SCIENCE CHINA
Life Sciences

FROM CAS & CAE MEMBERS
•REVIEW•

February 2019 Vol.62 No.2: 168–178
https://doi.org/10.1007/s11427-018-9423-3

Conservation metagenomics: a new branch of conservation biology

Fuwen Wei[1,2,3*], Qi Wu[1], Yibo Hu[1,3], Guangping Huang[1], Yonggang Nie[1,3] & Li Yan[1]

[1]CAS Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;
[2]University of Chinese Academy of Sciences, Beijing 100049, China;
[3]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China

Received August 30, 2018; accepted October 6, 2018; published online December 25, 2018

Multifaceted approaches are required to monitor wildlife populations and improve conservation efforts. In the last decade, increasing evidence suggests that metagenomic analysis offers valuable perspectives and tools for identifying microbial communities and functions. It has become clear that gut microbiome plays a critical role in health, nutrition, and physiology of wildlife, including numerous endangered animals in the wild and in captivity. In this review, we first introduce the human microbiome and metagenomics, highlighting the importance of microbiome for host fitness. Then, for the first time, we propose the concept of conservation metagenomics, an emerging subdiscipline of conservation biology, which aims to understand the roles of the microbiota in evolution and conservation of endangered animals. We define what conservation metagenomics is along with current approaches, main scientific issues and significant implications in the study of host evolution, physiology, nutrition, ecology and conservation. We also discuss future research directions of conservation metagenomics. Although there is still a long way to go, conservation metagenomics has already shown a significant potential for improving the conservation and management of wildlife.

microbiome, conservation biology, conservation metagenomics, endangered animal

Citation: Wei, F., Wu, Q., Hu, Y., Huang, G., Nie, Y., and Yan, L. (2019). Conservation metagenomics: a new branch of conservation biology. Sci China Life Sci 62, 168–178. https://doi.org/10.1007/s11427-018-9423-3

Introduction

The body surface and gastrointestinal tracts of humans have been colonized by a substantial number of indigenous prokaryotic and eukaryotic microbes, which is one order of magnitude more than human cells (Turnbaugh et al., 2007). The vast majority of the microbial cells reside in the gastrointestinal tract, occupying a large proportion of the total microbiota in the human body. The microbial communities in gut are composed of trillions of microorganisms, mainly including bacteria. These microbial genomes, microbiome, contain at least 100 times as many genes as the human genome involving many physiological processes such as host nutrition fermentation and intake (Gill et al., 2006). It has long been discovered that the survival of these microorganisms may be bi-directionally influenced by host gut homeostasis, metabolism, and physiology. Understanding this interaction between the gut microbiome and the host has become a topic of increasing interest during the recent decade.

Recent advances in sequencing techniques have broadened our insights into the intestinal microbiota in the last two decades. To unravel the mysteries of microbiome, metagenomics was first introduced before the Human Genome Project was accomplished, which denotes the set of genomes from all bacteria in soil (Handelsman, 2004). With the broad application of next-generation sequencing technique, more and more researchers have focused on the composition and function of human gut microbiota (Simpson et al., 2005; Zhernakova et al., 2016; Falony et al., 2016; Chu et al., 2016;

---

*Corresponding author (email: weifw@ioz.ac.cn)

Ferreiro et al., 2018). In addition, techniques of metatranscriptome (Booijink et al., 2010) and metaproteome (Klaassens et al., 2007) have also been introduced to study gene expression to illustrate intensive functions of certain microbiome. Subsequently, the virus and fungi in gut also attracted the interest of microbiologists, giving birth to concepts of metavirome (Reyes et al., 2010) and mycobiome (Ghannoum et al., 2010). In 2007, the National Institute of Health (NIH) launched the Human Microbiome Project (HMP) to investigate microorganisms living in association with human body (Turnbaugh et al., 2007). Characterized microbial communities included several different sites on the human body, among which gastrointestinal tract was one of the most concerned (Turnbaugh et al., 2007). The gut microbiome has been discovered to entangle a series of physiological and genetic process of health and disease, infant development, and food habits of their animal host. Besides the HMP project, other human microbiome projects have also been conducted, such as US National Microbiome Initiative (Bouchie, 2016), EU MetaHIT Project (Ehrlich, 2011), as well as Chinese Gut Microbiome Projects (http://www.metagenomics.cn).

The ongoing development in metagenomics technologies as well as the introduction of non-invasive sampling methods enables deep access to the gut microbiome of wild animals. Some pioneering research focused on the evolutionary relationship between phylogeny and diet of hosts and composition/function of gut microbiome in mammals (Ley et al., 2008; Muegge et al., 2011). Following these global patterns of mammals, some intensive work on individual species has also been reported, including the tammar wallaby (Pope et al., 2010) and the giant panda (Zhu et al., 2011). The potential role of gut microbiome in conservation biology has been gradually revealed, including host health and disease, habitat degradation and nutrition utilization of endangered animals (Stumpf et al., 2016). Great achievements have been made in this field during the past decade (Ding et al., 2017; Table 1).

In this review, we summarize the recent development of "metagenomics" approaches, particularly its application to conservation biology of wild animals. Beyond the technique level of applying metagenomic analyses to effective conservation, we propose a new discipline, conservation metagenomics, a genome-wide assessment of microbiome for

Table 1 Pioneering studies in application of gut microbiome approaches in conservation biology[a]

Taxa	Host	Host latin name	Scientific issues	Methods[1]	Microbiome focus[2]	References
Mammalia	60 species	–	Diet and phylogeny	T	D	Ley et al., 2008
	30 species human	–	Diet and phylogeny	T/S	D/F	Muegge et al., 2011
Marsupialia	Tammar wallaby	*Macropus eugenii*	Adaptive evolution	T/S	D/F	Pope et al., 2010
Carnivora	Giant panda	*Ailuropoda melanoleuca*	Adaptive evolution, coevolution, Survivorship	S/P	D/F	Zhu et al., 2011
			Diet seasonal variation	S/P	D/F	Wu et al., 2017
	Cheetah	*Acinonyx jubatus*	Oligotyping approach for diversity	T	D	Menke et al., 2014
	Black-backed jackal	*Canis mesomelas*				
	Brown bear	*Ursus arctos*	Hibernation	T/S	D/F	Sommer et al., 2016
Artiodactyla	Yaks	*Bos grunniens*	Convergent evolution	T/R	D/F	Zhang et al., 2016
	Tibetan sheep	*Ovis aries*				
	Cattle	*Bos taurus*				
	Ordinary sheep	*Ovis aries*				
Hominid	Human	*Homo sapiens*	Codiversification	T	D	Moeller et al., 2016
Primates	Chimpanzee	*Pan troglodytes*	Convergent evolution Codiversification	T	D/M	Moeller et al., 2013 Moeller et al., 2016
	Gorilla	*Gorilla gorilla*				
	Bonobo	*Pan paniscus*				
	Black howler monkey	*Alouatta pigra*	Habitat degradation	T	D	Amato et al., 2013
Rodentia	Desert woodrats	*Neotoma lepida*	Function to consume plant toxins	T/S	D/F	Kohl et al., 2014
Cetacea	Pacific Humpback Whales	*Megaptera novaeangliae*	Composition and functions	T/S	D/F	Sanders et al., 2015
	Atlantic white-sided dolphin	*Lagenorhynchus acutus*				
	Bottlenose dolphin	*Tursiops truncatus*	Composition	T	D	Soverini et al., 2016

a) 1, T means targeted sequencing (such as 16S rRNA); S means metagenomic shotgun sequencing; M means metabolomics methods. R means metatranscriptomic methods. P means (real time) PCR. 2, D means that the research focuses on the diversity of microbiome. F means that the research focuses on the function of microbiome. M means the metabolites of the microbiome.

wildlife conservation, and highlight the role of microbiota in conservation biology, including its implications in the evolution, ecology, physiology and metabolism of wild animals. Because gut microbiomes have been extensively studied and proved to play important roles in multiple aspects for host biology, evolution and conservation, we take the gut microbiome as examples to introduce the new concept of conservation metagenomics.

What is conservation metagenomics?

Conservation metagenomics is a new branch of conservation biology that applies metagenomics technologies to solve ecological, evolutionary, and conservation problems in wild animals. This new subdiscipline focuses on studying the composition and function of microbiome and its roles in evolution, ecology, health and conservation of the host animal. Within the last decade, this field has undergone a fast development, and assessing composition and function of microbiome of wild animals has become a technically feasible and cost-effective strategy. Therefore, conservation metagenomics can offer comprehensive insights into the relationship between host and its microbiota, and help guide conservation decisions and management policy for wild animals from the perspective of microbiome.

Scientific questions in conservation metagenomics

The composition and function of gut microbiota can be used to assess the fitness of wild populations of endangered species. It has therefore been argued what kinds of scientific questions conservation metagenomics can address. Here we summarize the relevant issues on health, nutrition, evolution, ecology, genetic diversity and conservation implications in wild animals.

Health and nutrition

The microbial communities colonize different parts of the gut, and influence many aspects of host health and nutrition during the lifespan. Because of the physiological connection between the gut, the liver and the brain, the gut microbiota is involved in regulating multiple metabolic pathways. The beneficial roles of microbial communities in human physiological, immunological and metabolic functions have become clear (Flint et al., 2012; Nicholson et al., 2012; Marchesi et al., 2016; Qin et al., 2018). Its role in the biology and fitness of wild animals has also attracted increasing research interests recently. Better understanding of the dynamics of gut microbiome will open up a new window for enhancing the host health and monitoring nutritional status.

Physiology and metabolism

Over the past decade, more attention has been given to the link of gut microbiome to many physiological processes such as host diet (Ley et al., 2008; Muegge et al., 2011; Sommer and Bäckhed, 2013). Gut microbiome strongly affected digestion and nutrition utilization, while host diet heavily influenced bacterial diversity. Thus, the host physiology and metabolism cannot be considered separately from gut microbiome.

Previous studies show that host diet and phylogeny both influence bacterial diversity. The herbivores have the most diversity of microbiota, the carnivores contain the fewest, and the omnivores are in the middle (Ley et al., 2008). To more exactly determine the relationship of host diet and gut microbiome, Amato et al. compared the gut microbiota of different species of Primates with different gut morphology, and found that host phylogeny appears to be a more dominant factor than diet influencing gut microbial composition and function (Amato et al., 2018). These results indicate that the effect of diet on the dynamics of mammalian gut microbiome is constrained by host phylogeny.

Additionally, the gut flora contributes to a range of metabolic pathways, such as carbohydrate, amino acid, energy, nucleotide, and lipid metabolisms. Understanding the effects of gut microbiome on host metabolism is one of the fundamental research objectives of conservation metagenomics. It is well-known that some host animals periodically select for different food resources in different seasons or different locations. A spatio-temporal variation in composition and function of gut microbiome may depend on host physiology or nutrition. A recent research using a metagenomic approach and nutritional analyses illustrated the association between the foraging pattern and variation of gut microbiome in giant pandas (Wu et al., 2017). The results demonstrated that the gut microbiomes exhibit significantly different patterns at different nutritional stages. This is supported by increased diversity of bacterial taxa associated with leaf-eating stage, and enrichment of genes associated with raw fiber utilization and cell cycle control, In the shoot-eating stage, this increased diversity is also associated with prokaryotic secretion and signal transduction activity. These data suggest that gut microbiome plays an important role in modulating host physiology and metabolism.

Hibernation

Hibernating animals undergo seasonal cycles of feeding and fasting to maintain the integrity of organ systems when dietary nutrients are lacking. Yet little is known about the gut microbiota response to periodic dietary switch. Understanding host hibernation mechanisms and gut microbiota response can provide insights into species conservation. Costello et al. investigated bacterial communities from the intestines of fasted and digesting Burmese python, and found

https://engine.scichina.com/doi/10.1007/s11427-018-9423-3

a marked postprandial change in bacterial community composition (Costello et al., 2010). These observations provide a unique opportunity for dissecting the role of gut microbiota in host hibernation. Comparison of gut microbiomes of brown bear in hibernated and active stages indicated a decreased diversity in hibernator's microbiota as well as a functional disruption in lipid metabolism (Sommer et al., 2016). Further investigation based on artificially inducing hibernation in amphibians showed similar diversity reduction and more disruption on host metabolism, disease, and immune responses (Weng et al., 2016; Wiebler et al., 2018). These data suggest that gut microbiota plays a key role in hibernation process, highlighting the importance of maintaining mutualistic gut microbiota configuration in conservation of wild animals.

Immunology and disease

The mammalian immune system plays an essential role in maintaining homeostatic relationships with the microbiota inhabiting the body. The dense bacterial community resides in the intestine and continually stimulates host immunity. The immune system has thus evolved adaptive mechanisms to tolerate the presence of potentially beneficial microbes, which is also a basic trait in mammalian evolution (Hooper et al., 2012). Disruption of their relationship can lead to diseases such as inflammatory bowel disease (Round and Mazmanian, 2009). In human and mouse models, gut microbiota has been proven to be required for the development and mature of immune system of young individuals (Palmer et al., 2007; Wall et al., 2009; Kau et al., 2011). As identified in wild and captive Antarctic seals, core members of gut microbiota are passed via vertical transmission and help to develop the immune system (Nelson et al., 2013). Understanding the potential role of gut microbiota of wild animals will provide exciting opportunities to improve host health.

Evolution

Recent investigations imply that host-associated microbiomes can profoundly influence the evolution of wild animals (Ingala et al., 2018; Sharpton, 2018). As the famous geneticist Theodosius Dobzhansky said, nothing in biology makes sense except in the light of evolution, conservation metagenomics provides a useful tool to enhance our understanding of the evolution of wild populations.

Adaptive evolution

Host special diet has long been regarded as a result of adaptation. Gut microbiome has been illustrated involving the host diet adaptation. For example, the giant panda, a threatened mammalian species exclusively living on bamboo diet, possesses carnivorous digestive system but lacks cellulose-digesting function. However, their gut microbiome has adapted to their bamboo diets and the presence of putative cellulose-digesting microbes indicates that giant pandas have evolved to overcome the anatomical and physiological challenges of digesting a high-fibrous diet (Zhu et al., 2011). Like giant pandas, red pandas also mainly depend on highly fibrous bamboo, and maintain high level of bacteria related to cellulose degradation (Kong et al., 2014; Li et al., 2015).

Pope et al. analyzed the foregut microbiome of the tammar wallaby (*Macropus eugenii*), and confirmed that Australian macropods harbor unique bacterial lineages facilitating plant biomass conversion, suggesting an adaptation to host herbivory (Pope et al., 2010). Koalas have a special diet consisting almost exclusively of eucalyptus leaves. Metagenomic analysis for both wild and captive koalas showed significant intra-individual variation, implicating the adaptation of gut microbiome for assisting with digestion and absorption of nutrients from this kind of food (Barker et al., 2013). A recent study about carnivoran gut metagenomes shows the microbiomes of carnivorous species such as Felidae and Canidae clustered in the same clade, and other clades clustered omnivorous and herbivorous ones (e.g., bears and pandas), revealing the gut microbial adaptation help carnivorous carnivorans to endure diets high in purines and fats (Zhu et al., 2018a). By contrast, the bamboo-eating giant and red pandas both significantly enrich putative cyanide-digesting gut microbes for adapting to their bamboo diet (Zhu et al., 2018b). These findings indicate adaptive change trends in the gut microbiome for special diet and physiological demands of the host.

Convergent evolution

Convergent evolution is a research hotspot in evolutionary biology, most often focusing on morphological and physiological convergences of host only and rarely on the microbiome resident in host body. Convergent evolution of gut microbiome is also an interesting issue in evolutionary biology: What is the functional evolution of the gut microbiome? Do animals with similar feeding patterns have similar microbial niches?

A previous study based on 16S rRNA sequences of different mammalian species revealed that diet drives convergent evolution in gut microbiome functions across the mammalian lineage with different dietary niches (Muegge et al., 2011). Similar observations were reported in myrmecophagous placental mammals (Delsuc et al., 2014). Specialized myrmecophagous (ant- and termite-eating) mammals are a good model for understanding the potential role of gut microbiota in convergent adaptation to myrmecophagy. Moreover, the gut microbiota communities of sympatric chimpanzees and gorillas who share similar dietary niches have converged in terms of community composition (Moeller et al., 2013). These results indicate that diet adaptation is a major driving factor of convergence in gut microbiome composition over evolutionary timescales.

Foregut fermentation that occurs in ruminants and hoatzin represents another example of evolutionary convergence. Despite their considerable divergences in phylogeny, body size, physiology and diet, the similarity of microbial community structure in phylogenetically distant hosts contributes to the convergent function of foregut (Godoy-Vitorino et al., 2012). A recent study reported high-altitude ruminants such as yaks (*Bos grunniens*) and Tibetan sheep (*Ovis aries*) produce higher volatile fatty acid with lower methane emissions than their low-altitude relatives including cattle and ordinary sheep. Further metagenomic analysis revealed that ruminal microbiomes of high-altitude ruminants show significant enrichment in amino acid metabolism, suggesting the microbiomes were convergently evolving with their host for energy harvesting (Zhang et al., 2016). Better understanding of the complex interplay between the host and its associated microbiome will help us to uncover the mechanisms of host adaptation to extreme environment and specific diet.

Coevolution and cospeciation

Close associations between hosts and microorganisms suggest that they may have shared similar evolutionary history. Coevolution and codiversification are therefore introduced accordingly. Of them, similar symbiont and host lineages showing matching phylogenetic trees are more preferred to be explained by codiversification, while the shared evolutionary history are usually considered as coevolution (Moran et al., 2015; Groussin et al., 2017).

Coevolution has been an important mechanism influencing the host-gut microbe relationship (Amato, 2013; Shapira, 2016). To explore the relationship of host and microbe, it is necessary to understand the evolutionary origins of the gut microbiota. However, past evolutionary processes can only be inferred from comparative analyses of extant mammalian gut microbial communities. An analysis of the fecal microbial communities of 106 mammal individuals representing 60 species from 13 taxonomic orders indicated that bacterial communities codiversified with their hosts (Ley et al., 2008). Moeller et al. compared the gyrB phylogenies of major bacterial lineages in humans, wild chimpanzees, bonobos, and gorillas, and revealed that they mostly match the apehominid phylogeny (Moeller et al., 2016). This result shows that gut microbial compositions can reflect the host phylogeny in a manner, indicating host and microbes have codiversified for millions of years.

The coevolution of mammals with their microbiome confers multiple benefits to host physiological functions. For example, huddling Brandt's voles (*Lasiopodomys brandtii*), a small herbivore, had higher bacterial α and β-diversity with higher concentrations of short-chain fatty acids (SCFAs) than those exposed to warm conditions (Zhang et al., 2018). The acquisition of a new diet is a fundamental driver for the coevolution of host-microbes. In giant pandas, the presence of putative cellulose and hemicellulose-digesting microbiota along with morphological, ecological and genetic adaptations coevolved with the highly specialized herbivory, contributing to the fitness of this specialized herbivore (Zhu et al., 2011; Wei et al., 2015).

Ecology

The pattern of interspecies interaction within community is one of the central topics in this field. In addition, numerous environmental factors can influence the community dynamics of gut microbiota. Thus, understanding the gut microbiota responding to ecological variations including seasonal, environmental and geographical factors will step toward wild animal conservation.

Seasonal variation

Seasonal variation in the availability and distribution of food resources is a dominant selective pressure for wild animals, affecting host digestive efficiency and ultimately host nutrition. Previous studies show that the gut microbial composition and function of Tibetan macaque (Sun et al., 2016) and black howler monkey (Amato et al., 2014) varied seasonally, which provided an effective buffer against seasonal changes in energy and nutrient intake.

Similar observation is found in *Chlorocebus* monkeys from southern Ethiopia, whose gastrointestinal microbiotas vary seasonally in response to the alternating dry and wet climate (Trosvik et al., 2018). These observations demonstrate the plastic nature of gut microbiome in response to variation in diet.

Environmental variation

Due to the diversity between water and land environments, aquatic animals have quite different gut microbiomes from their terrestrial relatives. Numerous studies have elucidated the ecological role of gut microbiome of many terrestrial animals. However, those of aquatic mammals still remain poorly studied.

The gut microbiomes of marine mammals such as dolphin and sea lions were shaped by the extreme sea environment, and were distinct from those of terrestrial mammals (Bik et al., 2016). The composition and function of marine mammal microbiomes varied with host habitat, diet and phylogeny. For example, baleen whales harbor unique gut microbiome but with surprising functional similarity with those of terrestrial herbivores and carnivores (Sanders et al., 2015). The carbon and energy metabolism related genes were more similar to those of terrestrial herbivores. In contrast, protein catabolism and essential amino acid synthesis pathways more closely resembled those of terrestrial carnivores (Sanders et al., 2015). These data indicate that the surveillance of microbiome in marine mammals may provide insights into the impact of environmental change on ocean ecosystem.

Geographical variation

Understanding the correlation between gut microbiome and geographical distribution can also help to investigate the local adaptation of endangered populations. Geographically isolated populations harbor distinct gut microbiome. Gomez et al. investigated the gut microbiome of wild lowland gorilla occupying different geographical ranges in the Dzanga-Sangha Protected Areas, Central African Republic, where the gorilla has different dietary sources because of the distinction of their respective geographic vegetation (Gomez et al, 2015). The fecal metagenomic analysis indicated that geographical scale plays a vital modulation role in the gut microbiomes and metabolomes of the gorilla. Further research showed that differences and convergences of diet-microbe co-metabolic fingerprints responding to specific environment changes stimuli were triggered by dietary constraints during their adaptive radiation (Gomez et al., 2016). Overall, these data suggest that gut microbiome composition and function of wild animals could potentially reflect the host-habituated environment.

Genetic diversity

Dissecting the mechanisms responsible for population genetic divergence is a central topic in wildlife ecology and conservation. To explore whether host genome correlates with the dynamic changes of gut microbiome, inbred mouse model has been used to determine to what extent host genetics control the gut microbiome variation. The result shows that only one single host gene deletion or addition in this model can tremendously affect the diversity and structure of the gut microbiota (Spor et al., 2011). Recently, a comparative analysis of the microbiome compositions of 112 species representing 14 mammalian orders revealed a strong host phylogenetic signal in the degree of microbiomes diverge (Nishida and Ochman, 2018). Fietz et al. also confirmed the correlation between population genetic variation of two species of sand lance (*Ammodytes tobianus* and *Hyperoplus lanceolatus*) and their gut microbiome. These data suggest that understanding the gut microbiome variation may infer the extent of host genetic divergence (Fietz et al., 2018).

Conservation

It is becoming clear that the microbiome plays an important role in multiple aspects for host biology and evolution. These effects have important implications for the conservation and management of wild animals.

Habitat fragmentation

Habitat degradation and fragmentation as a threat to the biodiversity, can be assessed using metagenomics approaches. Since gut microbiome are sensitive to environmental alteration and physiological perturbation, gut microbiome dynamics can mirror the habitat quality. Black howler monkey as an endangered species, has recently attracted many interests on its conservation biology (Amato et al., 2013). These populations living in fragmented habitats have considerably reduced diversity and abundance of gut microbiomes compared to those in pristine continuous rainforest (Amato et al., 2013).

Corridors are designed to facilitate the movement of wild animals in geographically fragmented habitats, and to promote gene exchange among subpopulations (Rosenberg et al., 1997). With the increase in the usage of corridors for wildlife conservation, it has therefore been argued whether corridors make conservation more effective. To evaluate corridor effectiveness, traditional molecular approaches based on host microsatellite markers are used. Recently evidence shows that the composition and function of gut microbiome provide a useful tool to rapidly and accurately assess the outcome of habitat loss and fragmentation (Srivathsan et al., 2016).

Human or livestock induced pathogenic transmission

Pathogenic transmission between wild animals and humans or livestock poses a threat for species conservation. It has been reported that *Escherichia coli* in humans living near forest fragments share more similarity to bacteria of primates in the same region than those of nearby primates (Goldberg et al., 2008), indicating that human behavior can induce interspecific transmission of pathogenic microbes.

Pathogen transmission between different host species is of importance from public health and species conservation perspectives. An integrated analysis of pathogen characteristics, host range and risk factors aim to determine disease emergence among humans, domestic and wild animals. Multi-host pathogens were highly prevalent in these three host groups. Better understanding the dynamics of infectious diseases in complex multi-host communities will be able to remove disease threats to human health, livestock economies, and wildlife survivorship (Cleaveland et al., 2001).

Survivorship in captivity and in the wild

Conservation metagenomics also has another application for survivorship management of wild and captive individuals by detecting and comparing their gut microbiomes. Some researchers found almost no difference in gut microbiome between wild and captive individuals, such as in koalas (Alfano et al., 2015); however, apparent composition differences in gut microbiome between wild and captive animals were observed in other species such as red panda, giant panda, as well as the Tasmanian devil. In wild giant pandas, majority of microbiota belonged to phylum Firmicutes, while in captive giant pandas gut microbiota mainly belonged to phylum Proteobacteria (Zhu et al., 2011). Nevertheless, in red pandas the situation is contrary: more Proteobacteria bacteria were found in wild individuals and more Firmicutes bacteria were found in captives (Kong et al., 2014). In Tasmanian devil, the compositional diversity of gut bacteria in captives was significantly lower than that in the wild, and the functional contribution of microbiome to metabolism of glycan, amino acids and cofactors and vitamins was also different between captive and wild individuals (Cheng et al., 2015). These investigations suggest the difference in the microbiome of captive and wild animals may have impacts on the ability to survive.

Conservation metagenomic approach

The composition and function of gut microbiome mainly reflect the host physiology and metabolism status (Nicholson et al., 2012). Therefore, an ideal approach of conservation metagenomics should include total nucleic acids of all microbiota in the sample followed by the targeted or shotgun sequencing (Figure 1). In addition, advanced bioinformatics analyses are needed to discover the comprehensive microbial information in samples. There are two kinds of sequencing strategies: targeted (such as 16S rRNA gene) sequencing, which can acquire information about the composition of microbiome, and shotgun sequencing, which can obtain functional information of microbiome. Distinct strategies need different experimental approaches (Table 2) along with different bioinformatics pipelines (Figure 1). For targeted sequencing strategy, computational tools such as QIIME (Caporaso et al., 2010) were developed to allow researchers to assign sequences to operational taxonomic units and infer phylogenetic relationships. For shotgun sequencing strategy, either softwares such as MEGAN (Huson et al., 2007) or online tools such as MG-RAST (Meyer et al., 2008) could be used. The sequencing reads can either be used directly as paired short fragments, or be assembled into larger contigs/scaffolds for gene annotation.

Prospects

By using metagenomic approaches, research on gut microbiome has shifted from composition assessment of microbial communities to functional annotation and validation. Over the past decades, metagenomics has attracted lots of attention worldwide, but conservation metagenomics is currently quite new and immature in the field of conservation biology.

As a new branch of conservation biology, conservation metagenomics offers rapid, convenient and cost-effective tools to guide conservation and management of wild animals from the perspective of gut microbiome. Further development may benefit from technical improvement on both empirical and computational approaches. For instance, more powerful genome sequencing technique could be expected to be essentially useful, involving higher accuracy and precision, longer read length, larger amounts of data products and shorter run time. Moreover, some kinds of portable and easy-operating genome sequencing equipment are also of great importance for rapid identification of samples used in the field. For the computational approach, analytical tools and computational performance also need to be improved for dealing with processing large volumes of data. Cheaper sequencing cost and improved bioinformatics tools for microbiota analysis will allow more researchers to use metagenomics along with metatranscriptomics and metaproteomics approaches for conservation-related studies.

Recent investigations have shown a profound relationship between the gut microbiota and host behavior or personality (Diaz Heijtz et al., 2011; Ezenwa et al., 2012; Foster and McVey Neufeld, 2013; Schmidt, 2015). Personality traits including several complex host behavior patterns constitute important elements of this bidirectional communication (Ezenwa et al., 2012). Despite few studies have shown the relationship between gut microbiota and host behaviors including mating preference and anxiety disorder in model species, we still lack the knowledge what the implications of the microbe-behavior associations are for host ecology and evolution. In addition, further research is needed to determine the underlying mechanisms for the connection between the gut and nervous system.

Gut microbiota has played a key role in the gastrointestinal homeostasis and physiological aging processes of the host. Recent studies have shown that germ-free animal models have developed age-associated physiological perturbation such as degraded immune system, chronic inflammation and deranged bowel motility. The interaction between biological aging and gut microbiota has been studied recently (O'Toole and Jeffery, 2015). For instance, diminished bowel enzymatic activity and impaired barrier function in the elderly has contributed to the dysbiosis of gut microbiome. However, the role of gut microbiota in the biological aging of endangered species is rarely known. Better understanding the relationship between gut microbiome and aging physiology may promote the development of novel therapy.

The microbiota in gastrointestinal tract can also influence metabolic processes, such as energy production and conversion, carbohydrate transport, and metabolism. Although the important role of gut microbiota in regulating pathological outcomes, such as obesity and diabetes, have been well

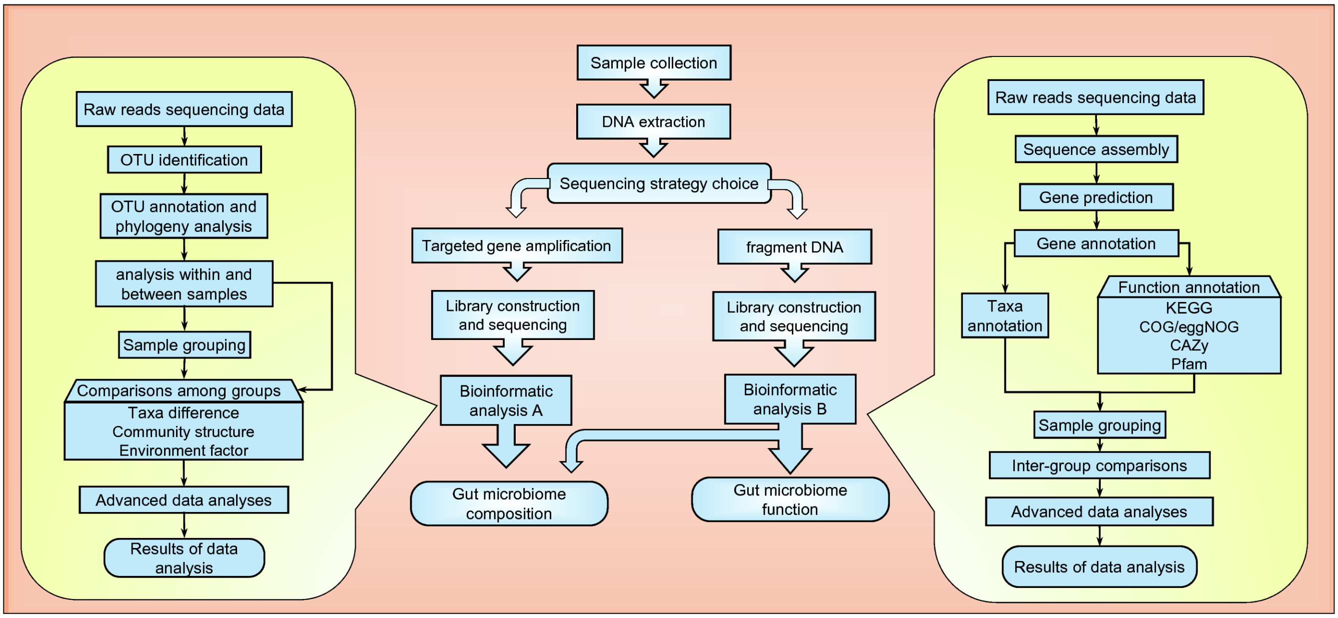

Figure 1 (Color online) The metagenomics approaches used for gut microbiome. Middle panel shows a total pipeline of metagenomic analysis, comprising the following steps in turn: sample collection, sample DNA extraction, sequencing strategy selection based on the research aim. Targeted or shotgun genome sequencing strategies are chosen to acquire information for composition or function of the microbiome sample. Generally speaking, 16S rRNA targeting sequencing is applied for the studies on composition of microbiome while shotgun genome sequencing is performed for functional analysis of microbiome. Detailed steps of bioinformatics analyses used for targeted and shotgun genome sequencing are shown in left and right panels, respectively.

Table 2 Comparison of different genome sequencing techniques applied in metagenomics[a)]

	ABI 3730	Illumina HiSeq 2000	Illumina HiSeq 2500 (Rapid Run)	Illumina HiSeq 3000/4000	Illumina MiSeq	PacBio
Maximum read length	800 bp	2×100 bp	2×150 bp	2×150 bp	2×300 bp	10–18 kb
Reads per run	96	3×10^9	6×10^8	2.5×10^9–5×10^9	2.5×10^7	–
Output range	70–80 kb	500–600 Gb	150–180 Gb	750–1,500 Gb	15 Gb	5–10 Gb/SMRT cell
Run time	2 h	11 d	40 h	1–3.5 d	4–55 h	0.5–6 h/SMRT cell
Raw error rate (%)	<0.001	<1	<1	<1	<1	12
Generational division	The first generation	The second generation	The second generation	The second generation	The second generation	The third generation

a) These parameters were cited from introductions of the commercial sequencer manufacturers

described both in human and mice models (Tremaroli and Bäckhed, 2012), it is unclear about the correlation between microbes and metabolism in wild animals. Therefore, understanding the gut microbiota in spatial and temporal scales will provide insights into the host metabolic states. Recently, transplantation of healthy microbiota into the sick recipients has been successful for the treatment of metabolic syndrome (de Groot et al., 2017). As a promising therapy method, more explorative works of the metabolic contributions of gut microbiota are needed.

Due to climate change and habitat fragmentation, endangered species have experienced rapid population declines and extirpations (Butchart et al., 2010). Microbiome analyses open a non-invasive insight into host physiology, immunology, metabolism, and pathology with important implications for conservation and management of wild animals, especially endangered species. Thus, incorporating microbial ecology into conservation strategies is important for protecting these sensitive species. Further understanding of the dynamics of gut microbiome of endangered individuals pre- and post-reintroduction will provide important clues for the assessment of conservation efforts. The patterns of microbial diversity and early signs of impending microbial disruption may facilitate therapeutic strategies design for diseases prevention and treatment.

In fact, metagenomics approaches may not be enough for the identification and characterization of rare bacterial species, even by high-coverage metagenomics sequencing. It is also not feasible if when we pursue the biotechnological application of a certain species such as fecal microbiota transplantation only using metagenomic sequencing methods. Recently, the emerging of culturomics and functional metagenomics could complement sequence-based metagenomics by overcoming the depth bias and functional analysis of a certain bacterial species (Lagier et al., 2012). In the future, once the most effective culture conditions are defined, culturomics will probably be more widely used in wildlife conservation. The information obtained from functional metagenomics will advance the development of new probiotics or therapeutic molecules towards wildlife conservation.

In summary, with the help of conservation metagenomics we can further improve conservation and management of wild, captive, and reintroduced individuals. We anticipate that conservation metagenomics will become more and more widely applied in conservation biology in the years to come.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB31000000), the National Key Program of Research and Development, Ministry of Science and Technology of China (2016YFC0503200), and the Creative Research Group Project of National Natural Science Foundation of China (31821001).*

References

Alfano, N., Courtiol, A., Vielgrader, H., Timms, P., Roca, A.L., and Greenwood, A.D. (2015). Variation in koala microbiomes within and between individuals: effect of body region and captivity status. Sci Rep 5, 10189.

Amato, K.R., Yeoman, C.J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Rex Gaskins, H., Stumpf, R.M., Yildirim, S., Torralba, M., et al. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. ISME J 7, 1344–1353.

Amato, K.R. (2013). Co-evolution in context: The importance of studying gut microbiomes in wild animals. Microbiome Sci Med 1, 10–29.

Amato, K.R., Leigh, S.R., Kent, A., Mackie, R.I., Yeoman, C.J., Stumpf, R. M., Wilson, B.A., Nelson, K.E., White, B.A., and Garber, P.A. (2014). The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (*Alouatta pigra*). Microb Ecol 69, 434–443.

Amato, K.R., G. Sanders, J., Song, S.J., Nute, M., Metcalf, J.L., Thompson, L.R., Morton, J.T., Amir, A., J. McKenzie, V., Humphrey, G., et al. (2018). Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. ISME J 23, doi: 10.1038/s41396-018-0175-0.

Barker, C.J., Gillett, A., Polkinghorne, A., and Timms, P. (2013). Investigation of the koala (*Phascolarctos cinereus*) hindgut microbiome via 16S pyrosequencing. Vet Microbiol 167, 554–564.

Bik, E.M., Costello, E.K., Switzer, A.D., Callahan, B.J., Holmes, S.P., Wells, R.S., Carlin, K.P., Jensen, E.D., Venn-Watson, S., and Relman, D.A. (2016). Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. Nat Commun 7, 10516.

Booijink, C.C.G.M., Boekhorst, J., Zoetendal, E.G., Smidt, H., Kleerebezem, M., and de Vos, W.M. (2010). Metatranscriptome analysis of the human fecal microbiota reveals subject-specific expression profiles, with genes encoding proteins involved in carbohydrate metabolism being dominantly expressed. Appl Environ Microbiol 76, 5533–5540.

Bouchie, A. (2016). White house unveils national microbiome initiative. Nat Biotechnol 34, 580.

Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J. P.W., Almond, R.E.A., Baillie, J.E.M., Bomhard, B., Brown, C., Bruno, J., et al. (2010). Global biodiversity: indicators of recent declines. Science 328, 1164–1168.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7, 335–336.

Cheng, Y., Fox, S., Pemberton, D., Hogg, C., Papenfuss, A.T., and Belov, K. (2015). The Tasmanian devil microbiome—implications for conservation and management. Microbiome 3, 76.

Chu, H., Khosravi, A., Kusumawardhani, I.P., Kwon, A.H.K., Vasconcelos, A.C., Cunha, L.D., Mayer, A.E., Shen, Y., Wu, W.L., Kambal, A., et al. (2016). Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. Science 352, 1116–1120.

Cleaveland, S., Laurenson, M.K., and Taylor, L.H. (2001). Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos Trans R Soc B-Biol Sci 356, 991–999.

Costello, E.K., Gordon, J.I., Secor, S.M., and Knight, R. (2010). Postprandial remodeling of the gut microbiota in *Burmese pythons*. ISME J 4, 1375–1385.

de Groot, P.F., Frissen, M.N., de Clercq, N.C., and Nieuwdorp, M. (2017). Fecal microbiota transplantation in metabolic syndrome: History, present and future. Gut Microbes 8, 253–267.

Delsuc, F., Metcalf, J.L., Wegener Parfrey, L., Song, S.J., González, A., and Knight, R. (2014). Convergence of gut microbiomes in myrmecophagous mammals. Mol Ecol 23, 1301–1317.

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., and Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 108, 3047–3052.

Ding, Y., Wu, Q., Hu, Y.B., Wang, X., Nie, Y.G., Wu, X.P., and Wei, F.W. (2017). Advances and prospects of gut microbiome in wild mammals. Acta Theriologica Sinica 37, 399–406.

Ehrlich, S.D. (2011). MetaHIT: The European Union Project on metagenomics of the human intestinal tract. In Metagenomics of the Human Body, K.E. Nelson, ed. (New York: Springer), pp. 307–316.

Ezenwa, V.O., Gerardo, N.M., Inouye, D.W., Medina, M., and Xavier, J.B. (2012). Animal behavior and the microbiome. Science 338, 198–199.

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., Kurilshikov, A., Bonder, M.J., Valles-Colomer, M., Vandeputte, D., et al. (2016). Population-level analysis of gut microbiome variation. Science 352, 560–564.

Ferreiro, A., Crook, N., Gasparrini, A.J., and Dantas, G. (2018). Multiscale evolutionary dynamics of host-associated microbiomes. Cell 172, 1216–1227.

Fietz, K., Rye Hintze, C.O., Skovrind, M., Kjærgaard Nielsen, T., Limborg, M.T., Krag, M.A., Palsbøll, P.J., Hestbjerg Hansen, L., Rask Møller, P., and Gilbert, M.T.P. (2018). Mind the gut: genomic insights to population divergence and gut microbial composition of two marine keystone species. Microbiome 6, 82.

Flint, H.J., Scott, K.P., Louis, P., and Duncan, S.H. (2012). The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9, 577–589.

Foster, J.A., and McVey Neufeld, K.A. (2013). Gut–brain axis: how the microbiome influences anxiety and depression. Trends Neurosciences 36, 305–312.

Ghannoum, M.A., Jurevic, R.J., Mukherjee, P.K., Cui, F., Sikaroodi, M., Naqvi, A., and Gillevet, P.M. (2010). Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6, e1000713.

Gill, S.R., Pop, M., Deboy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B. S., Gordon, J.I., Relman, D.A., Fraser-Liggett, C.M., and Nelson, K.E. (2006). Metagenomic analysis of the human distal gut microbiome. Science 312, 1355–1359.

Godoy-Vitorino, F., Goldfarb, K.C., Karaoz, U., Leal, S., Garcia-Amado, M.A., Hugenholtz, P., Tringe, S.G., Brodie, E.L., and Dominguez-Bello, M.G. (2012). Comparative analyses of foregut and hindgut bacterial communities in hoatzins and cows. ISME J 6, 531–541.

Goldberg, T.L., Gillespie, T.R., Rwego, I.B., Estoff, E.L., and Chapman, C. A. (2008). Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. Emerg Infect Dis 14, 1375–1382.

Gomez, A., Petrzelkova, K., Yeoman, C.J., Vlckova, K., Mrázek, J., Koppova, I., Carbonero, F., Ulanov, A., Modry, D., Todd, A., et al. (2015). Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (*Gorilla gorilla gorilla*) reflect host ecology. Mol Ecol 24, 2551–2565.

Gomez, A., Rothman, J.M., Petrzelkova, K., Yeoman, C.J., Vlckova, K., Umaña, J.D., Carr, M., Modry, D., Todd, A., Torralba, M., et al. (2016). Temporal variation selects for diet–microbe co-metabolic traits in the gut of Gorilla spp. ISME J 10, 514–526.

Groussin, M., Mazel, F., Sanders, J.G., Smillie, C.S., Lavergne, S., Thuiller, W., and Alm, E.J. (2017). Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. Nat Commun 8, 14319.

Handelsman, J. (2004). Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68, 669–685.

Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012). Interactions between the microbiota and the immune system. Science 336, 1268–1273.

Huson, D.H., Auch, A.F., Qi, J., and Schuster, S.C. (2007). MEGAN analysis of metagenomic data. Genome Res 17, 377–386.

Ingala, M.R., Simmons, N.B., Wultsch, C., Krampis, K., Speer, K.A., and Perkins, S.L. (2018). Comparing microbiome sampling methods in a wild mammal: fecal and intestinal samples record different signals of host ecology, evolution. Front Microbiol 9, 803.

Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L., and Gordon, J.I. (2011). Human nutrition, the gut microbiome and the immune system. Nature 474, 327–336.

Klaassens, E.S., de Vos, W.M., and Vaughan, E.E. (2007). Metaproteomics approach to study the functionality of the microbiota in the human infant gastrointestinal tract. Appl Environ Microbiol 73, 1388–1392.

Kohl, K.D., Weiss, R.B., Cox, J., Dale, C., and Dearing, M.D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. Ecol Lett 17, 1238–1246.

Kong, F., Zhao, J., Han, S., Zeng, B., Yang, J., Si, X., Yang, B., Yang, M., Xu, H., and Li, Y. (2014). Characterization of the gut microbiota in the red panda (*Ailurus fulgens*). PLoS ONE 9, e87885.

Lagier, J.C., Armougom, F., Million, M., Hugon, P., Pagnier, I., Robert, C., Bittar, F., Fournous, G., Gimenez, G., Maraninchi, M., et al. (2012). Microbial culturomics: paradigm shift in the human gut microbiome study. Clinical Microbiol Infection 18, 1185–1193.

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

Li, Y., Guo, W., Han, S., Kong, F., Wang, C., Li, D., Zhang, H., Yang, M., Xu, H., Zeng, B., et al. (2015). The evolution of the gut microbiota in the giant and the red pandas. Sci Rep 5, 10185.

Marchesi, J.R., Adams, D.H., Fava, F., Hermes, G.D.A., Hirschfield, G.M., Hold, G., Quraishi, M.N., Kinross, J., Smidt, H., Tuohy, K.M., et al. (2016). The gut microbiota and host health: a new clinical frontier. Gut 65, 330–339.

Menke, S., Wasimuddin, S., Meier, M., Melzheimer, J., Mfune, J.K.E., Heinrich, S., Thalwitzer, S., Wachter, B., and Sommer, S. (2014). Oligotyping reveals differences between gut microbiomes of free-ranging sympatric Namibian carnivores (*Acinonyx jubatus*, *Canis mesomelas*) on a bacterial species-like level. Front Microbiol 5, 526.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., et al. (2008). The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinf 9, 386.

Moeller, A.H., Peeters, M., Ndjango, J.B., Li, Y., Hahn, B.H., and Ochman, H. (2013). Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. Genome Res 23, 1715–1720.

Moeller, A.H., Caro-Quintero, A., Mjungu, D., Georgiev, A.V., Lonsdorf, E.V., Muller, M.N., Pusey, A.E., Peeters, M., Hahn, B.H., and Ochman, H. (2016). Cospeciation of gut microbiota with hominids. Science 353, 380–382.

Moran, N.A., and Sloan, D.B. (2015). The hologenome concept: helpful or hollow? PLoS Biol 13, e1002311.

Muegge, B.D., Kuczynski, J., Knights, D., Clemente, J.C., González, A., Fontana, L., Henrissat, B., Knight, R., and Gordon, J.I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332, 970–974.

Nelson, T.M., Rogers, T.L., Carlini, A.R., and Brown, M.V. (2013). Diet and phylogeny shape the gut microbiota of Antarctic seals: a comparison of wild and captive animals. Environ Microbiol 15, 1132–1145.

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. (2012). Host-gut microbiota metabolic interactions. Science 336, 1262–1267.

Nishida, A.H., and Ochman, H. (2018). Rates of gut microbiome divergence in mammals. Mol Ecol 27, 1884–1897.

O'Toole, P.W., and Jeffery, I.B. (2015). Gut microbiota and aging. Science 350, 1214–1215.

Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A., and Brown, P.O. (2007). Development of the human infant intestinal microbiota. PLoS Biol 5, e177–1573.

Pope, P.B., Denman, S.E., Jones, M., Tringe, S.G., Barry, K., Malfatti, S.A., McHardy, A.C., Cheng, J.F., Hugenholtz, P., McSweeney, C.S., et al. (2010). Adaptation to herbivory by the tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. Proc Natl Acad Sci USA 107, 14793–14798.

Qin, N., Dong, X., and Zhao, L. (2018). Microbiome: from community metabolism to host diseases. Sci China Life Sci 61, 741–743.

Reyes, A., Haynes, M., Hanson, N., Angly, F.E., Heath, A.C., Rohwer, F., and Gordon, J.I. (2010). Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466, 334–338.

Rosenberg, D.K., Noon, B.R., and Meslow, E.C. (1997). Biological corridors: Form, function, and efficacy. BioScience 47, 677–687.

Round, J.L., and Mazmanian, S.K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9, 313–323.

Sanders, J.G., Beichman, A.C., Roman, J., Scott, J.J., Emerson, D., McCarthy, J.J., and Girguis, P.R. (2015). Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores. Nat Commun 6, 8285.

Schmidt, C. (2015). thinking from the gut. Nature 518, S12–S14.

Sharpton, T.J. (2018). Role of the gut microbiome in vertebrate evolution. mSystems 3, e00174-17–17.

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. Trends Ecol Evol 31, 539–549.

Simpson, S., Ash, C., Pennisi, E., and Travis, J. (2005). The gut: inside out. Science 307, 1895.

Sommer, F., and Bäckhed, F. (2013). The gut microbiota — masters of host development and physiology. Nat Rev Microbiol 11, 227–238.

Sommer, F., Ståhlman, M., Ilkayeva, O., Arnemo, J.M., Kindberg, J., Josefsson, J., Newgard, C.B., Fröbert, O., and Bäckhed, F. (2016). The gut microbiota modulates energy metabolism in the hibernating brown bear *Ursus arctos*. Cell Rep 14, 1655–1661.

Soverini, M., Quercia, S., Biancani, B., Furlati, S., Turroni, S., Biagi, E., Consolandi, C., Peano, C., Severgnini, M., Rampelli, S., et al. (2016). The bottlenose dolphin (*Tursiops truncatus*) faecal microbiota. FEMS Microbiol Ecol 92, fiw055.

Spor, A., Koren, O., and Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol 9, 279–290.

Srivathsan, A., Ang, A., Vogler, A.P., and Meier, R. (2016). Fecal metagenomics for the simultaneous assessment of diet, parasites, and population genetics of an understudied primate. Front Zool 13, 17.

Stumpf, R.M., Gomez, A., Amato, K.R., Yeoman, C.J., Polk, J.D., Wilson, B.A., Nelson, K.E., White, B.A., and Leigh, S.R. (2016). Microbiomes, metagenomics, and primate conservation: New strategies, tools, and applications. Biol Conserv 199, 56–66.

Sun, B., Wang, X., Bernstein, S., Huffman, M.A., Xia, D.P., Gu, Z., Chen, R., Sheeran, L.K., Wagner, R.S., and Li, J. (2016). Marked variation between winter and spring gut microbiota in free-ranging Tibetan Macaques (*Macaca thibetana*). Sci Rep 6, 26035.

Tremaroli, V., and Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. Nature 489, 242–249.

Trosvik, P., Rueness, E.K., de Muinck, E.J., Moges, A., and Mekonnen, A. (2018). Ecological plasticity in the gastrointestinal microbiomes of Ethiopian Chlorocebus monkeys. Sci Rep 8, 20.

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., and Gordon, J.I. (2007). The human microbiome project. Nature 449, 804–810.

Wall, R., Ross, R.P., Ryan, C.A., Hussey, S., Murphy, B., Fitzgerald, G.F., and Stanton, C. (2009). Role of gut microbiota in early infant development. Clin Med Pediatr 2009, 45–54.

Wei, F., Swaisgood, R., Hu, Y., Nie, Y., Yan, L., Zhang, Z., Qi, D., and Zhu, L. (2015). Progress in the ecology and conservation of giant pandas. Conserv Biol 29, 1497–1507.

Wei, F., Wang, X., and Wu, Q. (2015). The giant panda gut microbiome. Trends Microbiol 23, 450–452.

Weng, F.C.H., Yang, Y.J., and Wang, D. (2016). Functional analysis for gut microbes of the brown tree frog (*Polypedates megacephalus*) in artificial hibernation. BMC Genomics 17, 1024.

Wiebler, J.M., Kohl, K.D., Lee Jr, R.E., and Costanzo, J.P. (2018). Urea hydrolysis by gut bacteria in a hibernating frog: evidence for urea-nitrogen recycling in Amphibia. Proc R Soc B 285, 20180241.

Wu, Q., Wang, X., Ding, Y., Hu, Y., Nie, Y., Wei, W., Ma, S., Yan, L., Zhu, L., and Wei, F. (2017). Seasonal variation in nutrient utilization shapes gut microbiome structure and function in wild giant pandas. Proc R Soc B 284, 20170955.

Zhang, X.Y., Sukhchuluun, G., Bo, T.B., Chi, Q.S., Yang, J.J., Chen, B., Zhang, L., and Wang, D.H. (2018). Huddling remodels gut microbiota to reduce energy requirements in a small mammal species during cold exposure. Microbiome 6, 103.

Zhang, Z., Xu, D., Wang, L., Hao, J., Wang, J., Zhou, X., Wang, W., Qiu, Q., Huang, X., Zhou, J., et al. (2016). Convergent evolution of rumen microbiomes in high-altitude mammals. Curr Biol 26, 1873–1879.

Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T., Mujagic, Z., Vila, A.V., Falony, G., Vieira-Silva, S., et al. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 352, 565–569.

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 USA 108, 17714–17719.

Zhu, L., Wu, Q., Deng, C., Zhang, M., Zhang, C., Chen, H., Lu, G., and Wei, F. (2018a). Adaptive evolution to a high purine and fat diet of carnivorans revealed by gut microbiomes and host genomes. Environ Microbiol 20, 1711–1722.

Zhu, L.F., Yang, Z.S., Yao, R., Xu, L.L., Chen, H., Gu, X.D., Wu, T.G., and Yang, X.Y. (2018b). Potential mechanism of detoxification of cyanide compounds by gut microbiomes of bamboo-eating pandas. MSphere 3, e00229-18.