Comparison of the gut microbiota composition between the wild and captive Tibetan wild ass (Equus kiang)

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Abstract

Aims: The gut microbiota has a great effect on the health and nutrition of the host. Manipulation of the intestinal microbiota may improve animal health and growth performance. The objectives of our study were to characterize the faecal microbiota between wild and captive Tibetan wild asses and discuss the differences and their reasons.

Methods and Results: Through high-throughput sequencing of the 16S rRNA V4-V5 region, we studied the gut microbiota composition and structure of Tibetan wild asses in winter, and analysed the differences between wild and captive groups. The results showed that the most common bacterial phylum in Tibetan wild ass faeces samples was Bacteroidetes, while the phylum Firmicutes was dominant in captive Tibetan wild ass faecal samples. The relative abundance of Firmicutes, Tenericutes and Spirochaetes were significantly higher ($P < 0.01$) than in the wild groups.

Conclusions: Captivity reduces intestinal microbial diversity, evenness and operational taxonomic unit number due to the consumption of industrial food, therefore, increasing the risk of disease prevalence and affecting the health of wildlife.

Significance and Impact of the Study: We studied the effect of the captive environment on intestinal micro-organisms. This article provides a theoretical basis for the ex-situ conservation of wild animals in the future.

Introduction

The Qinghai-Tibetan plateau provides one of the most extreme environments for the survival of humans and other mammals (Zhang \textit{et al.} 2016). The Tibetan wild ass (\textit{Equus kiang}) is a unique species on the Qinghai-Tibetan plateau and is widely distributed in Qinghai, Gansu, Xinjiang, Sichuan and Tibet (Wu and Yi 2000; Moehlman 2002). It is a key protected species in China and is listed in the International Union for Conservation of Nature Red List 2012 of threatened species. Intensive research has been performed regarding the conservation of this species (Joseph and Bard-Jorgen 2005; Yifan and Jianping 2006; Yin \textit{et al.} 2007; St-Louis and Côté 2009; Kefena \textit{et al.} 2012; Dong \textit{et al.} 2015; Guo \textit{et al.} 2018). With the development of wildlife protection plans, the change in environment during ex-situ conservation comes with a change in animal health.

The microbial community of the gastrointestinal tract remains balanced in terms of species, quantity and location in healthy organisms. Animal intestines have large, diverse and dynamically changing bacterial communities that play important roles in host immunity, nutrient metabolism and energy acquisition (Yun \textit{et al.} 2017). The composition of the mammalian gut microflora is associated with many environmental factors, among which living conditions are a major part (Guan \textit{et al.} 2016).
Intestinal microbial diversity of Equus kiang

H. Gao et al.

Captive environments affect the composition of gut microbes in wild animals (Xenoulis et al. 2010; Guan et al. 2016, 2017). Changes in the intestinal microbe composition are associated with host health and disease (Quigley 2010; Costa et al. 2012; Morgan et al. 2012; Qin et al. 2012). Diet is a key factor affecting microbial diversity in the host gut (Ley et al. 2008; Yin et al. 2017; Qin et al. 2018). As industrial food consumption increases in humans and wildlife, each dietary change is accompanied by an adjustment of intestinal microbes, resulting in the loss or extinction of certain intestinal microbes (Zhang et al. 2018). Recent studies have shown that diet-induced loss of microbial diversity can be amplified over generations, resulting in reduced intestinal microbial diversity and increased risk of population extinction (Sonnenburg et al. 2016).

Therefore, the objectives of our study were (i) to characterize the faecal microbiota between wild and captive Tibetan wild asses; (ii) to analyse the differences between faecal samples from different environments; (iii) discuss the causes for the differences, and finally, (iv) to explore the relationship between diet, gut flora and host health. The study of intestinal microbial diversity, which can be used to assess host health and related diseases, provide a theoretical basis for the future breeding or release of wild animals.

Materials and methods

Faecal samples from Tibetan wild asses living in the wild were collected from different regions of the headwaters of the Yellow River, Maduo County on Qinghai-Tibet Plateau in January 2018. A total of 140 wild Tibetan wild ass faecal samples were collected. All samples were collected after natural defecation. Animals were not scared, nor driven, and drugs were not used to promote defecation. Captive Tibetan wild ass faecal samples were collected from the Qinghai-Tibet Plateau wild animal park in January 2018. In total 28 captive Tibetan wild ass samples were collected.

None of the animals had received anti-inflammatory drugs or antimicrobials within the last 3 months.

All sample collection processes were performed in accordance with the requirements of the authorizing ethics committee.

Genomic DNA from the samples were extracted by the CTAB method. DNA purity and concentration were monitored on a 1% agarose gel. DNA samples were diluted to 1 ng μl⁻¹ using sterile water. Universal 16S PCR primers (515F, 5’-GTGCCAGCMGCCGCGGTAA-3’ and 907R, 5’-CCGTCAATTCCMTTGTAGTTT-3’) were used to amplify the V4 and V5 regions of the 16S rRNA. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA). The polymerase chain reaction was carried out using the following mixture in a final volume of 30 μl: 10 μl of template DNA, 3 μl of each primer (6 μmol l⁻¹), 15 μl of Phusion Master Mix (2×) and 2 μl of ddH₂O. Next, DNA was amplified using the following conditions: denaturation for 1 min at 98°C, followed by 30 cycles of 10 s at 98°C for denaturation, 30 s at 50°C for annealing and 30 s at 72°C for extension, as well as a final extension step at 72°C for 5 min. The yield

Figure 1 Tibetan wild ass rarefaction curves (a) and rank abundance curve (b). [Colour figure can be viewed at wileyonlinelibrary.com]
of PCR products was estimated using 2% agarose gel electrophoresis. PCR products were then purified with the GeneJET™ Gel Extraction Kit (Thermo Scientific, Waltham, MA).

The library was sequenced on an Ion S5™ XL platform and 400 bp single-end reads were generated. The single-end method was used to construct a small fragment library for single-end sequencing. By cutting and filtering reads, OTUs (operational taxonomic units) were clustered and species annotation and abundance analysis were performed to reveal sample species composition.

Novogene was commissioned to complete all experiments (DNA extraction, PCR amplification, library preparation and sequencing) and data analysis.

All diversity indices in our samples were calculated with QIIME (ver. 1.9.1) and displayed with R software (ver. 2.15.3). In R, NMDS analysis was displayed using the vegan package, principal coordinates analysis (PCoA) was displayed using the WGCNA package, stat package and ggplot2 package. Cluster analysis was preceded by principal component analysis (PCA), which was applied to reduce the dimensionality of the original variables using the factor Mine R package and ggplot2 package. Cross-group and intra-group differences were tested using the MRPP function in the vegan package.

Results

Eighty-one faecal samples from wild and captive Tibetan wild asses were selected for sequencing, of which 60 samples were from wild animals (DY, DC and DZ), classified as the wild group (DYW), and 21 samples were from captive animals (DD1, DD2, DD3), classified as the captive group (DDD). A total of 4,809,901 high-quality reads were obtained from wild group and classified into 3,542 OTUs, while 1,693,293 high-quality reads were obtained from the captive group and classified into 3,155 OTUs. The number of OTUs present in both the wild and captive groups was 2,928, with 614 unique OTUs in the wild group, and 227 unique OTUs in the captive group.

The rarefaction curves and rank abundance curves of the wild and the captive Tibetan wild ass faecal samples (Fig. 1) show the richness and evenness of the species in the samples. As the sample size increased, the number of observed species gradually stabilized and there were no further significant growth or fluctuations. The results show that the curve had reached a plateau and the sequencing data were reasonable. The number of samples in this study was sufficient to study the intestinal microbial diversity of Tibetan wild asses in the field and in captivity.

We detected a total of 27 phyla, 47 classes, 81 orders, 134 families and 241 genera from 81 Tibetan wild ass faecal samples. In the wild group, we detected 26 phyla, 44 classes, 74 orders, 117 families and 199 genera, while in the captive group, 26 phyla, 43 classes, 71 orders, 121 families and 204 genera were detected.

In the wild group, Bacteroidetes (42.59%) was the predominant phylum, and Anaerovorax (2.29%) was the predominant genus. In the captive group, Firmicutes (49.74%) was the predominant phylum, and Streptococcus (4.39%) was the predominant genus. In order to show the relative abundance of bacterial communities more intuitively, we have chosen the top 10 species for each sample or group and generated a percentage stacked histogram of relative abundance at the phylum and genus levels in Fig. 2.

Figure 2 Relative abundance histogram. A histogram of the relative abundance of gut microbiota among groups in wild and captive Tibetan wild asses at the phylum level (a) and genus level (b). [Colour figure can be viewed at wileyonlinelibrary.com]
# Table 1 Alpha-diversity of gut microbiota in faeces samples from wild and captive Tibetan wild asses

| Sample | Observed_species | Shannon | Simpson | Chao1  | ACE  | Goods_coverage |
|--------|------------------|---------|---------|--------|------|----------------|
| DD1-1  | 1705             | 8.560   | 0.993   | 1812.014 | 1822.969 | 0.997 |
| DD1-2  | 1613             | 8.244   | 0.990   | 1704.377 | 1723.025 | 0.997 |
| DD1-3  | 1684             | 8.354   | 0.990   | 1772.946 | 1786.140 | 0.997 |
| DD1-4  | 1376             | 8.472   | 0.994   | 1452.703 | 1455.215 | 0.998 |
| DD1-5  | 1750             | 8.344   | 0.986   | 1880.569 | 1889.458 | 0.996 |
| DD1-6  | 1720             | 8.667   | 0.993   | 1810.725 | 1819.446 | 0.997 |
| DD1-7  | 1750             | 8.688   | 0.994   | 1850.665 | 1869.866 | 0.997 |
| DD1-8  | 1818             | 8.868   | 0.995   | 1920.097 | 1933.872 | 0.997 |
| DD2-1  | 1770             | 8.708   | 0.994   | 1885.545 | 1904.017 | 0.996 |
| DD2-2  | 1005             | 5.957   | 0.930   | 1152.396 | 1171.362 | 0.997 |
| DD2-3  | 1660             | 8.634   | 0.993   | 1773.305 | 1775.951 | 0.997 |
| DD2-4  | 1764             | 7.811   | 0.953   | 1875.000 | 1888.276 | 0.996 |
| DD2-5  | 1715             | 7.687   | 0.946   | 1810.174 | 1820.362 | 0.997 |
| DD2-6  | 1769             | 8.072   | 0.972   | 1900.790 | 1904.063 | 0.996 |
| DD3-1  | 1693             | 8.503   | 0.992   | 1847.962 | 1837.006 | 0.996 |
| DD3-2  | 1612             | 8.198   | 0.989   | 1738.196 | 1738.003 | 0.997 |
| DD3-3  | 1650             | 8.370   | 0.991   | 1785.631 | 1772.260 | 0.997 |
| DD3-4  | 1674             | 8.503   | 0.990   | 1774.000 | 1784.444 | 0.997 |
| DD3-5  | 1664             | 8.609   | 0.993   | 1774.571 | 1789.636 | 0.997 |
| DD3-6  | 1626             | 8.596   | 0.994   | 1744.719 | 1737.675 | 0.997 |
| DD3-7  | 1693             | 8.727   | 0.994   | 1788.050 | 1797.103 | 0.997 |
| DZ1    | 1874             | 8.582   | 0.990   | 1986.267 | 2013.325 | 0.996 |
| DZ4    | 1409             | 8.124   | 0.988   | 1500.838 | 1500.093 | 0.997 |
| DZ6    | 1793             | 8.523   | 0.991   | 1900.505 | 1905.494 | 0.997 |
| DZ8    | 1891             | 8.675   | 0.992   | 2035.361 | 2022.190 | 0.996 |
| DZ11   | 1831             | 8.748   | 0.993   | 1930.100 | 1937.509 | 0.997 |
| DZ12   | 1817             | 8.514   | 0.989   | 1921.659 | 1936.995 | 0.997 |
| DZ13   | 1788             | 8.657   | 0.993   | 1928.041 | 1924.393 | 0.996 |
| DZ14   | 1709             | 8.687   | 0.994   | 1812.000 | 1824.012 | 0.997 |
| DZ16   | 1810             | 8.649   | 0.993   | 1896.900 | 1915.408 | 0.997 |
| DZ17   | 1680             | 8.896   | 0.995   | 1765.562 | 1762.498 | 0.997 |
| DZ18   | 1861             | 8.869   | 0.995   | 2008.877 | 2011.590 | 0.996 |
| DZ25   | 1842             | 8.886   | 0.994   | 1928.671 | 1941.348 | 0.997 |
| DZ27   | 1840             | 8.688   | 0.990   | 1975.591 | 1978.662 | 0.996 |
| DZ28   | 1814             | 8.378   | 0.988   | 1962.125 | 1980.656 | 0.996 |
| DZ29   | 1771             | 8.452   | 0.990   | 1910.183 | 1914.916 | 0.996 |
| DC3    | 1873             | 8.763   | 0.994   | 2035.515 | 2031.729 | 0.996 |
| DC5    | 1875             | 8.763   | 0.994   | 2035.515 | 2031.729 | 0.996 |
| DC8    | 1999             | 8.948   | 0.995   | 3207.036 | 2373.265 | 0.993 |
| DC9    | 1692             | 8.452   | 0.989   | 1811.929 | 1828.708 | 0.996 |
| DC11   | 1778             | 8.700   | 0.993   | 1882.046 | 1899.458 | 0.997 |
| DC15   | 1826             | 8.661   | 0.992   | 1918.130 | 1925.591 | 0.997 |
| DC17   | 1729             | 8.295   | 0.990   | 1898.375 | 1910.953 | 0.996 |
| DC20   | 1918             | 8.855   | 0.994   | 2045.401 | 2053.585 | 0.996 |
| DC23   | 1713             | 8.414   | 0.988   | 1827.895 | 1824.986 | 0.997 |
| DC24   | 1656             | 8.216   | 0.987   | 1791.264 | 1795.118 | 0.996 |
| DC25   | 1831             | 8.935   | 0.995   | 1960.242 | 1944.414 | 0.997 |
| DC28   | 1887             | 8.952   | 0.995   | 2027.000 | 2008.004 | 0.996 |

(Continued)
The alpha diversity indices (including Shannon, Simpson, Chao1, ACE, Goods_coverage) are shown in Table 1 (cut-off = 62 431). The Goods coverage index was above 99%, indicating a high level of diversity was found in the samples. The Shannon, Chao1 and ACE indices in the wild group were higher than in the captive group, indicating that the difference between the two groups was significantly different. The difference between the groups was greater than the difference within the groups, indicating that the study groups were reasonable. The significance of $P = 0.009368 < 0.01$, showed that the wild group and the captive group were significantly different.

The Metastat method was used to test the microbial species abundance data for wild and captive faecal samples. According to the $q$ value at the phylum level and genus there was a significant difference between the species ($P < 0.01$), and a plot of the difference between the species can be seen in the abundance distribution box map (Figs 4 and 5).

**Discussion**

In the analysis of alpha diversity, the Shannon, Chao1 and ACE indexes of the wild group were larger than

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**Table 1** (Continued)

| Sample | Observed_species | Shannon | Simpson | Chao1 | ACE | Goods_coverage |
|--------|------------------|---------|---------|-------|-----|----------------|
| DC29   | 1557             | 8.048   | 0.998   | 1668  | 647 | 1674-144       |
| DC30   | 1788             | 8.417   | 0.990   | 1896  | 005 | 1906-001       |
| DC31   | 1854             | 8.710   | 0.992   | 1963  | 000 | 1974-194       |
| DC34   | 1676             | 8.236   | 0.988   | 1812  | 573 | 1800-127       |
| DC36   | 1585             | 8.368   | 0.990   | 1706  | 731 | 1707-016       |
| DC41   | 1773             | 8.245   | 0.988   | 1921  | 479 | 1922-046       |
| DC45   | 1803             | 8.494   | 0.989   | 1949  | 250 | 1934-658       |
| DY1    | 1856             | 8.715   | 0.993   | 1935  | 377 | 1952-197       |
| DY3    | 1926             | 9.032   | 0.995   | 2054  | 211 | 2053-668       |
| DY4    | 1897             | 8.907   | 0.994   | 2026  | 814 | 2030-692       |
| DY6    | 1853             | 8.789   | 0.994   | 1955  | 511 | 1975-373       |
| DY8    | 1924             | 9.043   | 0.995   | 2036  | 718 | 2040-398       |
| DY11   | 1891             | 9.170   | 0.996   | 2002  | 216 | 1998-883       |
| DY22   | 2052             | 8.998   | 0.994   | 2177  | 845 | 2177-968       |
| DY23   | 1926             | 8.818   | 0.994   | 2084  | 049 | 2082-460       |
| DY32   | 1942             | 8.865   | 0.994   | 2116  | 527 | 2100-596       |
| DY37   | 1945             | 8.984   | 0.995   | 2072  | 467 | 2079-782       |
| DY43   | 1911             | 8.924   | 0.995   | 2040  | 868 | 2042-800       |
| DY44   | 1773             | 8.669   | 0.992   | 1865  | 205 | 1890-189       |
| DY45   | 1842             | 8.775   | 0.994   | 1951  | 120 | 1970-065       |
| DY46   | 1816             | 8.641   | 0.992   | 1979  | 125 | 1982-641       |
| DY59   | 1899             | 9.036   | 0.995   | 2006  | 622 | 2022-777       |
| DY65   | 1783             | 8.839   | 0.994   | 1902  | 691 | 1903-654       |
| DY70   | 1824             | 8.782   | 0.994   | 1947  | 142 | 1956-814       |
| DY72   | 1789             | 8.574   | 0.992   | 1900  | 500 | 1910-883       |
| DY74   | 1717             | 8.665   | 0.993   | 1816  | 969 | 1805-623       |
| DY75   | 1794             | 8.374   | 0.987   | 1927  | 526 | 1932-330       |
those of the captive group, which suggests that the bacterial diversity of gut microbes in the wild Tibetan wild ass population is significantly higher than for those individuals in captivity. Although the intestinal microbial diversity of the wild Tibetan wild ass was higher, fewer microbes were identified, and the exploration of wild animal intestinal flora has a broader prospect.

The Bacteroides and Firmicutes phyla made up more than 80% of the total bacterial content. This is consistent with previous studies of intestinal microbial diversity in mammals (Eckburg et al. 2005; Mariat et al. 2009; Middelbos et al. 2010; Qin et al. 2010; Van den Abbeele et al. 2010; Zhu et al. 2011; Guan et al. 2016) and these organisms facilitate the digestion of cellulose and hemicellulose in food (Wu et al. 2016). However, the numbers of bacteria from these two phyla were significantly different in the different host groups ($P < 0.01$). Bacteroidetes was the dominant phylum in the wild group, while Firmicutes was the dominant phylum in the captive group.

In winter, captive Tibetan wild asses are fed semi-dry oat grass (fiber content 353.1 g kg$^{-1}$), feed (protein 17.5%, fat 2%) and carrots (proportional to 8 : 2 : 1), and...
more fat and protein may reduce microbial diversity and lead to an increase in the number of Firmicutes and Actinobacteria (Zhang et al. 2012; He et al. 2013; Cani 2018). Thus the diversity of the gut microbiota was significantly lower in the captive group than in the wild group, with higher numbers of Actinobacteria and Firmicutes (Middelbos et al. 2010), and lower numbers of Bacteroidetes. The wild Tibetan wild asses feed mostly on Gramineae, Leguminosae and Cyperaceae plants, including pedicularis, Stipa purpurea, Bryllkinia caudate, Poa annua, Carex myosuroides and Potentilla chinensis (Yin et al. 2007; Dong et al. 2015). In the wild, due to food shortage, protein and fat intake decreased, and the Bacteroidetes content increased to help host to increase their nutrition.

A disruption of the symbiosis between the microbiota and host is known as dysbiosis and is described in multiple chronic diseases, such as obesity and malnutrition (Castaner et al. 2018; Zhang et al. 2018; Jeong et al. 2019), neurological disorders (Kurokawa et al. 2018), cancer and other diseases (Katsimichas et al. 2018; Lu et al. 2018; Panebianco et al. 2018; Pulikkan et al. 2018; Zitvogel et al. 2018). We presume that the health of the wild group of Tibetan wild asses was better than the captive group. On the one hand, in the case of captivity, the feeding density is high and there is long-term contact with human beings, with a higher probability of zoonosis among animals in captivity, and generally poorer health than animals in the wild. On the other hand, the intestinal microbial composition and content of the captive group was greatly altered, which can present as qualitative changes, such as increased proportions of harmful bacteria and reduced levels of beneficial bacteria. The captive Tibetan wild assed had more Spirochaetes, Proteobacteria and Campylobacter; groups of bacteria that contain pathogens (Ludwig et al. 2010), Proteobacteria is closely related to IBD and Clostridium difficile infection. Campylobacter is the most frequent cause of foodborne disease. At same time, the captive group samples had a lower content of Bacteroidetes, the basal microbiota, which is one of the richest phyla in a healthy human body and its levels can be a predictor of an animal’s health.

In summary, there were significant differences in gut microbial composition and structure between wild and captive Tibetan wild asses. We believe that food, bacterial content and animal health are connected and changes in the numbers of different bacteria play an important role for the host.

With the intake of large amounts of industrial food, the intestinal microbial diversity of captive Tibetan wild asses decreased, increasing the risk of disease. Other methods of

Figure 4 Box diagram of species differences between wild and captive Tibetan wild asses at the phylum level. [Colour figure can be viewed at wileyonlinelibrary.com]
feeding that better approximate nature should be chosen to protect rare and endangered wildlife in a captive environment. The gut microbiota of the Tibetan wild ass is complex and this study of its composition and function is of great significance to the protection of the Tibetan wild ass. In addition, it is important to conduct more research to understand how environmental differences directly affect the diversity of bacteria in stool samples.

Acknowledgements

We express our heartfelt thanks to the director of the Yellow-River-Sources Park Management Station at Three-River-Sources National Park in Maduo county and all breeders at the Qinghai-Tibet plateau wild animal park in Xining for their active cooperation and their valuable suggestions on the collection of faecal samples. This study was financially supported by National Key R&D Program of China (2017YFC0506405); The Strategic Priority Research Program of the Chinese Academy of Sciences (XDA20020302); Qinghai Key R&D and Transformation Program (2019-SF-150); Construction Fund for Qinghai Key Laboratories (2017-ZJ-Y23).

Statement on the welfare of animals

All procedures performed in studies involving animals were approved by the Ethics and Welfare of Experiment Animals Committee affiliated to Northwest Institute of Plateau Biology.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Cani, P.D. (2018) Human gut microbiome: hopes, threats and promises. Gut 67, 1716–1725.
Castaner, O., Goday, A., Park, Y.M., Lee, S.H., Magkos, F., Shiw, S.T.E. and Schroder, H. (2018) The gut microbiome profile in obesity: a systematic review. Int J Endocrinol 2018, 4095789.
Costa, M.C., Arroyo, L.G., Allen-Vercoe, E., Stampfl, H.R., Kim, P.T., Sturgeon, A. and Weese, J.S. (2012) Comparison of the fecal microbiota of healthy horses and...
horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. **PLoS ONE** 7, e41484.

Dong, S., Wu, X., Liu, S., Su, X., Wu, Y., Shi, J., Li, X., Zhang, X. *et al.* (2015) Estimation of ecological carrying capacity for wild yak, kiang, and Tibetan antelope based on habitat suitability in the Aerjin Mountain Nature Reserve, China. *Acta Ecol Sin* 35, 7598–7607.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., DeSilets, L., Sargent, M., Gill, S.R., Nelson, K.E. *et al.* (2005) Diversity of the human intestinal microbial flora. *Science* 308, 1635–1638.

Guo, X., Shao, Q., Li, Y., Wang, Y., Wang, D., Liu, J., Fan, J. and Yang, F. (2018) Application of UAV remote sensing for a population census of large wild herbivores—taking the Headwater Region of the Yellow River as an Example. *Remote Sens* 10, 1041.

He, X., Marco, M.L. and Slupsky, C.M. (2013) Emerging aspects of food and nutrition on gut microbiota. *J Agric Food Chem* 61, 9559–9574.

Jeong, M.Y., Jang, H.M. and Kim, D.H. (2019) High-fat diet causes psychiatric disorders in mice by increasing Proteobacteria population. *Neurosci Lett* 698, 51–57.

Joseph, L. and Bard-Jorgen, B. (2005) Density of Tibetan antelope, Tibetan wild ass and Tibetan gazelle in relation to human presence across the Chang Tang Nature Reserve of Tibet, China. *Acta Zool Sin* 51, 586–597.

Katsimichas, T., Ohtani, T., Motooka, D., Tsukamoto, Y., Guo, X., Shao, Q., Li, Y., Wang, Y., Wang, D., Liu, J., Fan, J. and Yang, F. (2018) Application of UAV remote sensing for a population census of large wild herbivores—taking the Headwater Region of the Yellow River as an Example. *Remote Sens* 10, 1041.

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegal, M.L., Tucker, T.A. *et al.* (2008) Evolution of mammals and their gut microbes. *Science* 320, 1647–1651.

Lu, L., Wan, Z., Luo, T., Fu, Z. and Jin, Y. (2018) Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. *Sci Total Environ* 631, 449–458.

Ludwig, W., Euzéby, J. and Whitman, W.B. (2010) Taxonomic outlines of the phyla Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadaetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. In *Bergey’s Manual of Systematic Bacteriology*, 21–24. New York, NY: Springer.

Mariat, D., Firmesse, O., Levenez, F., Guiamarés, V., Sokol, H., Dore, J., Corthier, G. and Furet, J.P. (2009) The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol* 9, 123.

Middelbos, I.S., Vester Boler, B.M., Qu, A., White, B.A., Swanson, K.S. and Fahey, G.C. Jr (2010) Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. *PLoS ONE* 5, e9768.

Moebliman, P.D.R. (2002) *Equids: Zebras, Asses, and Horses: Status Survey and Conservation Action Plan*. Gland, Switzerland: IUCN.

Morgan, X.C., Tickle, T.L., Sokol, H., Gevers, D., Devaney, K.L., Ward, D.V., Reyes, J.A., Shah, S.A. *et al.* (2012) Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 13, R79.

Panebianco, C., Andriulli, A. and Pazienza, V. (2018) Pharmacomicrobiomics: exploiting the drug-microbiota interactions in anticaner therapies. *Microbiome* 6, 92.

Pulikkun, J., Maji, A., Dhakan, D.B., Saxena, R., Mohan, B., Anto, M.M., Agarwal, N., Grace, T. *et al.* (2018) Gut microbial dysbiosis in indian children with autism spectrum disorders. *Microb Ecol* 76, 1102–1114.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichenah, C., Nielsen, T., Pons, N. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W. *et al.* (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60.

Qin, C., Gong, L., Zhang, X., Wang, Y., Wang, Y., Wang, B., Li, Y. and Li, W. (2018) Effect of *Saccharomyces boulardii* and *Bacillus subtilis* B10 on gut microbiota modulation in broilers. *Anim Nutr* 4, 358–366.

Quaglieriello, A., Del Chierico, F., Russo, A., Reddel, S., Conte, G., Lopetuso, L.R., Ianiro, G., Dallapiccola, B. *et al.* (2018) Gut microbiota profiling and gut–brain crosstalk in children affected by pediatric acute-onset neuropsychiatric syndrome and pediatric autoimmune neuropsychiatric developmental syndrome. *J Pediatr Psychol* 43, 67–85.
neuropsychiatric disorders associated with streptococcal infections. Front Microbiol 9, 675.
Quigley, E.M. (2010) Prebiotics and probiotics; modifying and mining the microbiota. Pharmacol Res 61, 213–218.
Roche-Lima, A., Carraquilho-Carrion, K., Gómez-Moreno, R., Cruz, J.M., Velazquez-Morales, D., Rogozin, I.B. and Baerga-Ortiz, A. (2018) The presence of genotoxic and/or pro-inflammatory bacterial genes in gut metagenomic databases and their possible link with inflammatory bowel diseases. Front Genet 9, 116.
Sonnenburg, E.D., Smits, S.A., Tikhonov, M., Higginbottom, S.K., Wingreen, N.S. and Sonnenburg, J.L. (2016) Diet-induced extinctions in the gut microbiota compound over generations. Nature 529, 212–215.
St-Louis, A. and Côté, S.D. (2009) Equus kiang (Perissodactyla: Equidae). Mammalian Species 304, 1–11.
Sun, M.F. and Shen, Y.Q. (2018) Dysbiosis of gut microbiota and microbial metabolites in Parkinson’s disease. Ageing Res Rev 45, 53–61.
Van den Abbeele, P., Grootaert, C., Marzorati, M., Possemiers, S., Verstraete, W., Gerard, P., Rabot, S., Bruneau, A. et al. (2010) Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. Appl Environ Microbiol 76, 5237–5246.
Wu, Z.S. and Yi, G.X. (2000) Status of wild ass in China. Chinese Biodiversity 8, 81–87.
Wu, X., Zhang, H., Chen, J., Shang, S., Wei, Q., Yan, J. and Tu, X. (2016) Comparison of the fecal microbiota of dholes high-throughput Illumina sequencing of the V3-V4 region of the 16S rRNA gene. Appl Microbiol Biotechnol 100, 3577–3586.
Xenoulis, P.G., Gray, P.L., Brightsmith, D., Palculict, B., Hoppes, S., Steiner, J.M., Tizard, I. and Suchodolski, J.S. (2010) Molecular characterization of the cloacal microbiota of wild and captive parrots. Vet Microbiol 146, 320–325.
Yifan, C. and Jianping, S. (2006) A new technique for temporary slide mounting in microscopic histological herbivore fecal analysis. Acta Theriologica Sinica 26, 407–410.
Yin, B., Huai, H., Zhang, Y., Le, Z. and Wei, W. (2007) Trophic niches of Pantholops hodgsonii, Procicapra picticaudata and Equus kiang in Kekexili region. J Appl Ecol 18, 766–770.
Yin, J., Han, H., Li, Y., Liu, Z., Zhao, Y., Fang, R., Huang, X., Zheng, J. et al. (2017) Lysine restriction affects feed intake and amino acid metabolism via gut microbiome in pigs. Cell Physiol Biochem 44, 1749–1761.
Yun, D., Qi, W., Yibo, H., Xiao, W., Yonggang, N., Xiaoping, W. and Fuwen, W. (2017) Advance and prospects of gut microbiome in wild mammals. Acta Theriologica Sinica 37, 399–406.
Zhang, C., Zhang, M., Pang, X., Zhao, Y., Wang, L. and Zhao, L. (2012) Structural resilience of the gut microbiota in adult mice under high-fat dietary perturbations. ISME J 6, 1848–1857.
Zhang, Z., Xu, D., Wang, L., Hao, J., Wang, J., Zhou, X., Wang, W., Qiu, Q. et al. (2016) Convergent evolution of rumen microbiomes in high-altitude mammals. Curr Biol 26, 1873–1879.
Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y.Y., Wang, X., Fu, H., Xue, X. et al. (2018) Gut bacteria selectedly promoted by dietary fibers alleviate type 2 diabetes. Science 359, 1151–1156.
Zhu, L., Wu, Q., Dai, J., Zhang, S. and Wei, F. (2011) Evidence of cellulose metabolism by the giant panda gut microbiome. Proc Natl Acad Sci 108, 17714–17719.
Zitvogel, L., Ma, Y., Raoult, D., Kroemer, G. and Gajewski, T.F. (2018) The microbiome in cancer immunotherapy: diagnostic tools and therapeutic strategies. Science 359, 1366–1370.