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Research Article

RESEARCH ARTICLE

# Effects of host traits and land-use changes on the gut microbiota of the Namibian black-backed jackal (*Canis mesomelas*)

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**One sentence summary:** Host traits have a bigger impact than environmental factors on the gut microbiota of the generalist black-backed jackal.

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## ABSTRACT

Host traits and environmental factors drive the natural variation in gut microbiota, and disruption in homeostasis can cause infections and chronic diseases. African wildlife is increasingly facing human-induced agricultural habitats, which also amplifies the contact probability with livestock with unknown consequences for wildlife gut microbiotas and the risk of transmission of potentially pathogenic bacteria. We applied high-throughput sequencing of bacterial 16S rRNA genes and microsatellite genotyping to investigate the impact of host traits and habitat use on the gut microbiotas of black-backed jackals (*Canis mesomelas*). This abundant carnivore inhabits livestock and game farms in central Namibia and is often persecuted as pathogen reservoir and vector. We further compared the gut microbiotas of black-backed jackals to other wild and domestic carnivores, herbivores and an omnivore, to disentangle the effects of environment, host species and dietary preference. In black-backed jackals, intrinsic host traits had a stronger impact in shaping the host–bacteria relationship than environmental factors. Nevertheless, the abundance of bacterial operational taxonomic units (OTUs) differed in individuals from livestock and game farms for specific bacterial genera such as *Lactobacillus* and *Clostridium*. We found, however, no evidence that black-backed jackals harbour abnormal levels of OTUs related to potential bacterial pathogens or that livestock farming has a negative impact on their health. We present here the first study investigating simultaneously the impact of host traits and environmental factors on gut microbiotas of a wildlife carnivore that occurs in a human-modified habitat.

**Keywords:** gut microbiota; 16S rRNA gene; wildlife; black-backed jackal (*Canis mesomelas*); livestock and game farming; Namibia

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## INTRODUCTION

Recent research that has investigated host–bacteria relationships in humans, domestic and laboratory animals emphasises the functional importance of gut microbial communities in food digestion (Hollister, Gao and Versalovic 2014), the synthesis of vital nutrients (LeBlanc *et al.* 2013), the development and performance of the immune system (Kau *et al.* 2011) and host behaviour (Archie and Tung 2015). Gut microbiotas are shaped by intrinsic host traits and extrinsic environmental factors influencing host–bacteria relationships. Changes in diversity and/or shifts in proportions of bacteria beyond the natural variation can cause functional dysbiosis of the gut microbiota leading to an increased susceptibility to infections or the development of chronic diseases (Turnbaugh *et al.* 2007; McKenna *et al.* 2008; Looft *et al.* 2012; Ellis *et al.* 2013). Host traits include not only sex (Org *et al.* 2016), age (Kim *et al.* 2011) and body mass (Dominianni *et al.* 2015), but also diet (Muegge *et al.* 2011), genetics (Goodrich *et al.* 2014) and behaviour (Ezenwa *et al.* 2012; Archie and Tung 2015; Moeller *et al.* 2016). Environmental factors encompass climatic conditions (Lesser *et al.* 2016), habitat type (Amato *et al.* 2013; Clayton *et al.* 2016) and the increasing human-induced shifts in land use that impact contact probabilities of wildlife and livestock and, thus, microbial transmission between species facilitating zoonotic infections (Daszak, Cunningham and Hyatt 2000; Kilpatrick, Gillin and Daszak 2009). General conclusions concerning the relative importance of these factors on the variation of gut microbiota are difficult to draw under standardised laboratory conditions (McKenna *et al.* 2008) because host–bacteria relationships might differ under natural selection regimes and, thus, studies in wildlife species within their natural habitats are required (Yildirim *et al.* 2010; Phillips *et al.* 2012; Amato *et al.* 2013; Menke *et al.* 2014; Roggenbuck *et al.* 2014b). Moreover, comparisons between gut microbiotas of wildlife species and their domestic counterparts are lacking; these could add important information about the characteristic features of 'wild' gut microbiotas (De Jesús-Laboy *et al.* 2011). Studies comparing free-ranging and captive wildlife populations have reported modified gut microbiota compositions associated with a loss in diversity in captive animals (Amato *et al.* 2013; Kong *et al.* 2014; Cheng *et al.* 2015); this has mainly been attributed to a modified diet and environmental, behavioural and physiological changes, all of which might also be true for wildlife species and their domestic counterparts.

So far, studies are still rare that investigate, in free-ranging individuals, the natural variation of gut microbiota attributable to host traits and the effect of environmental factors on the gut microbiota composition (Yuan *et al.* 2015; Moeller *et al.* 2016). Host factors have been found to have a dominant impact on gut microbiotas of wild southern elephant seals (*Mirounga leonina*) (Nelson *et al.* 2013) and reproductively active bats of the order Chiroptera (Phillips *et al.* 2012), whereas habitat degradation shape gut microbiotas to a great extent in wild black-howler monkeys (*Alouatta pigra*) (Amato *et al.* 2013), the wild mouse (*Apodemus sylvaticus*) (Maurice *et al.* 2015) and wild brown bears (*Ursus arctos*) (Sommer *et al.* 2016). In wild ring-tailed lemurs, however, both host factors, such as age and social group, and environmental factors, such as habitat disturbance, have an effect on specific microbial taxa in gut microbiotas (Bennett *et al.* 2016). In this context, the impact of host genetics on the phylogenetic structure of the host gut microbiota is still not understood. In the house mouse (*Mus musculus*), the impact of host genetic relatedness on the position of individual gut microbiotas in a phylogenetic tree is very low compared with the impact of geography (Linnenbrink *et al.* 2013), whereas in a hindgut-fermenting tortoise (*Gopherus polyphemus*) the degree of relatedness does shape patterns of gut microbiota diversity (Yuan *et al.* 2015). Thus, depending on the respective environment (specifically, the intensity of the anthropogenic disturbance), the relative impact of intrinsic and extrinsic factors might vary (Amato 2013).

Besides the gut microbiota diversity also the relative abundance of specific bacterial taxa might be an indicator of host health. The Firmicutes/Bacteroidetes ratio (Ley *et al.* 2006) has been found to be connected with the capacity to extract energy from the diet (Turnbaugh *et al.* 2006), and a high ratio has been associated to diseases such as immune dysfunctions, gastrointestinal mucositis, diarrhoea and obesity (Turnbaugh *et al.* 2009; De Filippo *et al.* 2010; Ren *et al.* 2014). To what extent host and environmental factors contribute to changes in this ratio in wildlife species and the way that this affects their health is barely understood. Initial investigations in wildlife have revealed that a higher Firmicutes/Bacteroidetes ratio is attributable to the need for the efficient extraction of energy from occasionally limited food sources (Cheng *et al.* 2015), facilitates nutrition during reproduction (black howler monkey females, *Alouatta pigra,* (Amato *et al.* 2014)) and might be associated with helminth infections (yellow-necked mouse, *Apodemus flavicollis*, (Kreisinger *et al.* 2015)). More studies are required to disentangle the main drivers shaping the natural range of variation of wildlife gut microbiota from unhealthy modifications attributable to human impact. An understanding of these complex host–bacteria relationships may provide the basis for conservation decisions in wildlife as the importance of the gut microbiota in species conservation is increasingly being recognised (Redford *et al.* 2012; Amato 2013; Bahrndorff *et al.* 2016; Stumpf *et al.* 2016).

In this study, we have investigated the impact of host traits, sampling location and shifts in land use on the host–bacteria relationship in a prominent African carnivore, the black-backed jackal (*Canis mesomelas*). We have combined high-throughput sequencing of gut bacterial 16S rRNA genes with estimates of individual genetic relatedness by using microsatellite markers and have compared the gut microbiota of this wild canid with domestic dogs and other mammals. In Namibia, this omnivorous territorial canid prospers particularly on commercially used farmland, such as livestock farms or game farms and is persecuted as livestock predator, pathogen reservoir and rabies vector (Mansfield *et al.* 2006; Bellan *et al.* 2012). Its adaptability to live in natural as well as disturbed habitats makes this species an interesting model for investigating the impact of host traits and anthropogenic environmental factors on gut microbial community composition and occurrence of potential bacterial pathogens in wildlife canids.

Specifically, we investigated (i) the impact of host traits and habitat on black-backed jackal gut microbiota alpha and beta diversity. Since black-backed jackals exhibit an opportunistic lifestyle and prosper particularly on commercially used farmland such as livestock farms or game farms, we expected that the natural variation of microbial alpha and beta diversity is primarily shaped by intrinsic host factors. Concerning environmental factors, we hypothesised no effect of sampling location, since all but one farm were situated within a radius of 100 km in the central Namibian cattle-ranching area and experienced similar abiotic conditions. We expected, however, significant effects of the land-use type on bacterial communities because of contact with different mammal communities. Furthermore, we investigated (ii) the drivers of host gut microbiota similarity. Since host traits are hypothesised to have a larger effect on the gut community than environmental factors in black-backed jackals,

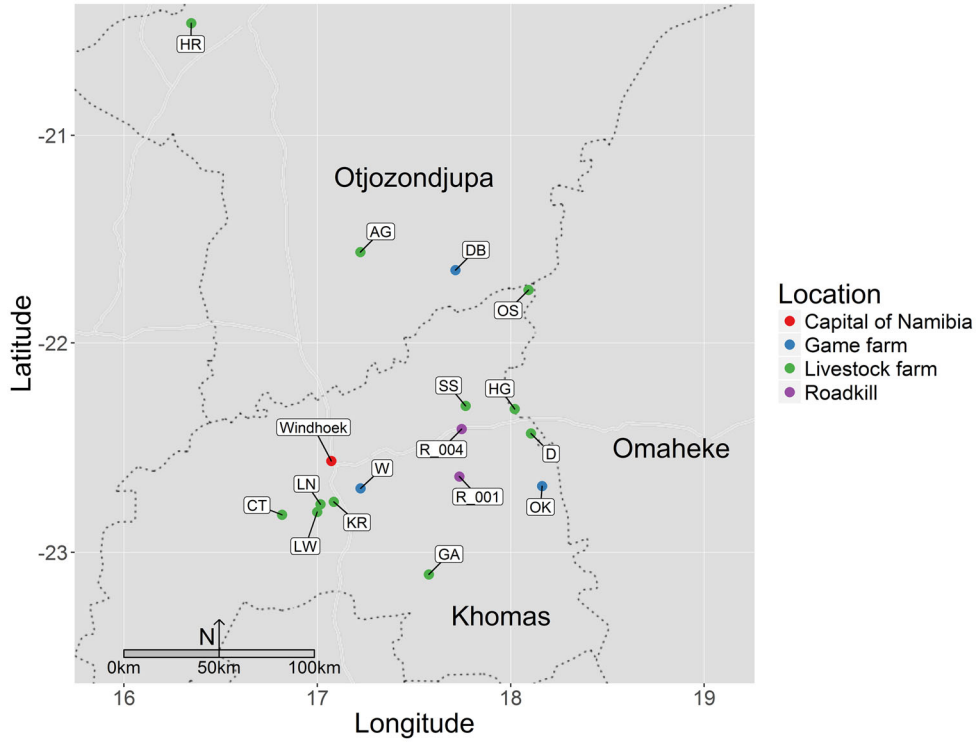


**Figure 1.** Sampling locations in central Namibia. Map showing the 16 sampling locations of black-backed jackals in central Namibia on livestock farms (AG, CT, D, GA, HG, HR, KR, LN, LW, OS, SS), game farms (DB, OK, W) and road kills (R_001 and R_004).

we expected that host genetic relatedness was positively associated with host gut microbiota similarity. We tested (iii) the usefulness of the Firmicutes/Bacteroidetes ratio as a predictor of physiological constrains and expected a higher ratio in individuals with a high energy demand, e.g. lactating females. Moreover, since black-backed jackals are blamed as being pathogen reservoirs and vectors, we investigated (iv) the impact of land-use types on the abundance of bacterial OTUs and whether they are related to potential bacterial pathogens. If the allegation is true, we would expect elevated levels of OTUs related to potential pathogens in black-backed jackals on livestock farms due to the disruption of natural host and pathogen interactions. Finally, to disentangle the effects of environment, host species and dietary preference, we compared (v) the gut microbiotas of black-backed jackals to other wild and domestic carnivores, herbivores and an omnivore. Our approach should help us to understand the main drivers of natural variation in gut microbiotas and to disentangle them from disturbances caused by human encroachment into former natural habitats and their potential consequences for wildlife health.

## MATERIAL AND METHODS

### Biology and sampling of black-backed jackals

Black-backed jackals are territorial and have a highly stable social and mating system. Breeding pairs are often accompanied by their offspring of the current year and non-breeding helpers, i.e. offspring of the previous year (Moehlman 1979; Walton and Joly 2003). In the wild, black-backed jackals can usually reach up to 8 years of life (Ferguson, Nel and de Wet 1983). They form breeding pairs with life-long bonds at the age of usually 2 years. Average home ranges range from 1.8 to 24.9 km$^2$ (Fuller *et al.* 1989; Kaunda 2001; Walton and Joly 2003) and usually do not differ between males and females (Fuller *et al.* 1989). Home ranges are usually smaller when prey abundance is high, such as on commercial farmland used for livestock or wildlife farming (mean core areas: 5.29 ± 0.32 km$^2$; Kamler *et al.* 2012). Although black-backed jackals can walk long distances during dispersal, they predominately stay within their territories. Their core ranges are far smaller than the average size of a Namibian farm (about 79.7 km$^2$, Engler and Baumgärtner 2014).

Samples used in this study were to a great extent collected from necropsies of individuals killed during legal predator control actions organised by farm owners and predator controllers (n = 48) or from fresh road kills (n = 2). The study period was conducted from mid-August 2012 until mid-January 2013 in central Namibia (Ministry of Environment and Tourism Research Permit 1723/2012). The 50 black-backed jackals consisted of 28 males and 22 females and originated from 16 sampling locations. Among these, 11 sampling locations were on livestock farms (locations AG (n = 3), CT (n = 1), D (n = 3), GA (n = 4), HG (n = 2), HR (n = 2), KR (n = 6), LN (n = 3), LW (n = 1), OS (n = 2), SS (n = 1)), 3 on game farms (DB (n = 14), OK (n = 1), W (n = 5)) and 2 on roads and therefore not assigned to any land-use type (R_001 (n = 1), R_004 (n = 1), Fig. 1). Livestock farms of the central Namibian cattle-ranching area are mainly used for cattle farming for meat production, whereas game farms are usually stocked with a mixture of hoofed animals such as impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*), oryx (*Oryx gazella*) and springbok (*Antidorcas marsupialis*) and in some cases more exclusive species such as sable antelopes (*Hippotragus niger*) or giraffes (*Giraffa camelopardalis*). These land-use types occur in a mosaic-like structure next to each other and are usually accessible by black-backed jackals by crawling under the fences. Based on preliminary results on black-backed jackal howl

responses in our study area (Krofel *et al.* 2014), black-backed jackals seem to prefer game farms over livestock farms probably due to the higher abundance of small game which is an ever-present prey, whereas calves of cattle are seasonal and adult cattle are typically not preyed on due to their body size. Both farm types, however, seem to be of good quality for this opportunistic canid which is also reflected in its abundance throughout the central Namibian cattle-ranching area. Since the territories of black-backed jackals are far smaller than the farms sizes and since no black-backed jackal was killed close to the boundary of a farm, the sampling locality was used to assign individuals to farm types. This was also supported by our field observations, since we regularly encountered the same black-backed jackals in the same area of a farm. We cannot exclude the possibility that we also sampled dispersing individuals but this is unlikely since hunting was focused on eliminating black-backed jackals that were present in a specific area of a farm.

It is well known that bacterial communities differ between fractions of the intestine as well between caecal and faecal samples (Bahrndorff *et al.* 2015). Therefore, to avoid the introduction of a sampling bias and to minimise environmental contaminations, we sampled faecal matter from the rectum of black-backed jackals during necropsy with sterile plastic spoons. Samples were then transferred to cryo tubes, stored in a car freezer until arrival at the field station and were finally deep-frozen in liquid nitrogen until isolation of DNA. Every dissected black-backed jackal was sexed, a picture of the teeth was taken for later age determination (Bingham and Purchase 2003) and body length (in m, measured from neck to tail root) and weight (in kg) were taken to determine body mass index (BMI, kg/m$^2$).

## 16S rRNA gene sequencing and initial processing

To study the effects of host traits and the habitat on the variation of the gut microbiota in free-ranging black-backed jackals, we used sequence reads that we had previously generated for another study in which we focused on a comparison of gut microbiotas of black-backed jackals and sympatric cheetahs. Briefly, we extracted DNA from faecal samples and amplified a target fragment of approximately 291 bp in length from the hypervariable V4 region of the 16S rRNA gene. After library preparation by using Fluidigm chemistry (Access Array System for Illumina Sequencing Systems, ©Fluidigm Corporation), sequencing was carried out on an Illumina MiSeq sequencing platform. A detailed description of the laboratory procedure can be found in our preceding publication (Menke *et al.* 2014); the respective sequencing data have been deposited at the sequence read archive (SRA) under the accession number SRP044660.

Our bioinformatic pipeline has previously been described in detail (Menke *et al.* 2014). Following the steps of primer cutting and quality and chimera checking, sequence reads were clustered into operational taxonomic units (OTUs with 97% similarity) by applying an open-reference OTU-picking approach with the USEARCH algorithm (Edgar 2010; Edgar *et al.* 2011). Taxonomy was assigned by using the ribosomal database project (RDP) classifier and the Greengenes database (version 13.5, http://greengenes.lbl.gov).

Sequence variation within bacterial families was investigated by oligotyping (Eren *et al.* 2013) on reads assigned to the 10 most abundant bacterial families. We followed the guide for best practice for oligotyping (http://merenlab.org/2013/11/04/oligotyping-best-practices/) and, in order to treat all individuals equally independent of sequencing depth, we used a minimum % abundance parameter (a = 5) as quality filter. As per bacterial family, we compared sequences of the three most abundant oligotypes with GenBank.

## Impact of host traits and habitat on the variation of gut microbiota diversity of black-backed jackals

We investigated the effects of the host traits sex, age and BMI on the natural alpha and beta diversity variation in gut microbial composition of Namibian black-backed jackals and the impact of the environmental factors sampling location and land-use type (livestock farm vs game farm).

We calculated alpha diversity by using phylogenetic diversity (PD) and applied a generalised linear mixed model (GLMM, function 'glmer' in R package 'lme4') by means of a Poisson distribution correcting for overdispersion. We implemented the host traits 'sex' (male vs female), 'age' divided into four categories (1 = 1–2 years, 2 = 3–4 years, 3 = 5–6 years, 4 = 7–8 years), 'BMI' divided into three equal categories (BMI 1 = 13.53–27.02, BMI 2 = 27.03–40.52, BMI 3 = 40.53–54.01) and environmental factors 'sampling locations' (given as GPS locations) and 'land-use type' (livestock farm vs game farm) in a GLMM treating 'sampling location' as a random factor. We tested for collinearity of factors in this model by using the variance inflation factor (VIF), which revealed that collinearity was not a problem (sqrt(VIF) < 2 for all factors). Black-backed jackals killed on roads were excluded from this model. In order to investigate solely the impact of sampling location on gut microbiotas, we reduced the full model and used only the explanatory variable 'sampling location' in a GLM. The significance of model results was calculated by using a type III analysis of variance (ANOVA; R package 'car' (function 'Anova (model, type = 'III')').

To test the effects of our host factors (sex, age and BMI) and environmental factors (land-use type and sampling location) on beta diversities (Bray-Curtis distance matrix, calculated by using R package 'phyloseq'), we performed a non-parametric multivariate analyses of variance (R package 'vegan' (function 'adonis')) (Oksanen *et al.* 2014). This statistical test was performed on an OTU table in which road kills were excluded because they were not assigned to a land-use type and in which counts were transformed into fractional abundances and only those OTUs were kept that occurred with a mean greater than 1e-05. We used 999 permutations for calculations of p-values.

## Drivers of host gut microbiota similarity

The genetic relatedness of all samples was investigated by using a commercially available kit (Canine Genotypes Panel 1.1, Thermo Scientific) that allows the co-amplification of 19 microsatellite markers in domestic dogs in a multiplex polymerase chain reaction. We isolated DNA from black-backed jackal liver samples and followed the manufacturer's protocol. The 16 marker-produced products in all black-backed jackals were sequenced on an automated electrophoresis instrument (ABI3130xl Genetic Analyser (Applied Biosystems, Foster City, California, United States)) and analysed with the Orange DNA Size Standard (MCLAB). The identification of peaks and detection of allele length (Table S1) were performed by using Gene Mapper v3.7 (Applied Biosystems). We constructed five groups from least to closely related (<–0.2; –0.2–0.0; 0.0–0.2; 0.2–0.4; >0.4), each representing a different range of host genetic relatedness (calculated on the microsatellite data by using the relatedness estimator W (Wang 2002) in kingroup-v2 (Konovalov, Manning and Henshaw 2004)). We tested whether pairwise Bray-Curtis distances were affected by host genetic

relatedness by using a one-way ANOVA. In addition, we applied a Wilcoxon rank sum test to test whether pairwise Bray-Curtis distances differed significantly between the groups of host genetic relatedness. To investigate whether genetic relatedness can explain the tips of the phylogenetic tree of the gut microbiota, we calculated a black-backed jackal pedigree using the likelihood ratio also implemented in kingroup-v2. We considered only results with a significance threshold of $P < 0.001$.

To test whether individuals also clustered significantly according to 'sampling location', we investigated whether gut microbiotas of black-backed jackals from the same sampling location clustered together in a phylogenetic tree and compared the real observations with a random assignment of individuals to sampling locations. We applied the 'minimum entropy decomposition' (MED) approach (Eren *et al.* 2014) on all sequences that have been previously assigned to the kingdom of Bacteria by the OTU approach. MED implements the principle of oligotyping (Eren *et al.* 2013) by using information uncertainty among sequence reads based on the Shannon entropy and applies it to the entire high-throughput sequencing marker gene dataset. Thus, MED iteratively decomposes a dataset until all entropy is explained. Subsequently, we subsampled 8000 sequences per individual from the matrix˙count.txt file (output of MED analyses) and computed the Bray-Curtis distance matrix (Hamady, Lozupone and Knight 2009) separately for each rarefied table. To assess the frequency with which cluster nodes were recovered, we applied a jackknife approach (100 times) for each distance matrix and used the unweighted pair group method with arithmetic mean (UPGMA) for tree building. Additionally, we created principal coordinate analysis (PCoA) plots of black-backed jackal gut microbiotas based on the Bray-Curtis distance metric to illustrate clustering according to individual characteristics (sex, BMI and habitat type). Finally, we tested whether beta diversities based on Bray-Curtis distances were greater between black-backed jackals from different locations compared with individuals sampled at the same location by using a Wilcoxon rank sum test. For this analysis, only sampling locations with at least two sampled individuals were used.

## Firmicutes/Bacteroidetes ratio and OTU relatedness to potential pathogens

We calculated the Firmicutes/Bacteroidetes ratio for all black-backed jackal individuals and applied a log transformation. We used a linear mixed effects model (R package 'lme4' (function 'lmer')) to test the impact of host traits (sex, age and BMI) and environmental factors (land-use type and sampling location) with sampling location as a random factor. Road kills were excluded from these analyses because no land-use type could be assigned. The significance of model results was calculated by using a type III ANOVA (R package 'car', (function 'Anova (model, type = 'III')').

To test whether individuals living predominantly on livestock or game farms differed in the abundances of specific bacterial OTUs, we applied the DESeq2 approach (R package 'phyloseq'; McMurdie and Holmes 2014) which estimates variance-mean dependence in count data from high-throughput sequencing (Love, Huber and Anders 2014). Basically, we converted our phyloseq class object into a DESeq2 object and applied the 'DESeq' function, which performs three analysis steps: (i) estimation of size factors, (ii) estimation of dispersion and (iii) negative binomial GLM fitting and Wald statistics. We tested 'DESeq' for all group comparisons by using various 'fitTypes' ('mean', 'local' and 'parametric') and investigated the type of fitting of dispersion to the mean intensity that worked best for our data. In our case, 'local', which fits a local regression of log dispersions over a log base mean and in which points are weighted by normalised mean counts, gave the best fit and was therefore used in all analyses. Here, we summarised OTUs that differed significantly. We used a significance threshold of 0.05 (after multiple-inference correction by using Benjamini-Hochberg) for the comparison between black-backed jackal gut microbiota from livestock or game farms at the bacterial genus level and present differences in abundance as a log2-fold change (a log2-fold change (B/A) of ± 1 means that B is twice as large/small as A, whereas a log2-fold change of ± 2 means that B is 4x as large/small as A). We assigned taxonomy to OTUs which were significantly different abundant between livestock and game farms by comparing them to GenBank (excluding uncultured and environmental samples).

## Comparison of gut microbial diversity of black-backed jackals with domestic dogs, and other mammals

To disentangle the effects of environment, host species and dietary preference, we compared the gut microbiotas of black-backed jackals to other wild and domestic carnivores, herbivores and an omnivore. We first solely compared gut microbiotas of black-backed jackals and domestic dogs to identify differences between the 'wild' and the 'domestic' canid gut microbiota. Therefore, we downloaded 16S rRNA gene sequencing reads from a study of 40 clinically healthy domestic dogs (Šlapeta *et al.* 2015) from the SRA under accession number PRJNA276586 using the SRA-Toolkit (https://www.ncbi.nlm.nih.gov/sra/docs/toolkitsoft/). Dog samples from this study originated from north Queensland and included free-roaming community dogs (n = 10), pound dogs (n = 20) and boarded privately owned dogs (n = 10).

Bacterial reads of these samples were trimmed, quality-filtered and chimera-checked as described above for black-backed jackals sequence reads. Their sequence reads were combined with sequence reads of black-backed jackals into a single file and clustered together against the Greengenes bacterial database using an open reference approach as integrated in QIIME (Caporaso *et al.* 2010). We tested whether black-backed jackal gut microbiota differed significantly from domestic dogs by using non-parametric multivariate analyses of variance on a weighted UniFrac matrix (R package 'vegan' (function 'adonis')) (Oksanen *et al.* 2014). We further tested whether differences in alpha diversities (PD) were significant by using a Wilcoxon rank sum test. To investigate those bacterial OTUs that were significantly different in abundance between black-backed jackals and domestic dogs, we again applied the DESeq2 approach (R package 'phyloseq' (McMurdie and Holmes 2014)) as described above for the comparison of the gut microbiota differences of black-backed jackals sampled on different land-use types.

In a second analysis, we compared the gut microbiotas (all based on high-throughput sequencing reads of the V4 region of the 16S rRNA gene) from black-backed jackals (omnivorous, Namibia, *Canidae*), domestic dogs (commercial pet food/scavenge, Australia, *Canidae*) (Šlapeta *et al.* 2015), cheetahs (carnivorous, Namibia, *Felidae*) (Menke *et al.* 2014), brown bears (omnivorous, Scandinavia, *Ursidae*) (Sommer *et al.* 2016), giraffes (herbivorous, Namibia, *Giraffidae*) (Menke, Meier and Sommer 2015) and springboks (herbivorous, Namibia, *Bovidae*) (Menke, Meier and Sommer 2015) as described above. We created a PCoA plot based on the weighted UniFrac distance metric to illustrate how black-backed jackal gut microbiota cluster in relation to the

gut microbiota of the other species with which they share at the most one of the factors (environment, phylogeny or dietary preference).

In addition, we compared the topological similarity between phylogenetic trees of selected mammalian species and their gut microbiotas (Brooks *et al.* 2016). The phylogenetic host tree was based on the taxonomy downloaded from NCBI (www.ncbi.nlm.nih.gov/taxonomy). For the construction of the microbiota phylogenetic tree, gut microbiota sequencing reads were merged on the species level and a weighted UniFrac distance matrix was created (McMurdie and Holmes 2013). The final tree was built using the UPGMA agglomeration method, and bootstrap values for tree nodes were calculated (nboot = 100) (Suzuki and Shimodaira 2006). We tested for the congruence between the host and the microbiota phylogenetic tree by applying the Robinson-Foulds (RF) tree metric (Robinson and Foulds 1981) and tested the null hypothesis of no topological similarity between the two phylogenetic trees using a permutation test (nsim = 1000) (Revell 2012). We further calculated the normalised RF (Schliep 2011). Finally, we plotted both trees next to each other in a 'tanglegram' (Galili 2015).

Microbiota sequencing data of brown bears was downloaded at the European Nucleotide Archive under the accession numbers ERS1023047–ERS1023051, sequencing data of cheetahs is available under the SRA accession number SRP044660 and sequencing data of giraffes and springbok under the SRA accession number SRP056240.

All data analyses, statistics and visualisations were conducted in 'quantitative insight into microbial ecology' (QIIME) (Caporaso *et al.* 2010), MED (Eren *et al.* 2014) and R (v. 3.0.3) (R Core Team 2013) by using the packages 'ade4' (Dray, Dufour and others 2007), 'ape' (Paradis, Claude and Strimmer 2004), 'car' (Fox, Weisberg and Fox 2011), 'DECIPHER' (Wright 2016), 'dendextend' (Galili 2015), 'ggmap' (Kahle and Wickham 2013), 'ggplot2' (Wickham 2009), 'ggrepel' (Slowikowski 2017), 'grid' (Murrell 2005),'ggsn' (Baquero 2016), 'lme4' (Bates *et al.* 2015), 'phangorn' (Schliep 2011), 'phyloseq' (McMurdie and Holmes 2013), 'phytools' (Revell 2012), 'pvclust' (Suzuki and Shimodaira 2006) and 'vegan' (Oksanen *et al.* 2014). Figures were partially finalised for publication using GIMP (https://www.gimp.org/) and Inkscape (https://inkscape.org/).

# RESULTS

## Gut microbial variation in black-backed jackals

Sequencing of the 16S rRNA gene of the gut bacterial communities resulted in 1 330 924 sequences after all filtering steps, with read abundances per individual ranging from 8718 to 40 466 with an average of 26 618 ± 8756. Rarefaction curves based on the alpha diversity measurements Shannon index and PD (Fig. S1, Supporting Information) support that the sequencing effort was sufficient.

Our results revealed that bacterial taxa varied largely in their proportions between individuals. The 10 most abundant bacterial families (mean of 20.5% in Fusobacteriaceae down to a mean of 2.3% in Mogibacteriaceae) were present in almost all individuals (Fig. S2, Supporting Information), whereas those bacterial families with lower mean proportions (<2%) were only present at low proportions (<0.1%) or absent in many individuals. Oligotyping of the 10 most abundant bacterial families revealed that the sequence variation within bacterial families was highest in the Lachnospiraceae (29 oligotypes) followed by the Ruminococcaceae (25 oligotypes) and lowest in the Enterobacteriaceae (9 oligotypes), Paraprevotellaceae (9 oligotypes) and Veillonellaceae (9 oligotypes) (Fig. S2). Comparing sequences of the three most abundant oligotypes per bacterial family to GenBank revealed that, in several families, oligotypes matched to various bacterial species (Table 1).

## Impact of host traits and environmental factors on gut microbiota diversity

Alpha diversity, i.e. PD, ranged from 26.83 to 102.77 with a mean of 68.23 ± 16.30 among all individuals (Fig. S3, Supporting Information) and varied between categories of host traits and environmental factors (Fig. 2, Table S2, Supporting Information). A GLMM used to investigate the drivers of gut microbial alpha diversity revealed that the host traits, sex ($X^2 = 20.14$, $P < 0.001$), age ($X^2 = 13.22$, $P = 0.004$) and BMI ($X^2 = 19.70$, $P < 0.001$), were the main drivers of gut microbial alpha diversities, whereas the environmental factor land-use type ($X^2 = 0.77$, $P > 0.05$) did not have an effect. In addition, alpha diversities differed between sampling locations ($X^2 = 41.28$, $P < 0.001$).

Non-parametric multivariate analyses of variance carried out on the beta diversity matrix revealed differences between the sexes ($R^2 = 0.04$, $P = 0.041$) and BMIs ($R^2 = 0.08$, $P = 0.011$), whereas age, sampling location and land-use type did not have an effect ($R^2 = 0.04$, $P = 0.881$; $R^2 = 0.265$; $P = 0.194$, $R^2 = 0.03$; $P = 0.193$, respectively) (Fig. S4, Supporting Information).

## Drivers of host gut microbiota similarity

We applied a panel of microsatellite markers to test whether the clustering of black-backed jackal gut microbiotas (i.e. phylogenetic relatedness) could be explained by host genetic relatedness. Results of the pedigree analysis revealed that several individuals sampled in close proximity were full siblings (DB_001/DB_011, DB_002/DB_014, DB_005/DB_013, GA_003/GA_004, KR_001/KR_005, KR_003/KR_004 and W_005/W_006) and one pair was a parental–offspring relationship (KR_002/KR_003). Out of these, the pairs DB_005/DB_013 and W_005/W_006 represented sister tips in the bacterial phylogenetic tree, and the individuals KR_002 and KR_003, which formed the parental–offspring pair, were placed in closely related sister clades in the phylogenetic analyses (Fig. 3). We found a significant relationship between genetic relatedness and gut microbiota similarity (Fig. 4, ANOVA: $F_{1,4} = 3.319$, $P = 0.010$), and all groups containing less closely related individuals showed significantly lower gut microbiota similarities than the group containing the more closely related individuals (Wilcoxon rank sum test: all $P < 0.05$).

We also detected a clustering of individuals according to sampling location ($P < 0.001$). Accordingly, the Bray-Curtis distances of black-backed jackals between and within sampling locations were different (Wilcoxon rank sum test: $W = 43\,879$, $P < 0.001$; Fig. S5, Supporting Information) showing that microbial communities within sampling locations were more similar to each other than to microbial communities of individuals from distant sampling locations.

## Firmicutes/Bacteroidetes ratio as predictor of physiological constrains and OTU relatedness to potential pathogens

The Firmicutes/Bacteroidetes ratio also varied greatly between individuals with values ranging from 0.40 to 132.75 and a mean of 11.93 ± 26.83 (Fig. 5). When we tested the combined effect of host traits and environmental factors, sex was the only factor significantly affecting the Firmicutes/Bacteroidetes ratio (linear

**Table 1.** GenBank blast results for the three most abundant bacterial oligotypes within the respective bacterial family.

| Bacterial family | Total oligotypes | Oligotype | Average proportion of oligotype within taxon (%) | GenBank blast hit | Identifier | Similarity (%) | E-score |
|---|---|---|---|---|---|---|---|
| | | CTACAAAGGCCTAGAGT | 62.29 | *Fusobacterium mortiferum* | LT574675.1 | 99 | 2.00E-124 |
| **Fusobacteriaceae** | 10 | CCGCAGGAATTCAGGAG | 14.22 | *Fusobacteriaceae bacterium* | HG326493.1 | 96 | 7.00E-113 |
| | | TTACTAGAATTCGAGAG | 5.13 | *Fusobacteriaceae bacterium* | EU728722.1 | 100 | 7.00E-128 |
| | | ATGTGTACGCTGCAAGATG | 41.13 | *Bacteroides dorei* | CP011531.1 | 98 | 9.00E-122 |
| **Bacteroidaceae** | 17 | ATGCGTACGCTGCAAGATG | 13.49 | *Bacteroides dorei* | CP011531.1 | 98 | 4.00E-120 |
| | | GCAATTAATTAATGGAGCT | 9.47 | *Bacteroides stercorirosoris* | NR˙113 207.1 | 96 | 4.00E-110 |
| | | TTTTGAGTCA | 58.22 | *[Clostridium] hiranonis* | AB971818.1 | 100 | 7.00E-128 |
| **Clostridiaceae** | 16 | GAGAACTGTT | 19.37 | *Clostridium perfringens* | KX826968.1 | 100 | 2.00E-128 |
| | | TTTTGAGTCT | 4.66 | *[Clostridium] hiranonis* | AB971818.1 | 99 | 2.00E-124 |
| | | TATGGCTCA | 15.41 | *[Ruminococcus] torques* | AB910746.1 | 100 | 2.00E-128 |
| **Lachnospiraceae** | 29 | AAATGGCAG | 14.03 | *[Ruminococcus] obeum* | NR_118 692.1 | 98 | 2.00E-118 |
| | | TATGACTTA | 9.54 | *[Ruminococcus] torques* | AB910746.1 | 99 | 4.00E-125 |
| | | CCTGCGCGGA–GG | 33.83 | *Prevotellaceae bacterium* | LT576394.1 | 97 | 4.00E-115 |
| **Paraprevotellaceae** | 9 | A-GTTCAACTCA– | 18.12 | *Alloprevotella* sp. | KM462157.1 | 91 | 4.00E-91 |
| | | TTTGCGCGGA–AG | 18.19 | *Prevotellaceae bacterium* | LT576394.1 | 96 | 2.00E-113 |
| | | -GGCTATGCAG | 89.23 | *Phascolarctobacterium* sp. | JN713316.1 | 100 | 7.00E-128 |
| **Veillonellaceae** | 9 | G-GCTATGCAG | 2.91 | *Phascolarctobacterium* sp. | JN713316.1 | 99 | 3.00E-126 |
| | | G-AGAGCAGTA | 3.88 | *Phascolarctobacterium* sp. | JN713316.1 | 96 | 7.00E-108 |
| | | CAAAAATATTTTTGTCCGCGAC | 15.76 | *Faecalibacterium prausnitzii* | KP005711.1 | 99 | 9.00E-127 |
| **Ruminococcaceae** | 25 | GGA–ATATGCACCATTATATG | 14.25 | *Ruminococcus* sp. | LT598596.1 | 99 | 3.00E-126 |
| | | TGAGACGC-TATCTCGTATAGA | 12.48 | Uncultured bacterium isolate | KX858551.1 | 97 | 4.00E-115 |
| | | GCTC-T-CCG | 45.50 | *Collinsella tanakaei* | LT223657.1 | 100 | 7.00E-128 |
| **Coriobacteriaceae** | 12 | CGCGTC-TGG | 23.78 | *Collinsella intestinalis* | KP233378.1 | 100 | 7.00E-128 |
| | | CCCCCGACGC | 13.39 | *Slackia faecicanis* | NR_042220.1 | 100 | 2.00E-128 |
| | | GTATACTTCGT | 83.81 | *Escherichia coli* | CP016404.1 | 100 | 2.00E-128 |
| **Enterobacteriaceae** | 9 | TTATACTTCGT | 3.28 | *Escherichia coli* | CP016404.1 | 99 | 9.00E-127 |
| | | GTATATAATAT | 4.14 | *Plesiomonas shigelloides* | KX828370.1 | 100 | 2.00E-128 |
| | | TTACCAA | 36.17 | Eubacteriaceae bacterium | LT576391.1 | 96 | 3.00E-111 |
| **Mogibacteriaceae** | 16 | AACAGTT | 22.48 | Bacterium NLAE-zl-G513 | JX048499.1 | 98 | 7.00E-118 |
| | | TTTCAGA | 18.76 | Peptostreptococcaceae bacterium | JN713238.1 | 95 | 3.00E-106 |

Uncultured and environmental samples were excluded for blast searches and only the closest match is presented.

mixed effects model: $X^2 = 4.715$, $P = 0.030$), with females having a higher ratio than males. We did not find a pattern regarding pregnancy because we detected high (W_004) as well as low (HG_001, HR_002) ratios in pregnant females. One female (D_003) with a high ratio suffered heavily from sarcoptic mange (*Sarcoptes scabiei*).

OTUs of the genera *Anaerobiospirillum* and *Blautia* (in two out of three cases) were more abundant on livestock farms, whereas OTUs of the genera *Clostridium*, *Ignatzschineria*, *Peptococcus* and *[Ruminococcus]* were more abundant on game farms (Table 2). Some OTUs that were differently abundant could not be assigned to the genus level by the RDP classifier. Comparing OTUs assigned to *Blautia* and *Lactobacillus* to GenBank revealed that they belonged to different bacterial species, whereby *Blautia glucerasea*, *Lactobacillus reuteri* and *L. gasseri* were more abundant on livestock farms and *B. obeum* and *L. coryniformes* were more abundant on game farms. Two OTUs that were more abundant on game farms were 100% identical to the potentially pathogenic *Ignatzschineria* sp. and *Clostridium tertium*.

## Comparison of gut microbial diversity of black-backed jackals with domestic dogs, and other mammals

Gut microbiota of black-backed jackals clustered significantly differently from those of domestic dogs (Fig. 6a; PERMANOVA: $R^2 = 0.29$, $P = 0.001$) and had higher alpha diversities (Fig. 6b; Wilcoxon rank sum test: $W = 1995$, $P < 0.001$). In addition, several bacterial genera were differently abundant between these two groups of canids (Fig. 6c). Black-backed jackals had significantly higher proportions of *Bacteroides*, *Clostridium* and *Fusobacterium*, whereas domestic dogs had higher proportions of *Blautia* and *Streptococcus*. Two bacterial classes, Clostridia and Fusobacteriia, were found to be important in scavenging animals (Roggenbuck *et al.* 2014a). Both were present at high proportions in black-backed jackals and dogs, with similar mean proportions for Clostridia (black-backed jackals: 39.0%; domestic dogs: 40.3%) but different mean proportions for Fusobacteriia (black-backed jackals: 21.8%; domestic dogs: 10.9%).

Clustering of black-backed jackal gut microbiotas together with gut microbiotas of other wild and domestic carnivores, herbivores and an omnivore (Fig. 7a) revealed that black-backed jackals clustered closest to sympatric cheetahs, whereas domestic dogs had the second most similar gut microbiotas in relation to black-backed jackals. Not surprisingly, brown bear gut microbiotas clustered closer to the other carnivores than gut microbiotas of giraffes and springbok. Results from the comparison of the microbiota dendrogram with the host dendrogram revealed that there was no topological similarity between the trees (Robinson-Foulds, nRF = 0.75, $P = 0.285$). Interestingly, phylosymbiosis, the congruence between the host tree and the tree of

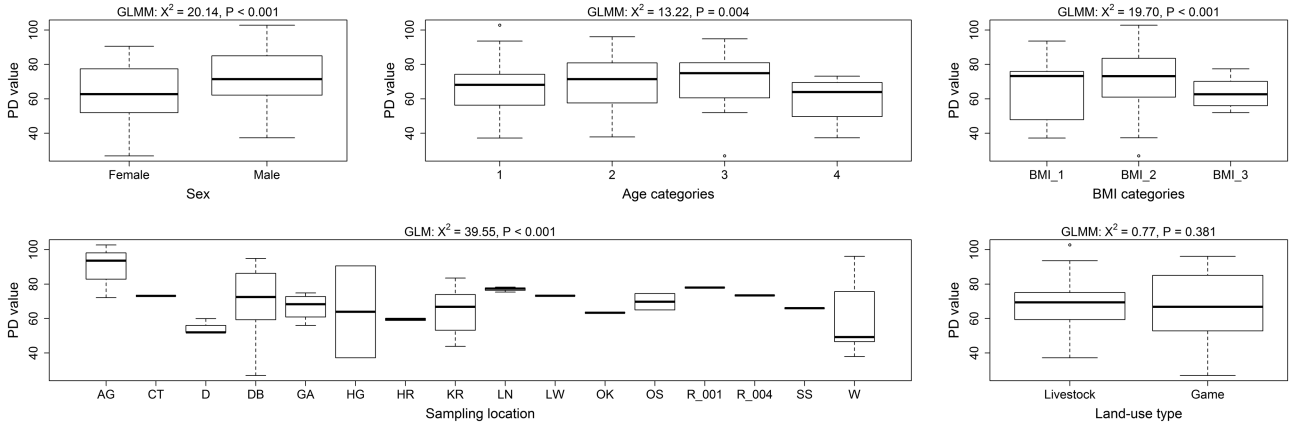


**Figure 2.** Alpha diversity in terms of PD for host traits and environmental factors. PD was averaged for the factors **(a)** sex, **(b)** age, **(c)** BMI, **(d)** sampling location and (e) land-use type for all individuals belonging to the respective category. Statistical results are based on GLMM full model data (only sampling location was tested solely by using a GLM, because it was a random factor in the GLMM).

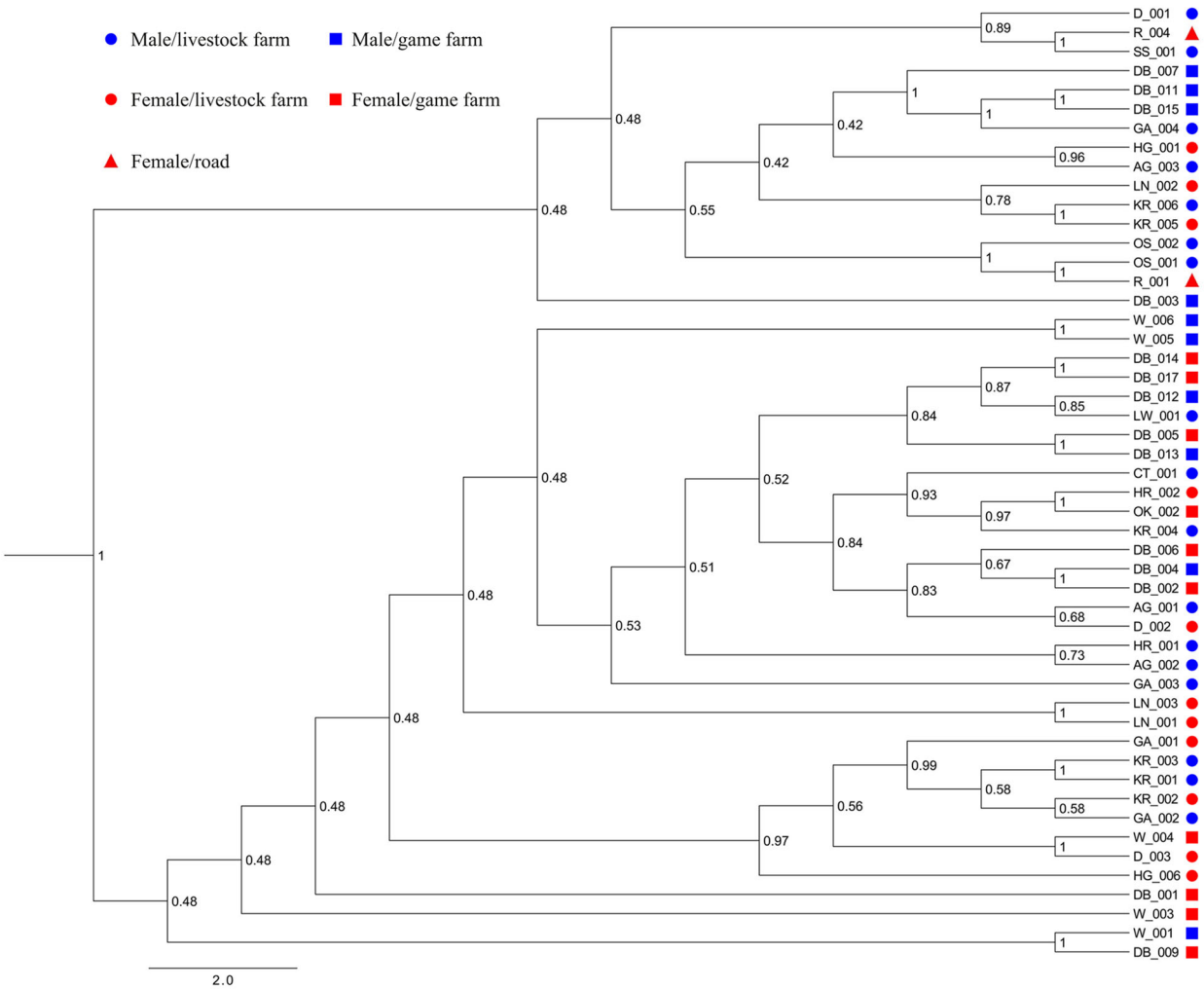


**Figure 3.** Gut microbiota similarity between individual black-backed jackals. The UPGMA tree was constructed based on a Bray-Curtis distance matrix calculated on the matrix_count.txt output file of the 'Minimum Entropy Decomposition' approach (Eren *et al.* 2014). Each individual is represented by 8000 sequences. Numbers at nodes represent the jackknife support values based on the comparison of 100 UPGMA trees.

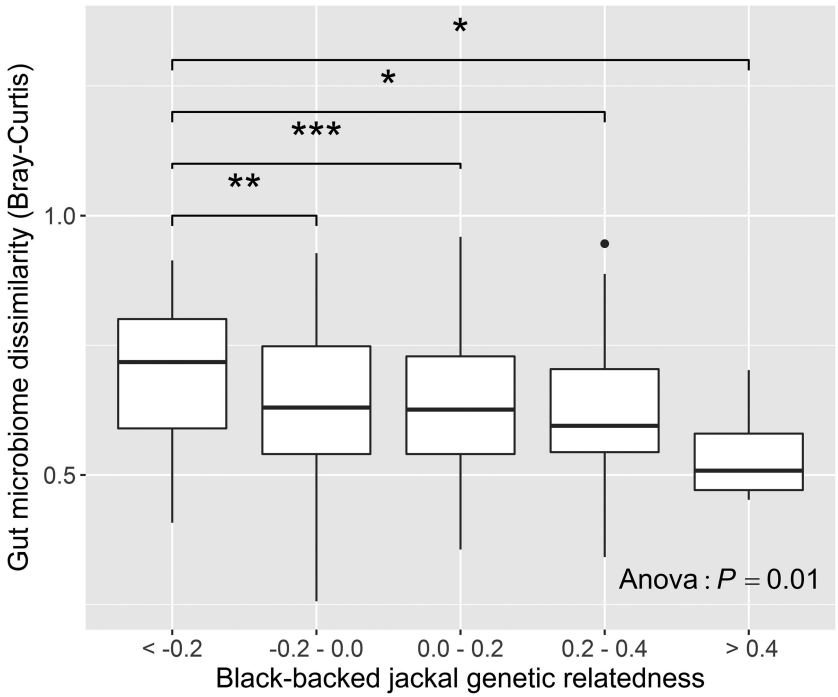


**Figure 4.** Effect of host genetic relatedness on gut microbiota similarity. The more closely related were the individuals, the more similar were their gut microbiotas, as measured by the Bray-Curtis distance metric (ANOVA: $F_{1,4} = 3.319$, $P = 0.010$).

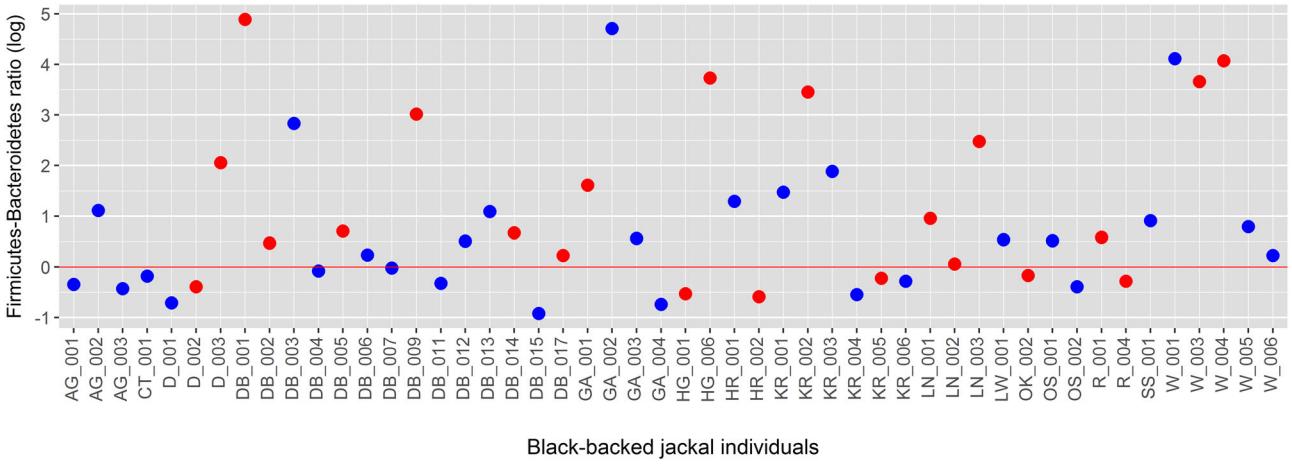


**Figure 5.** Log-transformed ratio of Firmicutes to Bacteroidetes of individual female (red) and male (blue) black-backed jackals. The red line represents the threshold above which the proportion of the phylum Firmicutes is higher than the proportion of Bacteroidetes.

microbial communities, was only present in giraffe and springbok (Fig. 7b).

## DISCUSSION

### Individual gut microbiota variation in black-backed jackals

Mammalian gut microbiota diversity is strongly related to host diet. Generally, gut microbiotas of herbivores contain high numbers of bacterial phyla (n = 14), omnivores lower numbers (n = 12) and carnivores the lowest numbers (n = 6; Ley *et al.* 2008). The number of bacterial phyla detected in the omnivorous black-backed jackal (n = 7, mean ≥ 0.1% abundance; Menke *et al.* 2014) is, however, more similar to that of a carnivorous than of an omnivorous nutrition. Similar to the number of bacterial phyla, alpha diversity measures such as PD should also decrease from herbivores to omnivores to carnivores. Our studies support this because values of alpha diversity in black-backed jackals are higher (mean PD: 39.21) than those in the carnivorous cheetah (mean PD: 20.66) (Menke *et al.* 2014) but lower than those in the herbivorous giraffe (*Giraffa camelopardalis*) (mean PD: 144.39) and springbok (*Antidorcas marsupialis*) (mean PD: 96.83) (Menke, Meier and Sommer 2015) (publications followed the same bioinformatic pipeline and mean PDs of herbivores remained higher than the mean PDs of carnivores when rarefied at the same sequence level (unpublished data)). Black-backed jackals are opportunistic feeders and occur in markedly different habitats. According to the optimal foraging theory, species with a diverse diet should select those prey animals or food items that

**Table 2.** Bacterial OTUs with significantly different abundance in individuals from livestock vs. game farms, as revealed by using DESeq2.

| | Game farm vs livestock farm | | | | | | |
|---|---|---|---|---|---|---|---|
| OTU | log2FoldChange | Padj | Family | Genus | Genbank | Similarity (%) | Identifier |
| **190577** | 1.904 | 0.008 | Lachnospiraceae | *[Ruminococcus]* | *Ruminococcus faecis* | 98 | ref\|NR_116747.1\| |
| **4482983** | 2.049 | 0.008 | Lachnospiraceae | *[Ruminococcus]* | *[Ruminococcus] torques* | 99 | dbj\|AB910746.1\| |
| **181226** | −2.802 | 0.035 | Lachnospiraceae | *Blautia* | *Blautia glucerasea* | 99 | ref\|NR_113231.1\| |
| **195166** | −2.589 | 0.003 | Lachnospiraceae | *Blautia* | *Blautia glucerasea* | 100 | ref\|NR_113231.1\| |
| **318949** | −4.838 | 0.019 | Lachnospiraceae | NA | *Bacterium YE62* | 95 | gb\|AY442826.1\| |
| **4481195** | 1.346 | 0.050 | Lachnospiraceae | *Blautia* | *Blautia obeum* | 97 | ref\|NR_118692.1\| |
| **4374663** | −4.923 | <0.001 | Lactobacillaceae | *Lactobacillus* | *Lactobacillus reuteri* | 99 | gb\|KU754503.1\| |
| **4349891** | 5.249 | 0.009 | Lactobacillaceae | NA | *Lactobacillus coryniformis* | 99 | dbj\|LC065033.1\| |
| **4453039** | 5.707 | <0.001 | Lactobacillaceae | NA | *Lactobacillus coryniformis* | 100 | dbj\|LC065033.1\| |
| **4428313** | −3.906 | 0.050 | Lactobacillaceae | *Lactobacillus* | *Lactobacillus gasseri* | 99 | gb\|KU726652.1\| |
| **New.CleanUp.ReferenceOTU460** | 2.869 | 0.032 | [Mogibacteriaceae] | NA | *Eubacterium* sp. | 94 | emb\|LN998061.1\| |
| **4330001** | 3.105 | 0.050 | [Mogibacteriaceae] | NA | *Eubacterium* sp. | 95 | emb\|LN998061.1\| |
| **New.ReferenceOTU142** | 3.207 | 0.029 | [Mogibacteriaceae] | NA | *Eubacterium* sp. | 95 | emb\|LN998061.1\| |
| **199182** | −4.769 | 0.010 | Ruminococcaceae | NA | *Faecalibacterium prausnitzii* | 94 | gb\|KP005553.1\| |
| **New.CleanUp.ReferenceOTU243** | 1.294 | 0.050 | Peptococcaceae | *Peptococcus* | *Peptococcus niger* | 94 | gb\|KU726680.1\| |
| **New.ReferenceOTU57** | −3.062 | 0.008 | Succinivibrionaceae | *Anaerobiospirillum* | *Anaerobiospirillum succiniciproducens* | 94 | gb\|EU863654.1\| |
| **New.CleanUp.ReferenceOTU520** | −2.949 | 0.019 | Succinivibrionaceae | *Anaerobiospirillum* | *Anaerobiospirillum succiniciproducens* | 95 | gb\|EU863654.1\| |
| **New.ReferenceOTU180** | −2.930 | 0.032 | Succinivibrionaceae | NA | *Succinivibrio dextrinosolvens* | 94 | ref\|NR_02 6476.1\| |
| **1142199** | 4.022 | 0.050 | Xanthomonadaceae | *Ignatzschineria* | *Ignatzschineria* sp. | 100 | emb\|AJ539230.1\| |
| **4452633** | 4.483 | 0.008 | Clostridiaceae | *Clostridium* | *Clostridium tertium* | 100 | dbj\|AB689162.1\| |

Significance threshold was set at 0.05 and p-value adjustment (Padj) for multiple testing was performed by using the Benjamin-Hochberg method. Negative log2-fold changes indicate a higher abundance on livestock farms, whereas positive log2-fold changes indicate a higher abundance on game farms. Resulting OTUs were compared against GenBank and the best result is presented.

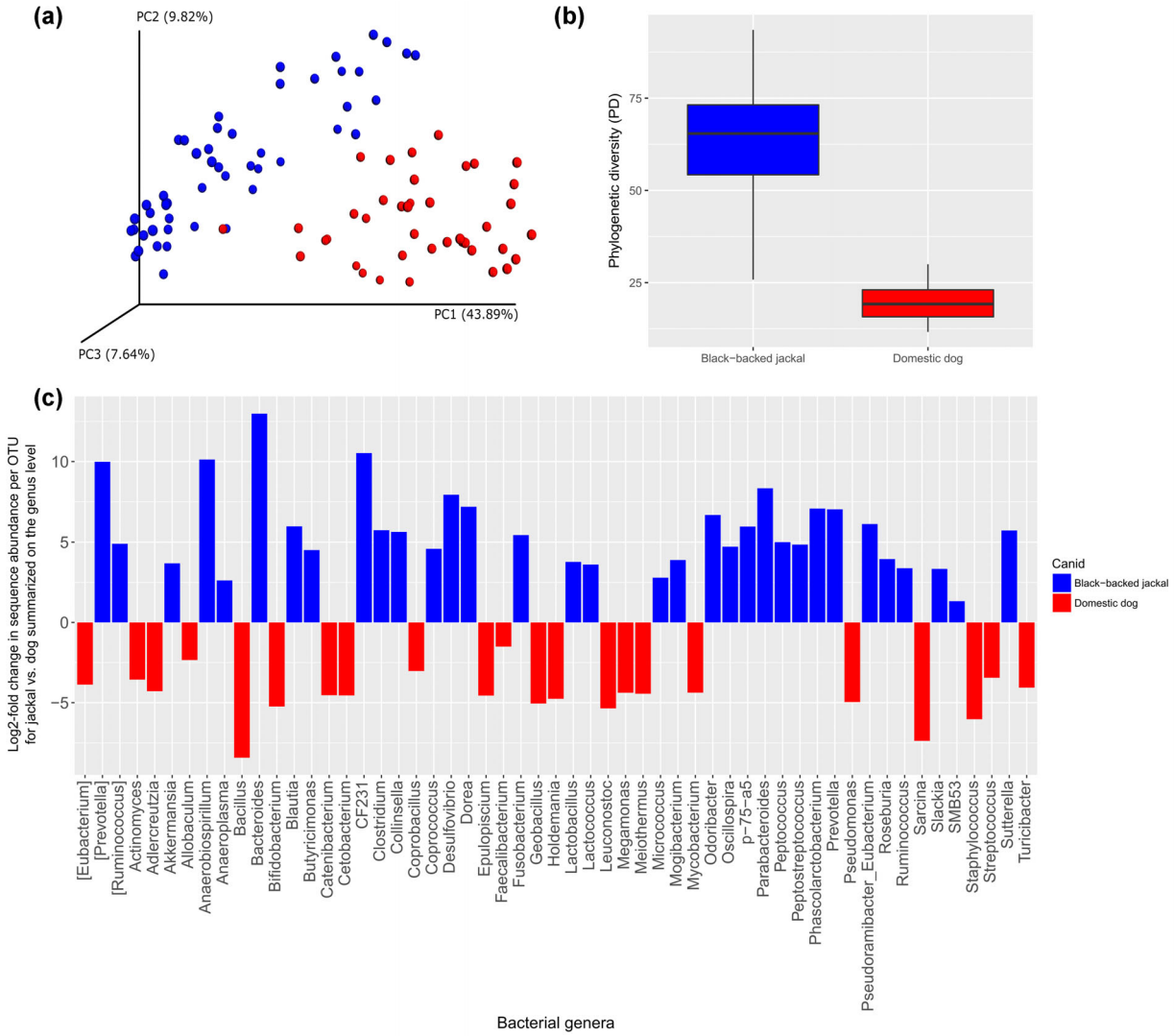


**Figure 6.** Comparison of black-backed jackal and domestic dog gut microbiotas. (**a**) PCoA plot of gut microbiotas of black-backed jackals (blue) and domestic dogs (red) based on the weighted UniFrac metric. 'Wild' and 'domestic' gut microbiotas differ in their bacterial communities (PERMANOVA: $R^2 = 0.29$, $P = 0.001$). (**b**) Alpha diversities (PD) of black-backed jackal and domestic dog gut microbiotas. Values of PD are higher and more variable in 'wild' than in 'domestic' gut microbiotas (Wilcoxon rank sum test: $W = 1995$, $P < 0.001$). (**c**) Differences in abundance of bacterial genera between microbiotas of black-backed jackals and domestic dogs. Red bars represent the log2fold-changes for genera in which black-backed jackals had significantly higher abundances, whereas blue bars represent bacterial genera in which domestic dogs had significantly higher abundances.

provide the highest energy outcome for the lowest cost investment (Krebs and Davis 1978). In our study area in central Namibia, game species of small body size such as the springbok, common duiker (*Sylvicapra grimmia*) and South African springhare (*Pedetes capensis*) are abundant. Black-backed jackals probably have a more mammal-based diet in central Namibia compared with their conspecifics in other habitats (Rowe-Rowe 1983; Kamler, Klare and Macdonald 2012), e.g. the less productive environment in the Namib desert in which their diet is complemented to a great extent by insects and invertebrates (Goldenberg *et al.* 2010).

## Impact of host traits and habitat on black-backed jackal gut microbiota diversity

We have revealed that host traits have a larger impact in shaping alpha and beta diversities in black-backed jackals than environmental factors in habitats such as livestock and game farms. In particular, the sex-dependent differences in bacterial community composition were strong, as has also been shown in humans (Schnorr *et al.* 2014) and other vertebrates (Bolnick *et al.* 2014). Differences in gut microbiotas between the sexes might result from differences in foraging and feeding behaviour (Grafton 1965; Davenport *et al.* 2015). However, this is a rather unlikely explanation for black-backed jackals because breeding pairs often forage together. Moreover, outside the breeding season, the differences in home range sizes are small with slight modifications according to the geographical region (Walton and Joly 2003). Only during lactation do mothers stay with their pups; males, and sometimes additional helpers, regurgitate or bring food to the mothers (Moehlman 1979). Sex-specific physiological effects might therefore be involved in shaping gut microbiotas in black-backed jackals, as is well known for other mammalian species (Ley *et al.* 2008; McCord *et al.* 2014; Wong, Dobson and Douglas 2014). In addition, sex hormones can modulate the gut microbiota (Koren *et al.* 2012) and, vice versa, gut microbiota can

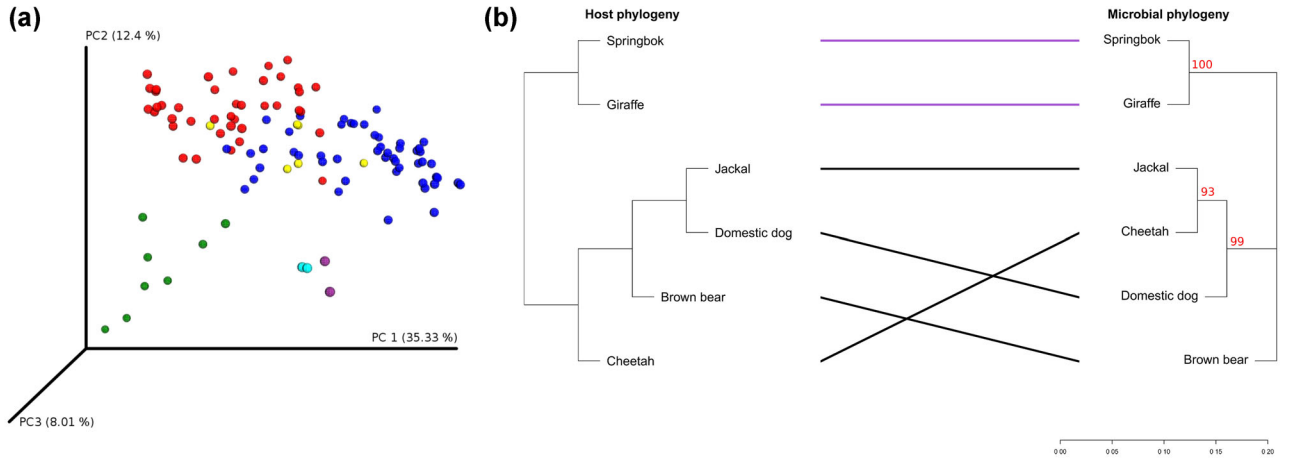


**Figure 7.** Comparison of host and gut microbial phylogeny in mammals. **(a)** PCoA plot based on weighted UniFrac distances between black-backed jackals (blue), cheetahs (yellow), domestic dogs (red), brown bears (green), giraffes (purple) and springboks (light blue). **(b)** Comparison between the host taxonomy tree (based on the NCBI taxonomy) and the respective gut microbiota tree. Coloured lines represent phylosymbiosis and red numbers are the bootstrap values for tree nodes (100 bootstraps).

also modulate hormones (Markle *et al.* 2013; Yurkovetskiy *et al.* 2013).

Changes in bacterial diversity during aging are expected because different life stages are linked to different nutritional requirements that cause shifts in microbial communities (Claesson *et al.* 2012; Lees *et al.* 2014). Age only had an effect on bacterial alpha diversity but not on beta diversity in black-backed jackals, indicating that age plays a minor role in shaping the gut microbiota compared with sex and BMI, at least in black-backed jackals.

Microbial alpha and beta diversities also differed with respect to the BMI of black-backed jackals. Individuals with a medium BMI, i.e. category BMI 2, carried the highest bacterial diversity. Individuals with a low BMI and individuals with a high BMI have lower bacterial diversities. In humans, a high bacterial diversity is associated with health (Clarke *et al.* 2014) and a loss of diversity can have negative consequences for host well-being (Le Chatelier *et al.* 2013; Fu *et al.* 2015). To date, almost nothing is known about optimal gut microbiota diversities for other species because this requires a profound understanding of their healthy gut microbiota variability and the way that deviations affect, for example, their BMIs. Nevertheless, our findings suggest that a deviation from an average BMI is connected with a lower bacterial diversity.

## Drivers of gut microbiota similarity between black-backed jackals

Gut microbiota similarity is partly determined by host genetic relatedness. Pedigree, but to a similar extent co-living on the same farm, explains the positioning of black-backed jackal gut microbiotas in a phylogenetic tree. Gut microbiotas of some individuals from different sampling locations are as similar to each other as closely related individuals from the same sampling location. Thus, host genetic relatedness did not overrule other host traits and environmental factors drive gut microbiota similarities when the genetic relationship is weak. Gut microbiotas of related individuals, particularly those that live socially and in the same environment, are often more similar compared with non-related individuals (Turnbaugh *et al.* 2009). In related individuals that have a similar genetic background but that also live together, the power of genetic versus environmental drivers is difficult to disentangle, since these individuals are exposed to similar environmental impacts, might share similar food sources and exchange bacteria between group members, all leading to similarities of gut microbiotas, in addition to genetic constraints (Theis, Schmidt and Holekamp 2012; Leclaire, Nielsen and Drea 2014).

## Firmicutes/Bacteroidetes ratio and OTU relatedness to potential pathogens

The Firmicutes/Bacteroides ratio has mainly been investigated in studies on humans and laboratory animals and individuals with a high ratio have been demonstrated to be more effective in releasing calories from food (Ley *et al.* 2006). Similar to humans (Koren *et al.* 2012; Dominianni *et al.* 2015), wild mice (Maurice *et al.* 2015) and black howler monkeys (Amato *et al.* 2014), black-backed jackal females had, on average, higher Firmicutes/Bacteroidetes ratios than males. No sex-dependent differences for this ratio, however, have been detected in American alligators, although free-ranging individuals with restricted availability of food during fasting seasons had a higher ratio compared with those raised in farms (Keenan, Engel and Elsey 2013). A higher ratio is assumed to cause a higher fermentation efficiency and increased production of energy-rich short-chain volatile fatty acids (Amato *et al.* 2014) which might be beneficial when food availability is low (Cheng *et al.* 2015) or when energetic demands are high as during pregnancy or during an ongoing infection. In contrast to the above-mentioned studies, a primary role of pregnancy in increasing the proportion of Firmicutes relative to Bacteroidetes could not be confirmed in black-backed jackals. Therefore, whether pregnant females have an increased ratio or not might depend on the combined effects of pregnancy and non-pregnancy-related fitness factors.

Although individuals sampled from livestock or game farms did not differ significantly in alpha and beta diversities, DESeq2 analysis revealed different abundances of OTUs within bacterial genera such as *Lactobacillus* and *Clostridium*. Notably, OTUs that matched the potentially pathogenic bacteria *Ignatzschineria* sp. and *Clostridium tertium* with 100% similarity were more abundant on game farms than on livestock farms. The genus *Ignatzschineria* consists of bacteria with a high pathogenic potential and occurs mainly in combination with maggot

infestation causing myiasis (Barker *et al.* 2014). *Clostridium tertium* is reported as a human pathogen and is involved in diseases such as bacteraemia, meningitis and pneumonia (Ray *et al.* 2003). In animals, this bacterium can cause enteritis in cattle (Silvera *et al.* 2003) and has been isolated from an abscess in a striped dolphin (Šeol *et al.* 2006). Despite the allegation that black-backed jackals represent pathogen vectors, we have found no evidence that black-backed jackals have elevated levels of OTUs related to potential pathogens or that livestock farming has a negative impact on their health. We are aware that the use of partial sequences of the 16S rRNA gene for the detection of potentially pathogenic bacteria has its limitations, although it has been applied in several studies (Leung, Wilkins and Lee 2015; Razzauti *et al.* 2015; Galan *et al.* 2016). Pathogen confirmation requires a better taxonomic resolution (i.e. on the strain level) and host specific tests to clarify their pathogenicity.

Previous studies investigating anthropogenic impacts in a different ecosystem (intact forests vs forest fragments) on the gut microbiota have provided similar results. The gut microbiota of non-human primates (red colobus (*Procolobus rufomitratus*), black-and-white colobus (*Colobus guereza*) and red-tailed guenons (*Cercopithecus ascanius*)) were relatively resistant to habitat perturbation and, thus, other factors such as phylogeny or gastrointestinal physiology have been suggested to be more important in driving shifts in bacterial communities than habitat features (McCord *et al.* 2014). However, gut microbiotas of black howler monkeys were sensitive to human-caused habitat changes (Amato *et al.* 2013). In this study, land-use type hardly affected gut microbiotas of black-backed jackals which is most likely attributable to their ability to cope with environmental changes and their omnivorous feeding behaviour.

### Comparison of gut microbial diversity of black-backed jackals with domestic dogs, and other mammals

To disentangle the effects of environment, host species and dietary preference, we compared the gut microbiotas of black-backed jackals to other wild and domestic carnivores, herbivores and an omnivore. Gut microbiotas of black-backed jackals had a higher diversity than those of domestic dogs and their bacterial genera differed in proportions. Although the samples of black-backed jackals and domestic dogs of this study originated from different regions of the world and experienced a different co-evolution with their gut microbes, some of the observed differences might be attributable to differences in their environment and resulting diet. Black-backed jackals feed on a variety of food sources and also scavenge, whereas most of the domestic dogs of the respective study were fed with commercial dog food. The genus [*Prevotella*], which is associated with hemicellulose and carbohydrates (Wu *et al.* 2011), was higher in black-backed jackals than in domestic dogs because of the omnivorous feeding behaviour of the former. Šlapeta *et al.* (2015) found that parasites affected gut microbiotas in dogs leading to higher proportions of genera of the phylum Firmicutes compared with non-infected dogs (Šlapeta *et al.* 2015). Black-backed jackals of this study had higher proportions of genera belonging to the phylum Firmicutes, potentially resulting from a greater variety of parasitic infections compared with that of domestic dogs (Walton and Joly 2003). On the bacterial class level, Clostridia and Fusibacteriia were found in high proportions in both groups, but Fusobacteriia were much higher in black-backed jackals. Both bacterial classes were identified at high proportions in carrion-feeding vultures because of their contact with these bacterial classes when feeding (Roggenbuck *et al.* 2014a). Similarly, the scavenging behaviour in combination with the omnivorous diet of black-backed jackals might also explain the high proportions of Clostridia and Fusobacteriia in this canid.

In a second analysis, we compared gut microbiotas of additional species to those of black-backed jackals. Within the investigated sympatric carnivores, shared environment seemed to have a strong impact on gut microbiota because black-backed jackals clustered closer to cheetahs than to domestic dogs, although domestic dogs are phylogenetically closer related to black-backed jackals than cheetahs. Although limited, our comparison of the gut microbiota of these species revealed an impact of the environmental bacterial community on interspecific gut microbiota similarity in sympatric carnivores. In case of deeper phylogenetic splits (herbivores vs carnivores), however, host phylogeny still remained the stronger driver for gut microbiome separation.

## CONCLUSION

Our current knowledge of gut microbiotas in wildlife species under natural conditions is extremely limited. The extent to which anthropogenic habitat modifications have an impact on wildlife gut microbiotas seems to depend on a species-specific susceptibility to environmental factors. We present here the first study investigating simultaneously the impact of host traits and environmental factors on gut microbiotas of a wildlife carnivore that occurs in a human-modified habitat. This combined information is lacking in many studies, despite being essential for understanding whether the observed gut microbial variation within a wildlife species lies within its natural range or is driven by external stressors that might cause functional dysbiosis affecting host health.

### Data accessibility

- 16S rRNA gene reads: Deposited at the sequence read archive (SRA) under the accession number SRP044660.

## SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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## AUTHOR CONTRIBUTION

Conceived and designed the experiments: SM, SS. Performed the experiments: SM, MM. Field logistics and sample collection: SM, MM, JM, JKEM. Analysed the genomic data: SM. Statistical analysis: SM. Wrote the manuscript: SM. Critically revised the manuscript: SS, BW. All authors read and approved the final manuscript.

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## CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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