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ORIGINAL ARTICLE

Composition and diversity of the bacterial community in snow leopard (*Uncia uncia*) distal gut

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Abstract Intestinal microflora influences many essential metabolic functions, and is receiving increasing attention from the scientific community. However, information on intestinal microbiota, especially for large wild carnivores, is insufficient. In the present study, the bacterial community in the feces of snow leopards (*Uncia uncia*) was described based on 16S rRNA gene sequence analysis. A total of 339 near-full-length 16S rRNA gene sequences representing 46 non-redundant bacterial phylotypes (operational taxonomical units, OTUs) were identified in fecal samples from four healthy snow leopards. Four different bacterial phyla were identified: *Firmicutes* (56.5 %), *Actinobacteria* (17.5 %), *Bacteroidetes* (13 %), and *Proteobacteria* (13 %). The phylum *Actinobacteria* was the most abundant lineage, with 40.4 % of all identified clones, but *Clostridiales*, with 50 % of all OTUs, was the most diverse bacterial order. The order *Clostridiales* was affiliated with four families: *Clostridiaceae* I, *Lachnospiraceae*, *Peptostreptococcaceae*, and *Ruminococcaceae. Lachnospiraceae* was the most diverse family with 17 OTUs identified. These findings were basically consistent with previous reports on the bacterial diversity in feces from other mammals.

Keywords Snow leopard (*Uncia uncia*) · 16S rRNA gene · Feces · Bacterial diversity

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Introduction

The animal gastrointestinal tract harbors a complex microbial ecosystem, as has been proven by both classical culture-based and molecular biology techniques. During the long-term co-evolution between host and microorganisms, indigenous microbial communities have become a crucial part of the host, and alterations of this complex ecosystem have been associated with the host's age, diet and health (Ley et al. 2008b; Tilg and Kaser 2011). A large number of clinical trials have shown that imbalances in the gut microbiota can be correlated to many gastrointestinal diseases, such as inflammatory bowel diseases (Frank et al. 2007a; Xenoulis et al. 2008). Consequently, research into the composition and diversity of host gut microbiota is imperative to understanding the developmental mechanisms of several gastrointestinal diseases (Frank et al. 2007b; Xenoulis et al. 2008).

Previous research, beginning with that of Rahner (1901), applied cultivation methods, which provided much information pertaining to the microbial diversity of gut ecosystems. However, culture-dependent methods are extremely time consuming, and inefficient in identifying the anaerobic bacteria that are dominant within intestinal microbiota. With the development of molecular biology techniques, some new molecular tools, such as denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP), PCR-amplification of 16S rRNA genes, and metagenomic approaches have been applied successfully to evaluating the microbial composition and diversity of natural ecosystems. Particularly, 16S rRNA gene sequence analysis has proved to be an efficient and sensitive molecular method, and is now frequently applied in gastrointestinal microbial research.

Recently, several studies have used molecular biology techniques to take a deep look inside the gastrointestinal tract, and have yielded valuable results. It has been recognized that the host's diet and phylogeny can both influence the composition and diversity of the gut bacterial community, which increases

from carnivory to omnivory to herbivory (Ley et al. 2008a). Recently, a study using massive parallel 16S rRNA gene pyrosequencing revealed that feline and canine intestinal tracts harbor quite different bacterial communities. At the same time, inter-individual differences in the relatives abundance of major bacterial groups were less in cats than in dogs (Handl et al. 2011). In addition, several potential pathogens, such as *Clostrium perfringens*, *Escherichia coli*, *Pseudomonas* spp., and *Enterococcus* spp., are commonly detected in healthy animal intestines. This finding should demonstrate that potential pathogens are possibly indigenous members of the intestinal microbiota of healthy animals (Handl et al. 2011; Wu et al. 2012). To date, several studies concerned the microbial ecosystem of humans and of domestic animals while the intestinal bacterial community of large wild carnivores remains poorly described.

The snow leopard (*Uncia uncia*) is a mysterious feline that inhabits the high, remote mountains of Central Asia at altitudes of 3,000–4,500 m (Jackson 1996). Unfortunately, increasing human activities have caused this species to meet the criteria for endangered status in the IUCN Red List. Until recently, studies on the snow leopard have focused mainly on habitat utilization, population conservation, and phylogenetic classification, but no study has assessed the microbial communities in the intestinal tract. Therefore, our objective in this study was to describe the microbial diversity in fecal samples from healthy snow leopards in comparison to gut microbiota of other animals using 16S rRNA gene-based analysis.

Materials and methods

Sample collection

Fresh fecal samples were collected (within half an hour after defecation) from four (three males and one female) unrelated snow leopards that were raised in Xining Zoo of Qinghai, China during May 2012. The age of the animals ranged from 4 to 8 years old, and their weight from 32 kg to 48 kg. The tested animals were raised semi-freely singly and fed a diet based on fresh raw meat (mutton and beef) and live hare. All snow leopards were fed by the same personnel at the same time each day. All the animals were clinically healthy with no history of antibiotic or probiotic therapy 3 months prior to sample collection. Fecal samples were collected immediately after spontaneous defecation using sterile sampling bags. Feces from the same individual were seen as one fecal sample. Samples were frozen immediately and preserved at −80 °C without any additives or pretreatment until further analysis.

DNA extraction

An aliquot from each fecal sample (250 mg) was homogenized in a sterile tube and genomic DNA was extracted using a QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) under the guidance of the QIAamp® DNA Stool Handbook. To avoid bias, genomic DNA was extracted in duplicate for each sample and extracts from the same sample were pooled. Purified DNA was stored at −20 °C until use.

PCR amplification and purification

PCR amplification was carried out individually on all samples. The 16S rRNA gene was amplified using universal bacterial primer F (5′-GAGAGTTTGATCCTGGCTCAG-3′, *E. coli* position 7–27) and primer R (5′-TACGGCTACCTT GTTACGAC-3′, *E. coli* position 1493–1511). Both primers were purchased from the Sunbiotech (Beijing, China). DNA was amplified using the following reaction conditions: 2 μL of each primer (10 μM), 3 μL template DNA, 5 μL 10×Ex PCR buffer, 4 μL of dNTPs (10 mM each), 1 μL Ex *Taq* DNA polymerase (5U/μL) and 1 μL 20 mg/ml bovine serum albumin in a 50 μL reaction volume. The above reagents were all purchased from TaKaRa (Dalian, China). The DNA was then amplified in a DNA Thermal Cycler (GeneAmp® PCR System 9700, Applied Biosystems, Foster City, CA), using the following PCR protocol: initial denaturing at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 1 min 30 s, and a final extension at 72 °C for 10 min. For all samples, between four and eight independent PCR reactions were performed.

PCR products belonging to the same sample were pooled and visualized on agarose gels electrophoresis (0.5×TBE buffer). The were extracted with a QIAquick® Gel Extraction Kit (Qiagen) following the manufacturer's instructions. Approximately equal amounts of purified PCR products of each sample were then pooled and stored at 4 °C in sterilized double distilled water.

Amplicons cloning and sequencing

PCR products were ligated into pMD^{TM}18-T Vector and then transformed into DH5α^{TM}-T1^{R} competent *E. coli* cells (TaKaRa) according to the manufacturer's instructions. Clones were grown and selected (blue/white) on Luria-Bertani medium, containing ampicillin (100 μg/mL), X-gal (100 μg/mL) and IPTG (0.5 mM) at 37 °C overnight. Positive clones were stored in glycerol at −80 °C for future plasmid extraction.

Plasmids were extracted using the Perfectprep® BAC 96 plasmid purification kit (Eppendorf, Shanghai, China). The 16S rRNA gene insert was sequenced using an ABI PRISM Big Dye Terminator Cycle Ready Reaction Kit and an ABI PRISM 3730 DNA Sequencer (Applied Biosystems) with the bacterial universal primers (*E. coli* 27F and 1492R). The sequences were assembled using vector NTI advance software (version 10.0).

Sequence analysis

All near-full-length sequences obtained were edited to exclude the PCR primer-binding sites using the VecScreen available on NCBI. Additionally, sequences were checked by BELLEROPHON (Huber et al. 2004) available through the Ribosomal Database Project (RDP), and putative chimeras were excluded from further analysis.

Sequences were aligned with the CLUSTAL_W, which is contained in MEGA software package (version 5.1 Bata) (Tamura et al. 2011). A PHYLIP distance matrix was generated and used as an input file for the DOTUR software (version 1.5) (Schloss and Handelsman 2005) to determine phylotypes (operational taxonomical units, OTUs) at 97 % similarity.

The non-chimeric sequences were compared with existing 16S rRNA gene sequences using GenBank and RDP on an 80 % confidence threshold, and the closest 1–3 neighbor(s) for each sequence was obtained. Distal gut bacterial diversity in the snow leopard was demonstrated by constructed phylogenetic trees based on the neighbor-joining algorithm using the MEGA software. Evolutionary distances were inferred by the Jukes-Cantor model and branch stability was assessed by 1,000 replicates bootstrap analysis. The Archaea *Aquifex pyrophilus* (GenBank accession number: M83548) was selected as out-group.

Statistical analysis

The coverage of the clone library was calculated using the formula $C=[1-(n/N)]\times 100$ according to Good (1953), where n is the number of OTUs represented by one sequence and N is the total number of sequences of the clone library.

Bacterial community diversity was calculated by the Shannon–Weaver diversity index, which is defined as $H' = -\sum p_i \ln (p_i)$, where p_i is the proportion of individual bacteria found in a certain species.

Nucleotide sequence accession numbers

Sequences submitted to the GenBank database in this study were prefixed by UUF (*Uncia uncial* fecal, e.g., UUF001). A total of 339 near-full-length 16S rRNA gene sequences were deposited with the GenBank database (accession numbers: KC245156–KC245494).

Results

A total of 500 clones were selected randomly. Of these, 382 clones contained an insert with a sequence of adequate quality, of which 43 were identified as possible chimeras and excluded from further analysis. With 97 % sequence similarity, a total of 46 OTUs representing 339 near-full-length sequences were used in the subsequent phylogenetic analysis. The Coverage C and Shannon Weaver index (*H'*) of the 16S rDNA clone library were 94.4 % and 2.75, respectively.

BLAST analysis revealed that 27 OTUs (58.7 % of all OTUs) showed <98 % sequence similarity with existing 16S rRNA gene sequences in the GenBank database. Four major phylogenetic lineages were identified: *Actinobacteria* (17.5 %), *Bacteroidetes* (13.0 %), *Firmicutes* (56.5 %), *Proteobacteria* (13.0 %). Additionally, further classification indicated that 23 OTUs (50.0 %) were affiliated with the order *Clostridiales* of the phylum *Firmicutes*. The phylogenetic positioning of OTUs in each phylum is shown in Figs. 1, 2, 3 and 4.

Actinobacteria

A total of 137 reads representing eight phylotypes were classified within the phylum *Actinobacteria* (Fig. 1). Two different bacterial genera within the family *Coriobacteriaceae* were identified. The genus *Collinsella* was the predominant subgroup with 136 clones representing seven OTUs. One OTU was affiliated within the genus *Slackia*. According to the Blast analysis, most clones affiliated within this phylum showed < 98 % sequence similarity with the GenBank database entries (Table 1).

Bacteroidetes

A total of 26 clones representing six individual OTUs were identified within the phylum *Bacteroidetes* (Fig. 2). This phylum was exclusively represented by the family *Bacteroidaceae*. One OTU represented by two clones had 99 % similarity to *Bacteroides massiliensis* type strain (AY126616); one OTU had 99 % similarity to *Bacteroides vulgatus* (CP000139) and represented nine clones (Table 2).

Firmicutes

More than half of all the OTUs (56.5 %) containing 98 clones were classified within the phylum *Firmicutes* (Fig. 3), which was the most diverse phylum in the feces of the snow leopard. Three different bacterial classes were identified: *Clostridia*, *Erysipelotrichia*, and *Negativicutes*. Further classification showed that these classes were respectively represented by the orders *Clostridiales*, *Erysipelotrichales*, and *Selenomonadales*. A total of 83 clones, representing 23 phylotypes, were affiliated to the order *Clostridiales*. Only one clone fell into the order

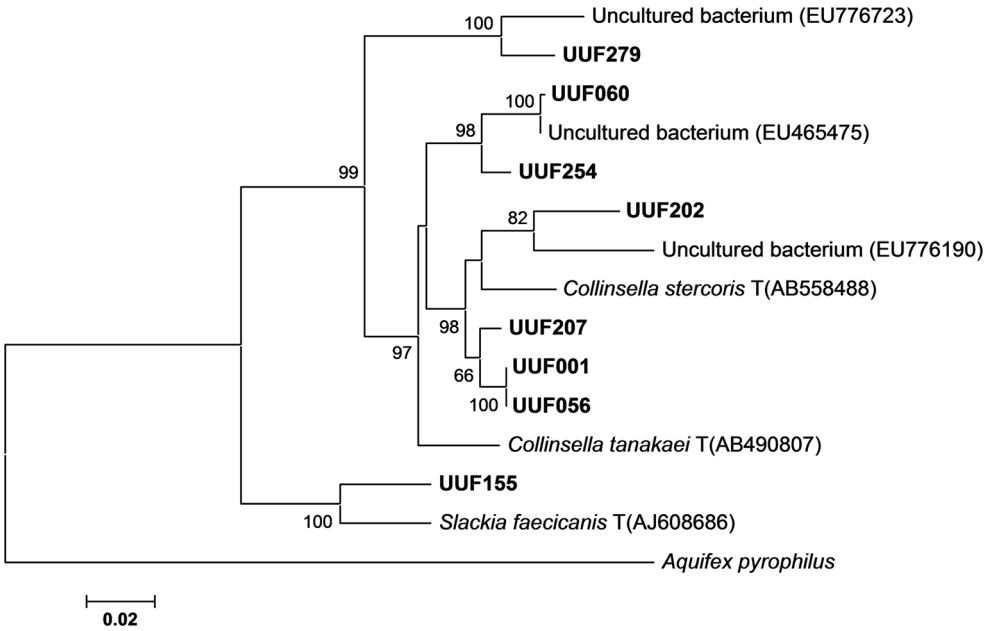

Fig. 1 Dendrogram illustrating the phylogenetic affiliation of operational taxonomical units (OTUs) isolated from the snow leopard fecal samples for *Actinobacteria*. The tree was inferred using neighbor-joining algorithm. Near-full-length 16S rDNA sequences were aligned to their closest neighbour(s) in RDP database. *Aquifex pyrophilus* was used as out-group

Erysipelotrichiales and it was ascribable to the family *Erysipelotrichaceae*. Finally, the order *Selenomonadales* was represented by the families *Acidaminococcaceae* and *Veillonellaceae*, each containing one OTU.

Clostridiales, the largest order in the *Firmicutes*, was divided into four families: *Clostridiaceae* I, *Ruminococcaceae*, *Peptostreptococcaceae*, and *Lachnospiraceae*. At the family level, *Lachnospiraceae* was predominant in this phylum with 63 clones representing 17 OTUs identified. In this family, one OTU showed 99 % similarity with *Blautia hansenii* (AB534168). In the family *Clostridiaceae*, two OTUs displayed 99 % and 98 % similarity with *Clostridium hiranonis* (JN713315), respectively, and one OTU showed 99 % similarity with *Clostridium perfringens* (CP000246) (Table 3).

Proteobacteria

A total of 78 clones representing six OTUs were affiliated with the phylum *Proteobacteria* (Fig. 4). Among four different identified classes in this phylum, the class *Gammaproteobacteria* was the most diverse group with 65 clones representing three individual OTUs. Within this class, the family *Pseudomonadaceae* was the predominant group with 64 clones representing two OTUs. At the genus level, *Pseudomonas* was the most common representative subgroup in family *Pseudomonadaceae*, followed by the genus *Sutterella* represented by nine clones in class *Betaproteobacteria*. The two last OTUs were affiliated to the class *Alphaproteobacteria* (one clone) and *Epsilonproteobacteria* (three clones), respectively.

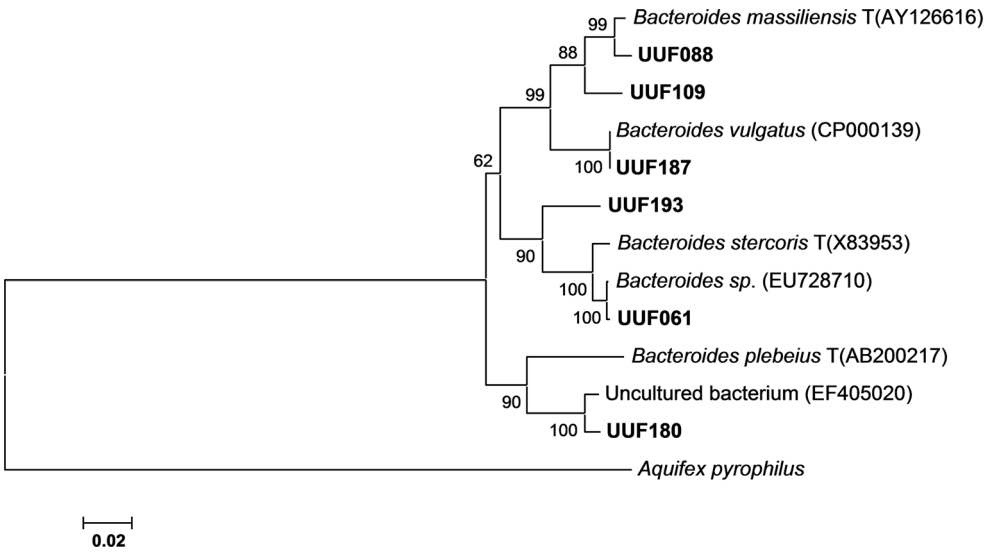

Fig. 2 Dendrogram showing the phylogenetic affiliation of OTUs isolated from the snow leopard gastrointestinal tract for *Bacteroidetes*. See legend of Fig. 1 for explanation

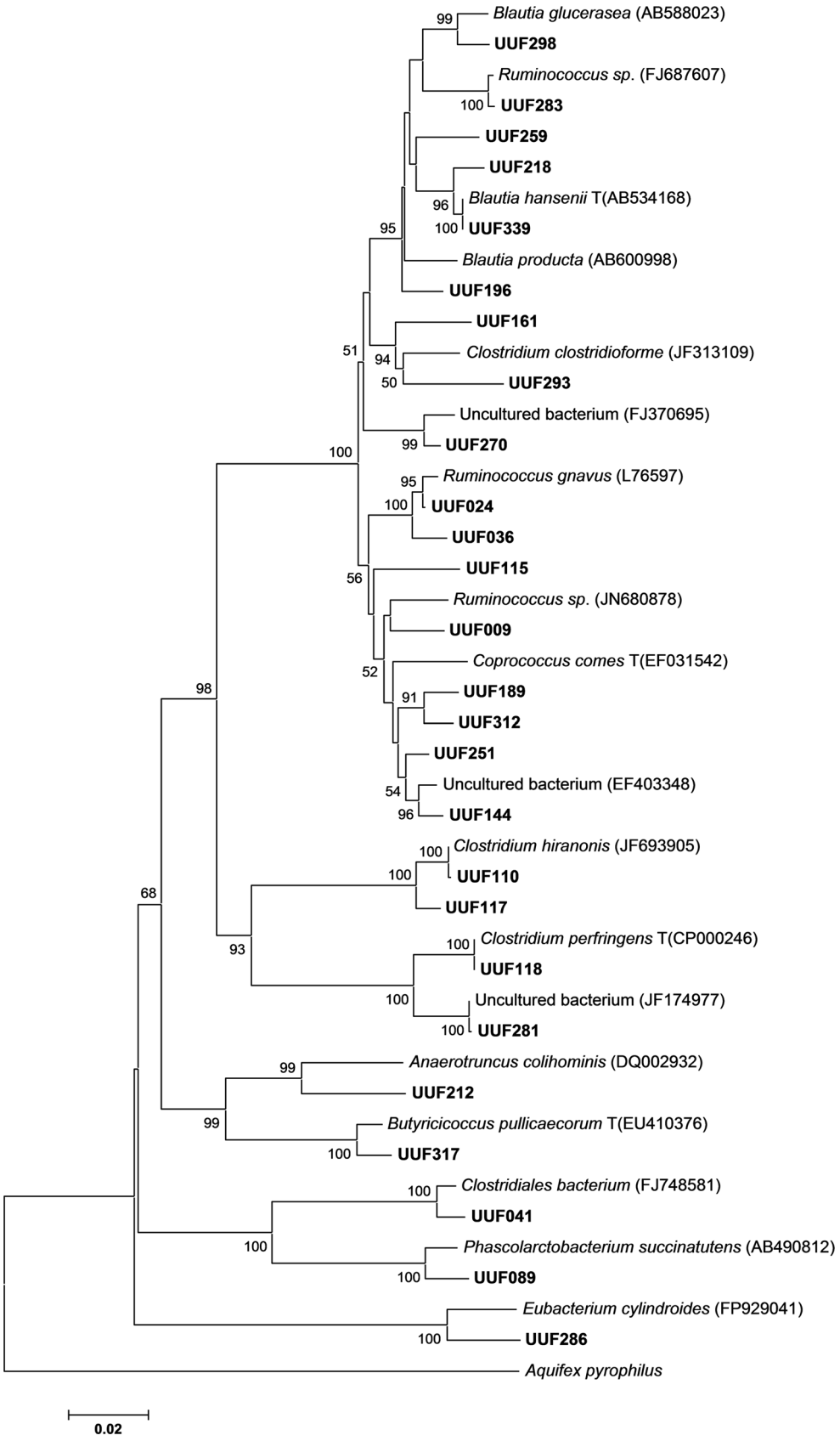

Fig. 3 Dendrogram showing the phylogenetic affiliation of OTUs isolated from the snow leopard gastrointestinal tract for *Firmicutes*. See legend of Fig. 1 for explanation

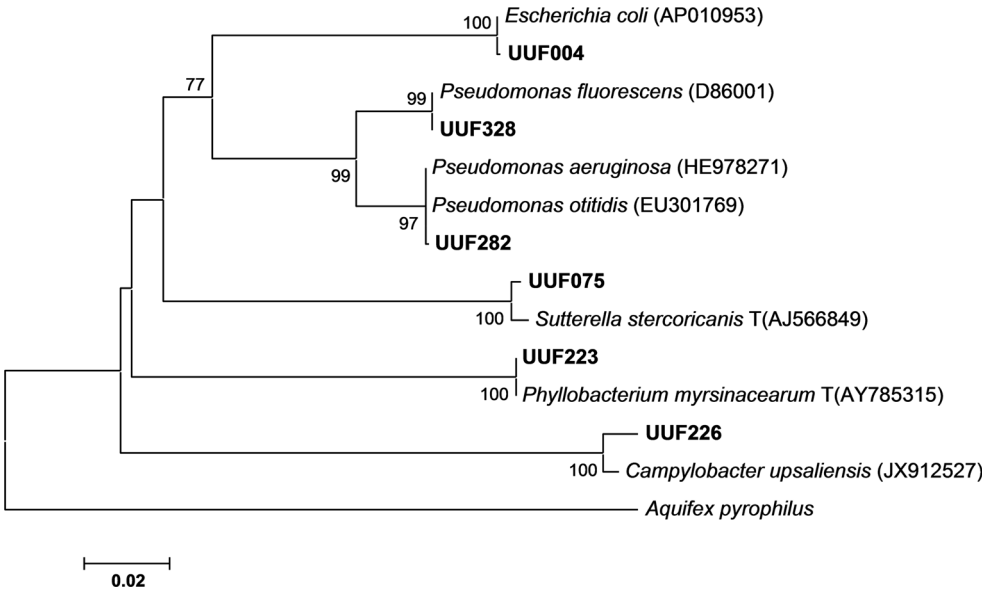

Fig. 4 Dendrogram showing the phylogenetic affiliation of OTUs isolated from the snow leopard gastrointestinal tract for *Proteobacteria*. See legend of Fig. 1 for explanation

Based on the BLAST results, OTUs in this phylum all showed >97 % sequence similarity to GenBank database strain entries. Within the most common class *Gammaproteobacteria*, the OTUs were closely related to previously cultured bacteria including *Escherichia coli* (AP010953), *Pseudomonas aeruginosa* (HE978271) and *Pseudomonas fluorescens* (D86001). Additionally, OTUs belonging to classes *Alphaproteobacteria*, *Betaproteobacteria* and *Epsilonproteobacteria* were classified as *Phyllobacterium myrsinacearum* (AY785315), *Sutterella stercoricanis* (AJ566849) and *Campylobacter upsaliensis* (JX912527), respectively (Table 4).

Discussion

According to our results, four major phylogenetic lineages were identified: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. In the present study, no *Fusobacteria* were identified in the clone libraries. However, this phylum was found as a major component of the intestinal microbial community of dogs (Suchodolski et al. 2008) and wolves (Zhang and Chen 2010), while it was less represented both in humans (Wang et al. 2005) and in different animal species such as cats (Ritchie et al. 2008), pigs (Leser et al. 2002), and horses (Daly et al. 2001). It could be hypothesized that *Fusobacteria*, if present in low numbers within the complex gut community of snow leopards, have been underestimated by the PCR procedure used here.

Phylogenetic analysis revealed that the *Firmicutes* was the most diverse phylum in the snow leopard distal intestinal tract and *Clostridiales* was the most diverse bacterial order. These findings are consistent with previous studies on other mammals (Mentula et al. 2005; Ritchie et al. 2008; Desai et al. 2009; Garcia-Mazcorro et al. 2012), in which *Firmicutes* has been frequently reported to be among the dominant bacterial groups in various segments of the gastrointestinal tract or in feces.

Furthermore, within the order *Clostridiales*, *Lachnospiraceae* was the most diverse family in the feces of the snow leopard, with 17 OTUs identified. This finding was consistent with a previous report in wolves (Zhang and Chen 2010) showing that *Lachnospiraceae* sequences were

Table 1 BLAST results and sequence analysis of *Actinobacteria* representative sequence

Sequence	Accession number	Identification	Accession number	Similarity (%)
UUF001	KC245156	*Collinsella intestinalis* T	AB558489	97
UUF056	KC245211	*Collinsella stercoris* T	AB558488	97
UUF060	KC245215	Uncultured bacterium	EU465475	99
UUF155	KC245310	*Slackia faecicanis* T	AJ608686	93
UUF202	KC245357	Uncultured bacterium	EU776190	94
UUF207	KC245362	*Collinsella intestinalis* T	AB558489	97
UUF254	KC245409	*Collinsella intestinalis* T	AB558489	95
UUF279	KC245434	Uncultured bacterium	EU776723	96

Table 2 BLAST results and sequence analysis of *Bacteroidetes* representative sequence

Sequence	Accession number	Identification	Accession number	Similarity (%)
UUF061	KC245216	*Bacteroides* sp.	EU728710	99
		Bacteroides stercoris	AB510708	99
UUF088	KC245243	*Bacteroidesmassiliensis* T	AY126616	99
UUF109	KC245264	*Bacteroides massiliensis*	EU136696	97
		Uncultured bacterium	EU530504	99
UUF180	KC245335	Uncultured bacterium	EF405020	99
UUF187	KC245342	*Bacteroides vulgatus*	CP000139	100
UUF193	KC245348	Uncultured bacterium	DQ800293	98

predominant within *Clostridiales* in wolf feces. On the contrary, data from humans (Eckburg et al. 2005), cats (Ritchie et al. 2010) and dogs (Mentula et al. 2005; Suchodolski et al. 2008) suggested that, within the phylum *Firmicutes*, *Clostridium* spp. was the most common genus in feces or in intestinal tract. Therefore, we speculate that the family *Lachnospiraceae* may account for a larger proportion of gut microorganisms in wild carnivores compared to humans or domestic animals.

In the present study, *Actinobacteria* was the most abundant phylum in the intestinal bacterial community (40 % of all identified sequences compared to *Firmicutes* 29 %,

Table 3 BLAST results and sequence analysis of *Firmicutes* representative sequence

Sequence	Accession number	Identification	Accession number	Similarity (%)
UUF009	KC245164	*Blautia* sp.	JN713310	98
UUF024	KC245179	*Ruminococcus gnavus*	L76597	98
UUF036	KC245191	*Ruminococcus gnavus*	L76597	96
UUF041	KC245196	*Clostridiales* bacterium	FJ748581	97
UUF089	KC245244	*Phascolarctobacterium* sp.	AB490812	100
UUF110	KC245265	*Clostridium hiranonis*	JF693905	99
UUF115	KC245270	Uncultured bacterium	EU462979	96
UUF117	KC245272	*Clostridium hiranonis*	JF693903	98
UUF118	KC245273	*Clostridium perfringens* T	CP000246	99
UUF144	KC245299	Uncultured bacterium	EF403348	97
		Dorea sp.	JX101687	95
UUF161	KC245316	Uncultured bacterium	HQ808112	97
UUF189	KC245344	Uncultured bacterium	EU772974	99
UUF196	KC245351	*Blautia producta*	AB600998	96
UUF212	KC245367	Uncultured bacterium	HQ795191	99
UUF218	KC245373	*Blautia hansenii*	AB534168	97
UUF251	KC245406	Uncultured bacterium	FJ370755	98
UUF259	KC245414	Uncultured bacterium	EF403112	99
UUF270	KC245425	Uncultured bacterium	HQ770584	98
UUF281	KC245436	Uncultured *Clostridium* sp.	GQ179695	99
UUF283	KC245438	*Ruminococcus* sp.	FJ687607	99
UUF286	KC245441	Uncultured bacterium	GQ898024	100
		Eubacterium cylindroides	FP929041	93
UUF293	KC245448	Uncultured bacterium	JN559609	98
UUF298	KC245453	*Blautia glucerasea*	AB588023	98
UUF312	KC245467	Uncultured bacterium	JN559620	98
		Clostridium sp.	AJ318890	95
UUF317	KC245472	Uncultured bacterium	HQ808319	99
		Butyricicoccus sp. T	EU410376	97
UUF339	KC245494	*Blautia hansenii* T	AB534168	99

Table 4 BLAST results and sequence analysis of *Proteobacteria* representative sequence

Sequence	Accession number	Identification	Accession number	Similarity (%)
UUF004	KC245159	*Escherichia coli*	AP010953	99
UUF075	KC245230	*Sutterella stercoricanis* T	AJ566849	99
UUF223	KC245378	*Phyllobacterium* sp. T	AY785315	99
UUF226	KC245381	*Campylobacter upsaliensis*	JX912527	98
UUF282	KC245437	*Pseudomonas aeruginosa*	HE978271	99
UUF328	KC245483	*Pseudomonas fluorescens*	D86001	99

Proteobacteria 23 %, and *Bacteroidetes* 8 %). However, this phylum generally makes up a small proportion of the gut bacterial community in humans and animals (Wang et al. 2005; Suchodolski et al. 2008; Middelbos et al. 2010). Only a limited number of studies, like the present one, have reported *Actinobacteria* as dominant in the mammal gut (Andersson et al. 2008; Desai et al. 2009). In the present study, *Collinsella* was the most frequent genus in the *Actinobacteria*.

Sequences belonging to the phylum *Bacteroidetes* were less abundant (7.7 % of all sequences) in snow leopard feces. Moreover, previous studies in humans and animals (Wang et al. 2005; Andersson et al. 2008; Handl et al. 2011; Tun et al. 2012) have shown that the relative abundance of *Bacteroidetes* varies significantly at intra- and inter-species level.

The *Proteobacteria* (including *E. coli*-like organisms) was the other phylum identified in the fecal microbial community of the snow leopard. These data support the results of studies on feline gut microbiota (Ritchie et al. 2008; Desai et al. 2009). Moreover, we evidenced the presence of potential pathogen species ascribable to the genus *Pseudomonas*. Interestingly, the four snow leopards participating in our study were all clinically healthy and showed no signs of any gastrointestinal diseases. Other authors (Handl et al. 2011) detected several potential pathogens species in feces of healthy cats and dogs and they hypothesized that these populations are indigenous components of the gut microbiota of healthy animals. However, the exact role of these microorganisms in gastrointestinal diseases requires further study.

It is recognized that differences in gastrointestinal microbiota might be due to adaption to the diet and gut morphology of the host. The snow leopard's principal natural prey species are blue sheep (*Pseudois nayaur*) and ibex (*Capra sibirica*), whose distribution coincides closely with snow leopard habitat. Snow leopard also preys on marmot (*Marmota* spp.), hare (*Lepus* spp.) and small rodents (Jackson 1996). The diet administered to the tested animals is basically consistent with natural feeding. Thus, we consider our results representative of the composition and diversity of wild snow leopard fecal microbiota.

In conclusion, in the present study, the composition and diversity of snow leopard fecal microbiota were evaluated using 16S rRNA gene analysis. We also compared our study results with related data from other mammals. The results obtained facilitate the next step in understanding of the composition and diversity of the microbial community in the snow leopard's intestinal tract. Moreover, further studies are warranted to provide a more detailed description of the intestinal microbiota of wildlife and of the contribution of different gastrointestinal bacterial populations to digestion, immunology and nutrition.

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References

Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L (2008) Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One 3(7):e2836

Daly K, Stewart CSFHJ, Shirazi-Beechey SP (2001) Bacterial diversity within the equine large intestine as revealed by molecular analysis of cloned 16S rRNA genes. FEMS Microbiol Ecol 38:141–151

Desai AR, Musil KM, Carr AP, Hill JE (2009) Characterization and quantification of feline fecal microbiota using cpn 60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. Vet Microbiol 137(1):120–128

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308(5728):1635–1638

Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR (2007a) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 104(34):13780–13785

Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR (2007b) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 104(34):13780–13785. doi:10.1073/pnas.0706625104

Garcia-Mazcorro JF, Dowd SE, Poulsen J, Steiner JM, Suchodolski JS (2012) Abundance and short-term temporal variability of fecal microbiota in healthy dogs. Microbiologyopen 1(3):340–347. doi:10.1002/mbo3.36

Good IJ (1953) The population frequencies of species and the estimation of population parameters. Biometrika 40(3–4):237–264

Handl S, Dowd SE, Garcia-Mazcorro JF, Steiner JM, Suchodolski JS (2011) Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiol Ecol 76(2):301–310

Huber T, Faulkner G, Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20(14):2317–2319

Jackson RM (1996) Home range, movements and habitat use of snow leopard (*Uncia uncia*) in Nepal. University of London, London

Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Moller K (2002) Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl Environ Microbiol 68: 673–690

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R (2008a) Evolution of mammals and their gut microbes. Science 320(5883): 1647–1651

Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI (2008b) Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 6(10):776–788

Mentula S, Harmoinen J, Heikkilä M, Westermarck E, Rautio M, Huovinen P, Könönen E (2005) Comparison between cultured small-intestinal and fecal microbiotas in beagle dogs. Appl Environ Microbiol 71(8):4169–4175

Middelbos IS, Boler BMV, Qu A, White BA, Swanson KS, Fahey GC Jr (2010) Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. PLoS One 5(3):e9768

Rahner R (1901) Bakteriologische mitteilungen ueber die darmbakterien der huehner. Zentralbl Bakteriol Parasitenkd 80:239–244

Ritchie LE, Steiner JM, Suchodolski JS (2008) Assessment of microbial diversity along the feline intestinal tract using 16S rRNA gene analysis. FEMS Microbiol Ecol 66(3):590–598

Ritchie LE, Burke KF, Garcia-Mazcorro JF, Steiner JM, Suchodolski JS (2010) Characterization of fecal microbiota in cats using universal 16S rRNA gene and group-specific primers for *Lactobacillus* and *Bifidobacterium* spp. Vet Microbiol 144(1):140–146

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71(3):1501–1506

Suchodolski JS, Camacho J, Steiner JM (2008) Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. FEMS Microbiol Ecol 66(3): 567–578

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

Tilg H, Kaser A (2011) Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest 121(6):2126

Tun HM, Brar MS, Khin N, Jun L, Hui RK-H, Dowd SE, Leung FC-C (2012) Gene-centric metagenomics analysis of feline intestinal microbiome using 454 junior pyrosequencing. J Microbiol Methods 88(3):369–376

Wang M, Ahrné S, Jeppsson B, Molin G (2005) Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. FEMS Microbiol Ecol 54(2): 219–231

Wu S, Wang G, Angert ER, Wang W, Li W, Zou H (2012) Composition, diversity, and origin of the bacterial community in grass carp intestine. PLoS One 7(2):e30440

Xenoulis PG, Palculict B, Allenspach K, Steiner JM, Van House AM, Suchodolski JS (2008) Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. FEMS Microbiol Ecol 66(3): 579–589

Zhang H, Chen L (2010) Phylogenetic analysis of 16S rRNA gene sequences reveals distal gut bacterial diversity in wild wolves (*Canis lupus*). Mol Biol Rep 37(8):4013–4022. doi:10.1007/s11033-010-0060-z