superficial ulceration (Aslam *et al.*, 1992; Deplancke & Gaskins, 2003).

TM7 candidate phylum

The TM7 phylum represents a recently recognized, widely distributed group of yet uncultured filamentous bacteria (Hugenholtz *et al.*, 2001). These bacteria can be detected in the human oral cavity and the gastrointestinal tract, while a recent study of the microbiota along the gastrointestinal tract has shown that TM7 bacteria are one of the common microorganisms, widely distributed among humans (Stearns *et al.*, 2011). Although the presence of these bacteria is not determinative of the health status, it has been shown that different TM7 bacteria inhabit the gastrointestinal tracts of inflammatory bowel disease patients and healthy controls (Kuehbacher *et al.*, 2008).

Verrucomicrobia

Currently, only two species within the *Verrucomicrbia* phylum have been detected in the human gastrointestinal tract. *Akkermansia muciniphila* was described in 2004 as a unique human gastrointestinal bacterium that is able to grow on intestinal mucus as a sole carbon source (Derrien *et al.*, 2004). This bacterium is widely distributed and can be detected in fecal material of humans of all age groups (Collado *et al.*, 2007), and although its abundance varies between subjects, it is probably one of the members of the core microbiota. Recent literature shows that *A. muciniphila* is important for a healthy host as its decreased abundance is associated with compromised health including acute appendicitis (Swidsinski *et al.*, 2011), ulcerative colitis (Vigsnæs *et al.*, 2012; Rajilić-Stojanović *et al.*, 2013a), autism (Wang *et al.*, 2011a), and atopic diseases (Candela *et al.*, 2012). Finally, the abundance of *A. muciniphila* is inversely correlated with obesity (Karlsson *et al.*, 2012). A recent study suggests that *A. muciniphila* plays a pivotal role in obesity as its duodenal delivery regulates fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance in an animal model experiment (Everard *et al.*, 2013). Given the very short history of the research related to *A. muciniphila*, the wealth of data that support its beneficial role provides evidence of the remarkable importance of this bacterium. In addition, one other species of the *Verrucomicrobia* — *Prosthecobacter fluviatilis* — has been detected in the study of bacteria of a patient with ileal pouch (McLaughlin *et al.*, 2010), while sequences classified within this genus were detected in the study of the microbiota of infants (Palmer *et al.*, 2007).

Other gastrointestinal bacterial phyla

There are a few gastrointestinal bacterial phyla with low diversity that have not yet been discussed, including the *Deinococcus-Thermus* bacteria, the *Melainabacteria* the *Gemmatimonadetes* and the *Planctomycetes* (Fig. 26). The *Deinococcus-Thermus* bacteria were for the first time associated with the human gastrointestinal microbiota in 2006, when uncultured phylotypes within this phylum were recovered in a molecular survey of the microbiota of the human stomach (Bik *et al.*, 2006). Several studies have confirmed the presence of these bacteria in the gastrointestinal microbiota, while a recent high-throughput culturing study detected a single cultivated bacterium of this phylum (Lagier *et al.*, 2012a). Currently, no particular function has been assigned to this bacterial group, although there is evidence that these bacteria are active in the distal parts of the gastrointestinal tract (Peris-Bondia *et al.*, 2011).

Molecular studies have retrieved SSU rRNA gene sequences from the gastrointestinal tract of humans and other animals that cluster into a distinct clade, related to, but separate from cultured photosynthetic *Cyanobacteria* (Ley *et al.*, 2005). A novel name – *Melainabacteria* — was recently proposed for this group of bacteria (Di Rienzi *et al.*, 2013). Sequences representing these bacteria were detected in the stomach (Andersson *et al.*, 2008), while two independent studies have shown that these bacteria are active in the distal part of the human gastrointestinal tract (Rajilić-Stojanović *et al.*, 2008; Peris-Bondia *et al.*, 2011), which indicates their wide distribution along the

gastrointestinal tract. The role of these bacteria in the ecosystem is currently undetermined and although no cultured representatives are available now, the genomes of these bacteria have been sequenced and annotated. Based on the genomic information, it was concluded that these are motile, strictly anaerobic, fermentative bacteria (Di Rienzi *et al.*, 2013).

In addition to the bacterial phyla that are reproducibly detected by many studies of the human gastrointestinal microbiota, some bacterial phyla are only occasionally found. For instance, some studies have reported the presence of the *Gemmatimonadetes* in the human gastrointestinal tract (Andersson *et al.*, 2008), but even when present, these bacteria constitute an extremely minor fraction, as indicated by the fact that only one of over 15 000 SSU rRNA gene sequences in the study of the microbiota of inflammatory bowel disease patients and controls originated from a *Gemmatimonadetes* bacterium (Frank *et al.*, 2007). Furthermore, a few pyrosequencing studies have reported *Nitrospira* bacteria in the human gastrointestinal tract (Hung *et al.*, 2011). It is interesting to note that in a recent study of the microbiota of pediatric irritable bowel syndrome patients only one *Nitrospira* phylotype was detected by pyrosequencing. However, the further attempts to retrieve this bacterium revealed that the detected sequence was actually retrieved from a novel taxon related to the genus *Ruminococcus* (Saulnier *et al.*, 2011). This example testifies to the fact that identification of sequences based on very short reads, such as those produced by currently used high-throughput sequencing technologies, are not always reliable (Werner *et al.*, 2012).

The presence of the *Planctomycetes* in the human gastrointestinal tract was reported only in molecular-based studies (Wilson & Blitchington, 1996; De Filippo *et al.*, 2010; Hong *et al.*, 2011). A sequence of an uncultured bacterium (Gene Bank Accession Number U58225) was reported in one of the first molecular studies of the human gastrointestinal microbiota, and was designated as an uncultured *Plantomycetes* bacterium (Wilson & Blitchington, 1996). However, the detected SSU rRNA gene sequence was probably retrieved from a representative of a new genus within the *Lentisphaerae* phylum, since it has the highest similarity (88%) with *V. vadensis* (that was isolated and described after publication of the molecular study). Nevertheless, *Plantomycetes* might be a part of the human gastrointestinal microbiota, as a recent review indicated the detection of a diverse community of the *Planctomycetes* in the human gastrointestinal tract (Lagier *et al.*, 2012b). When analyzing the publicly available data, we found that only one (JQ287572) had high similarity (98%) to the SSU rRNA gene sequence of a cultured bacterium—*Schlesneria paludicola*. However, based on the characteristics of this bacterium (e.g., it grows in the temperature range 4–32 °C), it is not likely that this bacterium resides in the human gastrointestinal tract.

Archaea

Bacteria are the dominant but not an exclusive component of the human gastrointestinal microbiota. *Archaea*, primarily the methanogenic ones, can be relatively abundant component of the gastrointestinal microbiota with densities of up to 10^{10} cells g^{-1} of feces (Bond *et al.*, 1971; Miller & Wolin, 1986). In total, eight archaeal species have been associated with the human gastrointestinal tract (Fig. 26, Table S2). In an early cultivation study, which dates from 1968, a single methanogenic species, isolated from four of five individuals, was identified as *Methanobrevibacter ruminantium* (Nottingham & Hungate, 1968). Today, *Methanobrevibacter smithii* is recognized as the most abundant, and often an exclusive methanogen of human gastrointestinal microbiota (Miller & Wolin, 1986; Dridi *et al.*, 2009), which suggests a possible misidentification of the isolates in the earlier study. In addition to *M. smithii, Methanosphaera stadtmaniae* is a relatively prevalent, but atypical methanogenic archaea that reduces methanol and that can be found in human feces in low concentrations (Miller & Wolin, 1985; Dridi *et al.*, 2009). Similar to *M. stadtmaniae,* a recently isolated *Methanomassiliicoccus luminyensis* can also utilize methanol in the presence of hydrogen, but these two gastrointestinal archaeal species are phylogenetically distant (Dridi *et al.*, 2012). Methanogenic archaea have been extensively studied as the process of methane synthesis from carbon dioxide and hydrogen results in a significant gas removal in the gastrointestinal tract. The role of methanogenic archaea might be particularly relevant for bloating, which is one of the symptoms of irritable bowel syndrome, and a recent study has shown a highly significant (fourfold) reduction of methanogenic archaea in irritable bowel syndrome patients relative to controls (Rajilić-Stojanović *et al.*, 2011). In addition to methanogenic archaea, two cultured species of halophilic archaea have been detected in the study of the microbiota of Korean subjects (Nam *et al.*, 2008), while the presence of low numbers of these organisms was confirmed by the analysis of the colonic mucosa of inflammatory bowel patients (Oxley *et al.*, 2010). In addition to the confirmed presence of the *Euryarchaeota* phylum members, the human gastrointestinal archaea might also include a number of yet uncultured species within the *Thermoplasma* and the *Crenarchaeota* phylum and putative novel orders, as detected in molecular-based studies and recently reviewed (Dridi *et al.*, 2011). The presence of *Crenarchaeota* phylum representatives was detected by retrieving the specific partial SSU rRNA gene sequences in a study that dates

from 2005 (Rieu-Lesme *et al.*, 2005). One of the amplified SSU rRNA gene sequences (AY887079) shows 97% sequence similarity with SSU rRNA gene sequence of *Sulfolobus solfataricus*, and another (AY887074) 99% sequence similarity with the SSU rRNA gene of Candidatus *Nitrososphaera gargensis*. It should be noted that both *Sulfolobus solfataricus* and *Nitrososphaera gargensis* are hyperthermophilic species, and it is highly unlikely that these species inhabit the gastrointestinal tract of humans. Until cultured representatives or at least the full SSU rRNA gene sequences of the *Crenarchaeota* species are obtained in the future independent studies, the presence of the this archaeal phylum in the human gastrointestinal tract remains questionable.

Eukarya

Different microeukaryal species can be detected in the human gastrointestinal tract, and although this group of organisms is subdominant, it is widely distributed component of the gastrointestinal microbiota. The first molecular-based study of this component of the gastrointestinal microbiota was published only recently (Scanlan & Marchesi, 2008). Very few other studies have been reported since, and the results of these have been recently summarized (Hamad *et al.*, 2013). The most prevalent human gastrointestinal Eukarya are yeasts, while a number of different microeukaryal intestinal parasites can be detected in the human gastrointestinal samples. These species have been excessively studied by epidemiologists, and although their presence is in most cases the result of an infection with contaminated food or water, some species establish in healthy humans and are probably a part of the normal gastrointestinal microbiota of some humans (Scanlan & Marchesi, 2008).

Fungi

The most prevalent *Eukarya* in the human gastrointestinal tract are yeast-like fungi and in total 57 intestinal species distributed between the two phyla, *Ascomycota* and *Basidiomycota*, have been detected (Fig. 27). The first report of yeasts in the human gastrointestinal tract dates from 1901 when *Candida albicans* was isolated from feces of patients infected with tropical sprue (Kohlbrugge, 1901). A thorough analysis of the yeasts diversity in the gastrointestinal tract in the early twentieth century showed that yeasts can be detected in about one out of five subjects and that the detected yeast community is similar in healthy subjects and patients suffering from various gastrointestinal disorders (Anderson, 1917). A recent molecular analysis of the fungal diversity allowed for detecting low amounts of fungi in any studied subject (Ott *et al.*, 2008). Among yeasts, *Candida* spp. are the most prevalent and there is considerable evidence that *C. albicans* and *C. rugosa* are part of the normal gastrointestinal microbiota, while other *Candida* spp. are scarcely detected in gastrointestinal samples (Fig. 27, Table S3). *Candida albicans* is early established in the ecosystem, as illustrated by the fact that it can be detected in over 95% of 1-month-old infants (Kumamoto & Vinces, 2005). *Candida* spp. are subdominant in the ecosystem and typically present in densities lower than 10^6 cells g^{-1} of intestinal content (Anderson, 1917; Finegold *et al.*, 1974; Finegold *et al.*, 1977). Although the natural environment of *Candida* spp. is the gastrointestinal tract, where they are either symbiotic or commensal to the human host, under specific circumstances these organisms can cause a variety of candidiasis in different organs of the human body. It has been proposed that in the developed world the increased intake of drugs, processed foods and pollutants can cause overgrowth of *Candida* spp. and trigger a *Candida*-associated complex of symptoms (Schulze & Sonnenborn, 2009). This might be relevant for gastrointestinal health, as it has been shown that Crohn's disease patients and their first relatives have a significantly higher abundance of *Candida* spp. compared to the controls (Standaert-Vitse *et al.*, 2009). Furthermore, there is a considerable overlap between the symptoms of the irritable bowel syndrome, and the symptoms of intestinal candidiasis (Santelmann & Howard, 2005), although the association between the irritable bowel syndrome and *Candida* spp. has not been adequately studied. Other yeasts in the gastrointestinal tract include several *Saccharomyces* spp., of which *Saccharomyces cerevisiae* was the most reproducibly detected in molecular studies (Nam *et al.*, 2008; Ott *et al.*, 2008; Scanlan & Marchesi, 2008). Furthermore, *Galactomyces geotrichum* has been detected in the human fecal samples using molecular techniques (Scanlan & Marchesi, 2008) and cultivation (Gouba *et al.*, 2013; Hamad *et al.*, 2013). In contrast to *Candida* spp. that have the gastrointestinal tract as their natural niche, both *Saccharomyces* spp. and *G. geotrichum* are yeasts that are used in food production and their detection in fecal samples of humans could be a result of the dietary intake prior to sampling. This hypothesis is supported by the results of the recently published gastrointestinal microbiota analysis of adults on extreme diets, as a number of foodborn microorganisms (including yeasts) were detected in the gastrointestinal tract of subjects on animal-based (meat and cheese rich) diet (David *et al.*, 2014).

Filamentous fungi are another group of *Eukarya* that can be present in the human gastrointestinal tract. Although they are not widely distributed, their presence was noticed in a number of studies (Finegold *et al.*, 1974; Finegold *et al.*, 1977; Benno *et al.*, 1986). Several *Aspergillus* spp. and *Penicillium* spp. have been identified

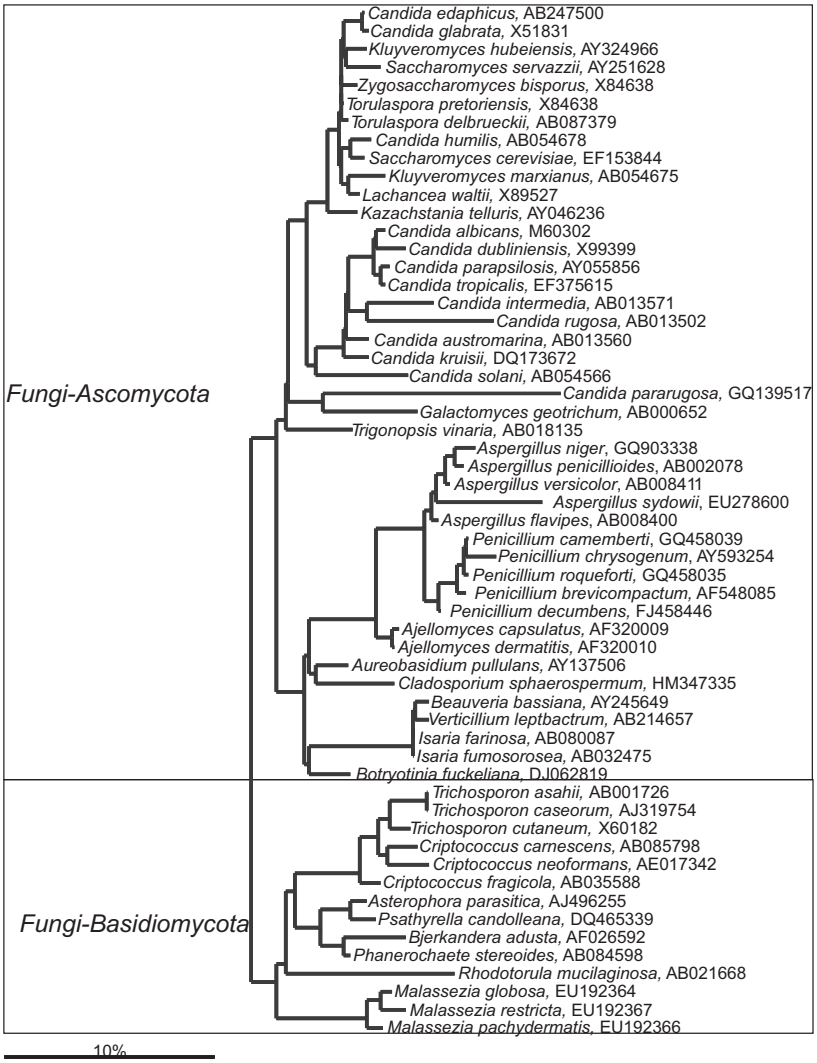

Fig. 27. Phylogenetic tree the human gastrointestinal Fungi. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the phylum names are indicated.

during a 9-year long culture-based monitoring of an immunodeficient child's microbiota (Taylor *et al.*, 1985), while different species of these two genera have been detected in recent years using both cultivation on various media (Gouba *et al.*, 2013), or molecular-based methods (Scanlan & Marchesi, 2008). Many of these species are associated with dietary sources, as they are used as starters for cheese production or ripening, whereas some are just food contaminants. Having in mind their source, and instability (as illustrated in the results of longitudinal follow up of fungal microbiota; Scanlan & Marchesi, 2008), it is likely that these fungi are not a constant and functionally relevant part of the gastrointestinal microbiota.

Eukarya-intestinal parasites

The gastrointestinal tract of humans can be inhabited by a number of different micro-eukaryotes that belong to the phyla: *Apicomplexa, Amoebozoa*, *Ciliophora*, *Metamonada*, *Micosporidia, Parabasalia,* and *Stramenopiles* (Fig. 28). Some of these organisms are pathogenic infectious agents, which after ingestion (through contaminated water or food) can cause gastrointestinal symptoms, most frequently diarrhea. Because of their clinical significance, these organisms, which are often referred as intestinal protozoa, are relatively thoroughly studied as infections agents. Little attention has been given to these organisms in terms of their role in the ecosystem, but as several studies have shown that some of these species can be present in the healthy human gastrointestinal tract, it is reasonable to expect that future research will reveal the true role of these organisms in the gastrointestinal microbiota.

A number of Eukarya classified within the phylum of *Apicomplexa* in addition to *Micosporidia* form a group of intestinal spore-forming protozoa that cause intracellular

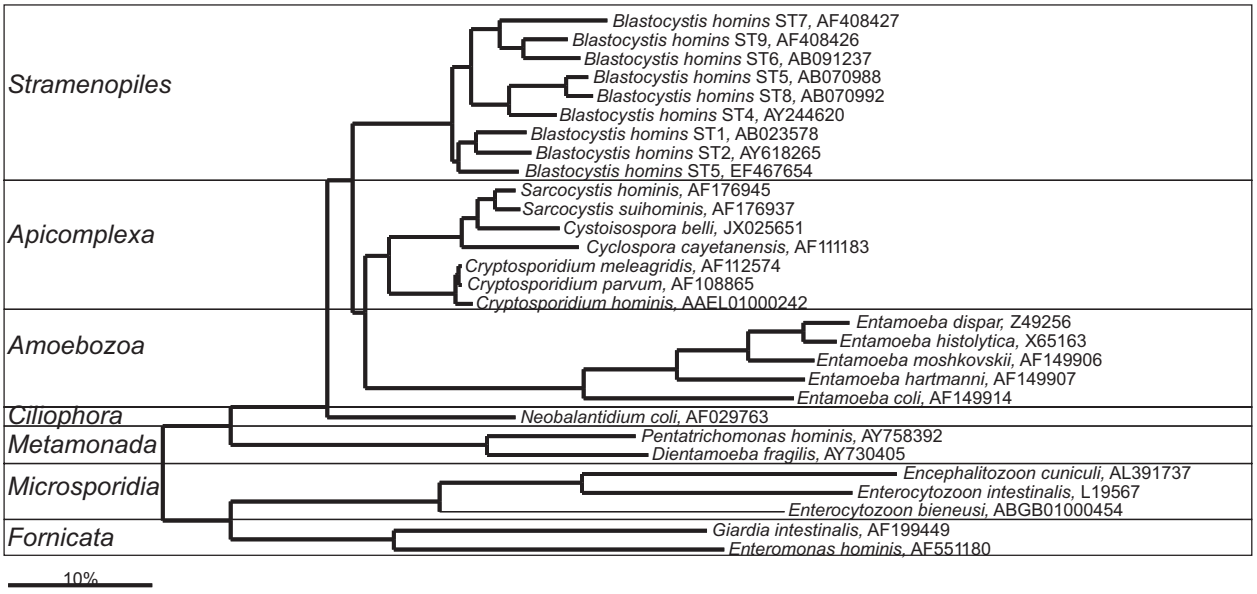

Fig. 28. Phylogenetic tree the human gastrointestinal microeukarya. GenBank Accession Numbers of the SSU rRNA gene sequence are provided for each species and the phylum names are indicated.

infections, primarily in the epithelial cells of the gastrointestinal tract. All *Apicomplexa* species that can be retrieved from the human gastrointestinal tract are considered to be pathogenic (Farthing, 2006). *Cryptosporidium parvum* is the most widely distributed representative of the group, and it has a high prevalence of infection in children of the developing world (Checkley *et al.*, 1997). Although infections with *Cryptosporidium parvum* can be asymptomatic, even when it does not cause diarrhea, this organism affects absorption of nutrients and has a negative effect on weight gain of children (Checkley *et al.*, 1997).

Blastocystis spp. (classified within the *Stramenopiles*) are single-celled protozoan organisms. *Blastocystis* spp. was for the first time isolated from the human gastrointestinal tract in 1911 and was reported under name *Blastocystis enterocola*, which was, at the time, designated as yeast (Alexieff, 1911). According to the current convention, all *Blastocystis* isolated from humans are identified as *Blastocystis hominis*, although their SSU rRNA gene sequence analysis showed that nine different phylotypes of these organisms can be detected in the human gastrointestinal tract (Arisue *et al.*, 2003; Scanlan & Marchesi, 2008). This 'within species' diversity of the *B. hominis* can explain the fact that the role of *Blastocystis* in human disease is still not defined (Zierdt, 1991). Longitudinal study of the Eukarya in the human gastrointestinal tract has shown that *Blastocystis* spp. are stable and frequently detected organisms in healthy subjects (Scanlan & Marchesi, 2008). Although some authors have suggested the link between *Blastocystis* and intestinal diseases such as diarrhea, irritable bowel syndrome and inflammatory bowel disease, the detection of *Blastocystis* in 105 patients suffering from various gastrointestinal diseases and 96 healthy controls, indicated that *Blastocystis* is equally frequent in patients and healthy subjects, although different phylotypes of *Blastocystis* are associated with different health status (Dogruman-Al *et al.*, 2008). Another recent study could establish a significantly higher incidence of *Blastocystis* in ulcerative colitis patients, when compared to controls (Cekin *et al.*, 2012).

Neobalantidium coli is the only representative of the *Ciliophora* phylum. *Neobalantidium coli* is the largest protozoan parasite that infects humans, but its natural hosts are pigs. Although the organism can reproduce within the intestinal lumen of humans without attacking the tissues and therefore remain asymptomatic, the infection with this species is typically followed by diarrhea and bloody stools (Katz *et al.*, 1982).

The *Amoebozoa* that can be detected in the human gastrointestinal tract include *Endolimax nana* and *Iodamoeba bütschlii* and six *Entamoeba* spp. (Table S3). The SSU rRNA gene sequence is available only for five *Entamoeba* spp. (Fig. 28). While most of the intestinal *Amoebozoa* are nonpathogenic, there is sufficient evidence that *Entamoeba histolytica* is pathogenic for humans and causes amebiasis — dysentery or amebic colitis with a high mortality rate (Fotedar *et al.*, 2007). *Entamoeba histolytica* was the first described 1875, although the species name *Entamoeba histolytica* was assigned later, in 1903 by Fritz Schaudinn

(Saklatvala, 1993). There are several studies that show the successful detection of *Entamoeba* spp. in clinical samples using molecular methods, typically in stool samples taken from patients with diarrhea. Molecular studies of the *Eukarya* as part of the ecosystem in the human gastrointestinal tract already confirmed the presence of two *Entamoeba* species: *Entamoeba coli* (Scanlan & Marchesi, 2008) and *Entamoeba hartmanni* (Hamad *et al.*, 2013) in the gastrointestinal tract of a healthy man.

Members of the phyla *Micosporidia*, *Parabasalia* and *Metamonada* are micro-eukaryotic organisms that contain flagella and are often commonly termed as flagellates. *Micosporidia* are obligate intracellular protozoan parasites that spread in the environment via spores. Four species of *Micosporidia* can infect the human gastrointestinal tract — *Enterocytozoon bieneusi* (Desportes *et al.*, 1985), *Encephalitozoon intestinalis* (Weber *et al.*, 1994), *Encephalitozoon cuniculi* (Franzen *et al.*, 1995), and *Retortamonas intestinalis* (Jones-Engel *et al.*, 2004; Fig. 28, Table S3). *Retortamonas intestinalis*, for which SSU rRNA gene sequence is not available, is the oldest isolate of this group that was for the first time cultured in 1879 (Hogue, 1933). Other *Micosporidia* were isolated from the gastrointestinal tract of a subject infected with HIV (Desportes *et al.*, 1985). The presence of *Micosporidia* in the gastrointestinal tract is typically associated with diarrhea, mostly in immuno-suppressed patients, although spores of these organisms can be detected in gastrointestinal samples of asymptomatic subjects (Cegielski *et al.*, 1999; Mungthin *et al.*, 2005; Wichro *et al.*, 2005). If asymptomatic, the presence of *Micosporidia* in the gastrointestinal tract is associated with malnutrition.

Among *Parabasalia*, two species can be associated with the human gastrointestinal tract. *Pentatrichomonas hominis* is generally regarded as a harmless commensal organism, although it is occasionally designated as a causal agent of diarrhea. This organism, which actually represents the oldest gastrointestinal isolate retrieved in 1854, has a low prevalence of the human gastrointestinal tract colonization that varies from 0.1% to 30.9% depending on the geographical location (Honigberg, 1990). In 1918 another member of this group, *Dientamoeba fragilis* was reported as a commensal in the human gastrointestinal tract. However, the latter research has suggested that *D. fragilis* might be associated with a number of diseases including diarrhea, abdominal pain, anorexia, irritable bowel syndrome or allergic colitis (reviewed in Johnson *et al.*, 2004). The uncertainty of the pathogenicity of *D. fragilis* might be, similar to *B. hominis*, due to the presence of different phylotypes, since two different phylotypes of this species, with 2% SSU rRNA gene sequence divergence, have been identified (Johnson & Clark, 2000).

The *Metamonada* phylum includes *Giardia lamblia* and two rarely detected and principally nonpathogenic species — *Enteromonas homins* and *R. intestinalis* (Katz *et al.*, 1982). *Giardia lamblia* is the most common flagellate of the human gastrointestinal tract. When ingested, typically via contaminated food or water, *G. lamblia* attaches to the mucosal surface of the duodenum or jejunum and multiplies by binary fission (Wolfe, 1992). Infection with *G. lamblia* is termed giardiasis, and although it may remain asymptomatic, giardiasis can be followed by a range of symptoms that include steatorrhoea, diarrhea and weight loss (Wolfe, 1992). It is not clear why some infections are asymptomatic, but already in the 1970s, it was suggested that the symptoms might depend on the relation between the parasite and the enteric gastrointestinal microbiota (Tandon *et al.*, 1977). Postinfective to the giardiasis, patients might develop a range of novel symptoms that resemble those of the irritable bowel syndrome (Hanevik *et al.*, 2009). This condition, which can be developed after infection with other infectious agents, such as *Campylobacter* spp., is recognized as postinfectious irritable bowel syndrome (Spiller & Garsed, 2009). A recent study showed that the bacterial fraction of the gastrointestinal microbiota of the postinfectious irritable bowel syndrome patients have a distinct composition relative to controls, which most likely reflects a consequence of an intensive interaction between the ecosystem and the infectious agent (Jalanka-Tuovinen *et al.*, 2013). This illustrates that infectious agent, although not true members of the gastrointestinal microbiota should be kept in mind when studying the gastrointestinal microbiota, as their short- and long-term impact on the microbiota composition and function can be profound.

Concluding remarks

The knowledge generated during more than a century of studying the human gastrointestinal microbiota has shown that this ecosystem is indeed a forgotten organ of the human body. The wealth of data about the gastrointestinal microbiota is highly scattered in time as well as in space — a caveat that this review aims to correct in an attempt to systematize the generated knowledge. We have given particular attention to the diversity and the defined functions of the abundant and important microbiota groups. Our inventory reports 1057 cultured gastrointestinal species, while many more are still expected to be cultured. Although cultivation of the gastrointestinal microbiota is laborious, it is an essential step for the detailed physiological and biochemical characterization of the individual gastrointestinal isolates that is needed for the progress of this research field. This has been

increasingly recognized and the recent high-throughput culturing studies have proven that cultivation can be used as a powerful methodology in discovery of currently unknown gastrointestinal inhabitants (Lagier *et al.*, 2012a; Dubourg *et al.*, 2013; Pfleiderer *et al.*, 2013). The future cultivation of the remaining majority of the gastrointestinal microbiota is expected to improve our understanding of this ecosystem, while this review can serve as a baseline for the gastrointestinal microbiota diversity and function when the first 1000 intestinal species had been discovered.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of *bacterial* species that can be members of the human gastrointestinal microbiota, with a reference that provides the link between the species and gastrointestinal tract as ecological niche.

Table S2. List of *archaeal* species that can be members of the human gastrointestinal microbiota, with a reference that provides the link between the species and gastrointestinal tract as ecological niche.

Table S3. List of *eukaryal* species that can be members of the human gastrointestinal microbiota, with a reference that provides the link between the species and gastrointestinal tract as ecological niche.