The gut microbiota in the common kestrel (*Falco tinnunculus*): a report from the Beijing Raptor Rescue Center

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As a complex microecological system, the gut microbiota plays crucial roles in many aspects, including immunology, physiology and development. The specific function and mechanism of the gut microbiota in birds are distinct due to their extremely special body structure, physiological attributes and life history. Data on the gut microbiota of the common kestrel, a second-class protected animal species in China, are currently scarce. With high-throughput sequencing technology, we characterized the bacterial community of the gut from 9 fecal samples from a wounded common kestrel by sequencing the V3-V4 region of the 16S ribosomal RNA gene in this study. Our results showed that *Proteobacteria* (41.078%), *Firmicutes* (40.923%) and *Actinobacteria* (11.191%) were the most predominant phyla. *Lactobacillus* (20.563%) was the most dominant genus, followed by *Escherichia-Shigella* (17.588%) and *Acinetobacter* (5.956%). Our results could also offer fundamental data and novel strategies for the protection of wild animals.

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Abstract

As a complex microecological system, the gut microbiota plays crucial roles in many aspects, including immunology, physiology and development. The specific function and mechanism of the gut microbiota in birds are distinct due to their extremely special body structure, physiological attributes and life history. Data on the gut microbiota of the common kestrel, a second-class protected animal species in China, are currently scarce. With high-throughput sequencing technology, we characterized the bacterial community of the gut from 9 fecal samples from a wounded common kestrel by sequencing the V3-V4 region of the 16S ribosomal RNA gene in this study. Our results showed that *Proteobacteria* (41.078%), *Firmicutes* (40.923%) and *Actinobacteria* (11.191%) were the most predominant phyla. *Lactobacillus* (20.563%) was the most dominant genus, followed by *Escherichia-Shigella* (17.588%) and *Acinetobacter* (5.956%). Our results could also offer fundamental data and novel strategies for the protection of wild animals.

Keywords: Common kestrel (*Falco tinnunculus*), Gut microbiota, 16S rRNA gene, High-throughput sequencing, Noninvasive

Introduction

With the rapid progress of sequencing techniques, more and more researches on gut microbiota have revealed its important roles in immunology, physiology and development (Guarner & Malagelada 2003; Nicholson et al. 2005), as well as several basic and critical process, such as nutrient absorption, vitamins synthesis and diseases in both human and animals (Fukuda & Ohno 2014; Kau et al. 2011; Omahony et al. 2015) . The analysis of gut microbiota for wild animal

gradually becomes a new method that could potentially inform animal conservation and husbandry. Reports concerning the gut microbiota of other avian species, such as Cooper's hawk (*Accipiter cooperii*) (Taylor et al. 2019), bar-headed geese (*Anser indicus*) (Wang et al. 2017), hooded crane (*Grus monacha*) (Zhao et al. 2017), Western Gull (*Larus occidentalis*) (Cockerham et al. 2019), herring gulls (*Larus argentatus*) (Fuirst et al. 2018) and black-legged kittiwakes (*Rissa tridactyla*) (van Dongen et al. 2013), have increased rapidly. Although these species have been studied in regards to their microbiome, there isn't a positive trend for each of these species. The specific function and mechanism of the gut microbiota in birds are distinct due to their body structure, physiological attributes and life history. For instance, the stable body temperature that higher than ambient temperature could ensure a high metabolic rate for birds, which meet the requirements of flight. The streamlined body, method of high-efficiency breathing and the relative short gastrointestinal tracts are their other special attributes. Meanwhile, the ability to fly brings additional unique differences for birds compared to other animals, as well as the changes of their intestinal microbiota to some extent. However, as a research focus, data on the gut microbiota of the common kestrel are currently very scarce.

The common kestrel (*Falco tinnunculus*) is a small raptor that belongs to *Falconidae*, which is a family of diurnal birds of prey, including falcons and kestrels. A total of 12 subspecies for common kestrel are distributed widely from the Palearctic to Oriental regions (Cramp & Brooks 1992). Although listed in the least concern (LC) class by the International Union for Conservation of Nature (IUCN) (BirdLife International. 2016), the common kestrel was listed as state second-class protected animals (Defined by the LAW OF THE PEOPLE'S REPUBLIC OF CHINA ON THE PROTECTION OF WILDLIFE, Chapter II, Article 9) in China. The common kestrel is a typical opportunistic forager that catches small and medium-sized animals, including small mammals, birds, reptiles and some invertebrates (Anthony 1993; Aparicio 2000; Village 2010). Insects such as grasshoppers and dragonflies were also identified in the diet of the common kestrel (Geng et al. 2009). As generalist predators, common kestrels choose distinct

predatory strategies when non-breeding and breeding to minimize the expenditure of energy, such as the strategy of the low-cost low-profit technique of perch-hunting in winter, while maximized daily energy gain in summer (Costantini et al. 2005; Masman et al. 1988). Previous studies on common kestrels were comprehensive, such as those on diet and prey selection (Geng et al. 2009; Kirkwood 1980; Korpimäki 1985; Lihu et al. 2007; Souttou et al. 2007; Van Zyl 1994), behavior and diseases (Aschwanden et al. 2005; Bustamante 1994; Hille et al. 2007), and genetic variation and diversity (Nesje et al. 2000; Padilla et al. 2009; Riegert et al. 2010; Zhang et al. 2008). As the common raptors around the whole world, as well as the top predators in the food chains, the common kestrels should be studied more deeply with the newer methods and techniques.

The aim of this study was to characterize the bacterial community of the gut by sequencing the V3-V4 region of the 16S rRNA gene of a wounded common kestrel. The data we obtained could provide basic information for further protection and rescue of wild common kestrels.

Materials & Methods

Sample collection

All fecal samples were collected from an injured common kestrel in the Beijing Raptor Rescue Center (BRRC). The injured common kestrel that could not fly was found first in the Fengtai district by a rescuer on June 22nd, 2019 and then taken to the BRRC for professional rescue. The wounded common kestrel was carefully treated with several surgeries and drug therapies. Nine fecal samples (E1-E9) that may reflect the actual state of its health were collected from the common kestrel after relevant treatments on different days. The samples collection information and medical records of the common kestrel were shown in *Table S1-S3* respectively. All samples were transported immediately into the laboratory in an ice box and ultimately stored at -80°C for further bacterial studies.

105 DNA extraction and PCR amplification

106 Microbial DNA was extracted from fresh fecal samples using an E.Z.N.A.® Stool DNA Kit
107 (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The V3-V4
108 region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95 °C for 3 min;
109 followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension
110 at 72 °C for 5 min) using the primers 338F (5'-barcode-ACTCCTACGGGAGGCAGCAG-3')
111 and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), where the barcode is an eight-base
112 sequence unique to each sample. PCRs were performed in triplicate in a 20 μL mixture
113 containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM),
114 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.
115
116 Illumina MiSeq sequencing
117 Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel
118 Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's
119 instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). Purified amplicons were
120 pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform
121 according to standard protocols.
122
123 Processing of sequencing data
124 Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17) (Caporaso et
125 al. 2010) with the following criteria. (i) The 300 bp reads were truncated at any site receiving an
126 average quality score <20 over a 50 bp sliding window, discarding the truncated reads that were
127 shorter than 50 bp. (ii) Exact barcode matching, 2 nucleotide mismatches in primer matching,
128 and reads containing ambiguous characters were removed. (iii) Only sequences that overlapped
129 longer than 10 bp were assembled according to their overlap sequence. Reads that could not be
130 assembled were discarded.

Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU115)16S rRNA database using a confidence threshold of 70% (Amato et al. 2013).

Data analysis

All the indices of alpha diversity, including Chao, ACE, Shannon, Simpson, and coverage, and the analysis of beta diversity were calculated with QIIME. The rarefaction curves, rank abundance curves, and stacked histogram of relative abundance were displayed with R (version 2.15.3) (R Core Team. 2013).

The hierarchical clustering trees were built using UPGMA (unweighted pair-group method with arithmetic mean) based on weighted and unweighted distance matrices at different levels. Principal coordinate analysis (PCoA) was calculated and displayed using QIIME and R, as well as hierarchical clustering trees.

This study was performed in accordance with the recommendations of the Animal Ethics Review Committee of Beijing Normal University (approval reference number: CLE-EAW-2019-026).

Results

Overall sequencing data

A total of 28 phyla, 70 classes, 183 orders, 329 families and 681 genera were detected among the gastrointestinal bacterial communities. There were altogether 389,474 reads obtained and classified into 1673 OTUs at the 0.97 sequence identity cut-off in 9 fecal samples from a common kestrel.

Alpha diversity indices (including Sobs, Shannon, Simpson, ACE, Chao and coverage) of each sample are shown in *Table 1*. The Sobs and Shannon index of all samples were shown in *Fig. 1*.

Additionally, the rarefaction curves (A) and the rank abundance curves (B) are shown in *Fig. S1*, which indicated that the number of OTUs for further analysis was reasonable, as well as the abundance of species in common kestrel feces. The total sequences, total bases and OTU distributions of all samples are shown in *Table S4* and Table *S5*.

Bacterial composition and relative abundance

At the phylum level of the gut microbiota in the common kestrel, the most predominant phylum was *Proteobacteria* (41.078%), followed by *Firmicutes* (40.923%), *Actinobacteria* (11.191%) and *Bacteroidetes* (3.821%). In addition to *Tenericutes* (0.178%) and *Verrucomicrobia* (0.162%), *Patescibacteria* (0.543%) and *Deinococcus-Thermus* (0.504%) were also ranked in the top 10 species in the common kestrel fecal microbiota (*Table* 2).

The top 5 families in the gut microbiota were *Lactobacillaceae* (20.563%), *Enterobacteriaceae* (18.346%), *Moraxellaceae* (6.733%), *Bifidobacteriaceae* (5.624%) and *Burkholderiaceae* (4.752%).

At the genus level, *Lactobacillus* (20.563%), *Escherichia-Shigella* (17.588%) and *Acinetobacter* (5.956%) were the most dominant genera. These were followed by *Bifidobacterium* (5.624%) and *Enterococcus* (4.024%) (*Table* 3). These five genera in the total gut microbiota of several samples accounted for a small proportion, such as for E5 (28.755%) and E6 (10.905%) and especially for E4 (2.861%), while the largest proportion was 98.416% in E1.

The stacked histogram of relative abundance for species is also demonstrated in *Fig. 2* at the phylum (A) and genus (B) levels, which could intuitively represent the basic bacterial composition and relative abundance. The community structures of E1 and E9 were more similar than those of the other feces samples at both levels.

The hierarchical clustering trees showed the similarity of community structure among different samples, which were generated by UPGMA (unweighted pair-group method with arithmetic mean) with the unweighted UniFrac (*Fig. 3A*) and weighted UniFrac (*Fig. 3B*) distance matrixes.

Although the fecal samples were collected from the common kestrel in chronological order (E1-E9) of therapy treatments, no distinct or obvious clustering relationships are discernable in *Fig. 3*.

Discrepancy of community composition

To further demonstrate the differences in community composition among the nine samples, principal coordinates analysis (PCoA) was applied and depicted in *Fig. 4*. For PCoA, we chose the same two distance matrices (unweighted UniFrac in *Fig. 4A* and weighted UniFrac in *Fig. 4B*) as above to analyze the discrepancies. The results in *Fig. 4* were similar to those in *Fig. 3*, in which all samples scattered dispersedly, suggesting that the variation in the composition of the gut microbiota of the common kestrel was not obvious in this case over time.

Discussion

Knowledge and comprehension concerning the gut microbiota have continued to progressively develop with relevant techniques over the past decade (Guarner 2014; Li et al. 2014; Qin et al. 2010). The application of analysis for intestinal microecology was also a research focus in the field of wild animal protection.

The common kestrel (*Falco tinnunculus*) is listed as a second-class protected animal species in China. Although research concerning avian species, including the common kestrel, has been increasing gradually, the available data on the gut microbiota in the common kestrel are currently unknown.

We characterized the basic composition and structure of the gut microbiota from a wounded common kestrel in this study, which was rescued by the Beijing Raptor Rescue Center (BRRC). In general, the overall community structure of the gut microbiota in this common kestrel was in accordance with previous relevant characterizations in avian species, such as Cooper's hawks (Taylor et al. 2019), bar-headed geese (Wang et al. 2017), hooded cranes (Zhao et al. 2017) and

swan geese (Wang et al. 2016), which included *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*.

The most predominant phylum in the fecal gut microbiota of the common kestrel was *Proteobacteria* (41.078%), which ranked after *Firmicutes* in other birds, such as cockatiels (*Nymphicus hollandicus*) (Alcaraz et al. 2016) and black-legged kittiwakes (van Dongen et al. 2013). This crucial phylum plays many valuable roles. For instance, *Proteobacteria* is beneficial for the giant panda, which can degrade lignin in its major food resource (Fang et al. 2012). Additionally, it has been reported that *Proteobacteria* is also the most dominant phylum in obese dogs (Park et al. 2015). The specific function of this phylum could be distinct in birds due to their unique physiological traits, as well as their developmental strategies (Kohl 2012). However, the high relative abundance of *Proteobacteria* in the total bacterial community was observed mainly in several samples that were collected during surgeries or drug treatments, such as E1 and E4. Sample E1 was collected on 23th June that the day after kestrel rescued from the wild. On 22th June, the kestrel was bandaged with silver sulfadiazine cream (SSD), also given subcutaneously 10 ml and orally 4ml lactated ringer's solution (LRS) respectively. The increased level of *Proteobacteria* was associated with some cardiovascular events, inflammation and inflammatory bowel disease (Amar et al. 2013; Carvalho et al. 2012). Although the kestrel's weight increased 34 grams when E4 was collected, it just ate a mouse's head. Combining the status when kestrel was rescued, we speculated that the increased proportion of *Proteobacteria* may reflect its food consumption or gastrointestinal status to some extent. Environmental influential factors, as well as dietary changes, should also be considered an important index that could result in variations in the relative abundance of species in the gut microbiota (De Filippo et al. 2010; Scott et al. 2013).

Furthermore, the dominant genera within *Proteobacteria* in our study were *Escherichia-Shigella* (17.588%), *Acinetobacter* (5.956%), *Paracoccus* (2.904%) and *Burkholderia-Caballeronia-Paraburkholderia* (2.408%). *Escherichia-Shigella* is a common pathogenic bacterium that can

cause diarrhea in humans (Hermes et al. 2009). The main cause for the high relative abundance of *Escherichia-Shigella* was the E1 (88.610%) sample, which suggested indirectly that the physical condition of the common kestrel was not normal when it was rescued by staff from the BRRC. This result was also consistent with the actual state of this wounded common kestrel that we observed (*Table S3*).

Although *Firmicutes* (40.923%) ranked after *Proteobacteria*, its actual relative abundance was slightly lower than that in the common kestrel. As a common phylum of the gut microbiota, *Firmicutes* exists widely in both mammals and birds, and this ancient symbiosis may be linked to the common ancestor of amniotes (Costello et al. 2010; Kohl 2012). *Firmicutes* can provide certain energy for the host through catabolizing complex carbohydrates and sugar and even digesting fiber by some species (Costa et al. 2012; Flint et al. 2008; Guan et al. 2017).

The dominant genera in *Firmicutes* were *Lactobacillus* (20.563%), *Enterococcus* (4.024%) and *Clostridium_sensu_stricto_1* (3.586%). The relative abundance of *Enterococcus* in E5 (15.026%) contributed to the highest ranking of this genus. *Enterococcus* is not regarded as a special pathogenic bacterium due to its harmlessness and can even be used as a normal food additive in related industries (Fisher & Phillips 2009; Moreno et al. 2006). *Enterococcus* species are also considered common nosocomial pathogens that can cause a high death rate (Lopes et al. 2005). Meanwhile, these species are also associated with the kinds of infections, including neonatal infections, intraabdominal and pelvic infections, as well as the nosocomial infections and superinfections (Murray 1990). Coincidentally, before the sample E5 was collected, to dealing with the wound on its right tarsometatarsus, the kestrel was treated under anesthesia. The kestrel's right digit tendon was exposed and has no function. Although ensuring the sterile conditions, we inferred that the kestrel was infected by certain bacteria during the surgery. The BRRC might be a specific location similar to hospitals for raptors to some extent, which could explain the high proportion of *Enterococcus* in the fecal samples of this common kestrel. However, this genus should be given sufficient attention in subsequent studies with additional

261 samples from different individuals. The abundance of *Clostridium* increases as more protein is
262 digested (Lubbs et al. 2009). Some species, like *Clostridium difficile*, that belong to *Clostridium*
263 was reported that might have related to certain diseases, such as diarrhea and severe life-
264 threatening pseudomembranous colitis (Kuijper et al. 2006; Pepin et al. 2004). The high relative
265 abundance of this genus also resulted primarily from certain samples (E8, 28.177%), similar to
266 the *Enterococcus* mentioned above. And, more remarkable, the collection of sample E8 was in
267 the same situation as E5. On 13th July, the kestrel also underwent the surgery under anesthesia.
268 While as E5 collected, the kestrel's status was still normal according to relevant records. These
269 results indicated that the high relative abundance of certain pathogens may not
270 show any symptoms of illness for kestrel. In general, the abnormal situation of E5 and E8 still
271 need to be paid enough attention. Moreover, to minimize the influences due to the individual
272 differences, more samples from different individuals should be collected for further study.
273 The third dominant phylum in the gut microbiota in our study was *Actinobacteria* (11.191%),
274 which was also detected in other species, such as turkeys (*Meleagris gallopavo*) (Wilkinson et al.
275 2017) and Leach's storm petrel (*Oceanodroma leucorhoa*) (Pearce et al. 2017). The abundance
276 of *Actinobacteria* varied in different species, such as house cats (7.30%) and dogs (1.8%) (Handl
277 et al. 2011), but only accounted for 0.53% in wolves (Wu et al. 2017). Within this phylum,
278 *Bifidobacterium* (5.624%) and *Glutamicibacter* (1.840%) were the primary genera. The presence
279 of *Bifidobacterium* is closely related to the utilization of glycans produced by the host, as well as
280 oligosaccharides in human milk (Sela et al. 2008; Turroni et al. 2010). Noticeably,
281 *Bifidobacterium thermophilum* was reported to be used through oral administration for chickens
282 to resist *E. coli* infection (Kobayashi et al. 2002). The detection and application of
283 *Bifidobacterium*, especially for the rescue of many rare avian species, would be worth
284 considering for curing various diseases in the future.
285 Additionally, the relative abundance of *Bacteroidetes* was 3.821% in this study, which consisted
286 mainly of *Sphingobacterium*. *Bacteroidetes* is another important component of the gut

287 microbiota that can degrade relevant carbohydrates from secretions of the gut, as well as high
288 molecular weight substances (Thoetkiattikul et al. 2013). The proportion of *Bacteroidetes*, which
289 was stable in most samples we collected except E5 (18.166%), would increase correspondingly
290 with weight loss for mice or changes in fiber content in rural children's daily diet (De Filippo et
291 al. 2010; Ley et al. 2006; Turnbaugh et al. 2008). However, the weight of kestrel was increasing
292 during the collection of E5 and E8. Additionally, although underwent surgery on 4th July, the
293 reason of the high proportion of *Bacteroidetes* in sample E5 still unknown. To characterize the
294 basic composition and structure of the gut microbiota for the common kestrel more accurately,
295 additional fresh fecal samples from healthy individuals should be collected in follow-up studies.
296 Furthermore, additional attention should be paid to the high ranking of *Patescibacteria* (0.543%)
297 and *Deinococcus-Thermus* (0.504%) at the phylum level. *Patescibacteria* might be related to
298 basic biosynthesis of amino acids, nucleotides and so on (Lemos et al. 2019). Members of
299 *Deinococcus-Thermus* are known mainly for their capability to resist extreme radiation,
300 including ultraviolet radiation, as well as oxidizing agents (Cox & Battista 2005; Griffiths &
301 Gupta 2007). The specific function of certain species in these phyla for the common kestrel
302 should be studied by controlled experiments, detailed observations or more advanced
303 approaches, as molecular biological techniques are developed.
304 In addition to the quantity of samples, living environment, age, sex and individual differentiation
305 should also be considered as influencing factors, which would cause a degree of discrepancies at
306 all levels in the gut microbiota. In addition, A comparison of wounded and healthy samples for
307 the bacterial composition in the intestinal microbiota is another essential research direction that
308 may provide additional information for wild animal rescue, such as important biomarkers that
309 indirectly indicate potential diseases.
310

311 **Conclusion**

In summary, using high-throughput sequencing technology in this study, we first characterized the elementary bacterial composition and structure of the gut microbiota for a wounded common kestrel in the BRRC, which could provide valuable basic data for future studies. Further research on *Enterococcus*, *Patescibacteria* and *Deinococcus-Thermus* should be conducted in the future with additional samples. The integration of other auxiliary techniques or disciplines, such as metagenomics and transcriptomics, could offer a deeper understanding of the function and mechanism of the gut microbiota, as well as the protection of wild animals.

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Figure 1

Sobs index and the Shannon index of samples

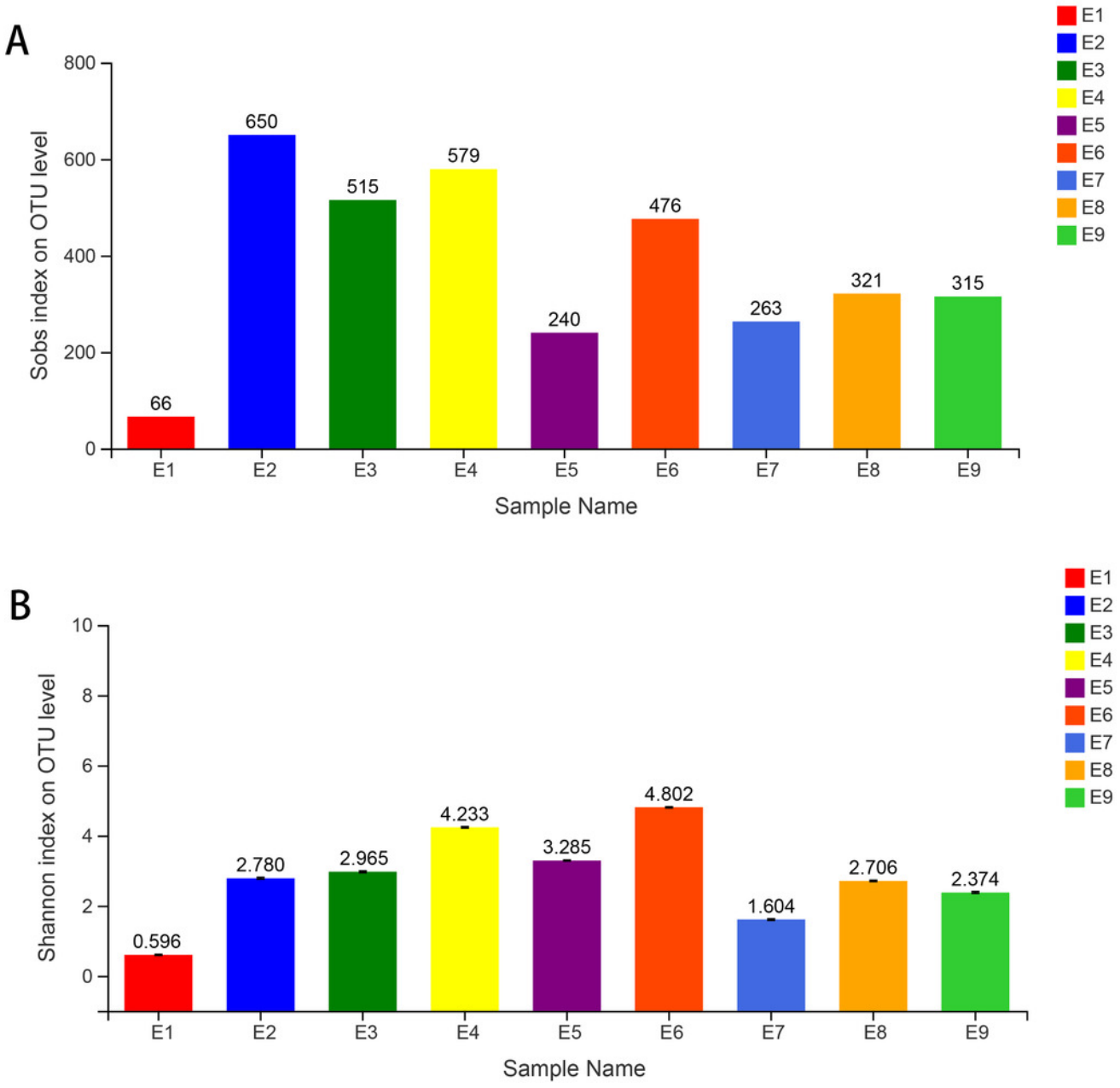

Figure 2

The histogram of relative abundance for species in Common Kestrel at phylum (A) and genus (B) level.

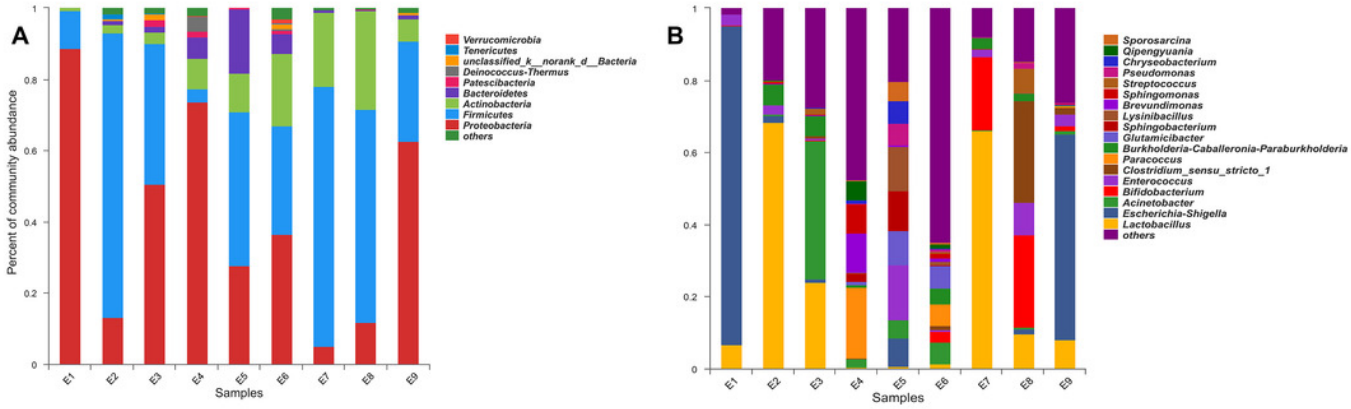

Figure 3

The hierarchical clustering trees

(A) and (B) were generated based on unweighted and weighted distance matrix at phylum level respectively.

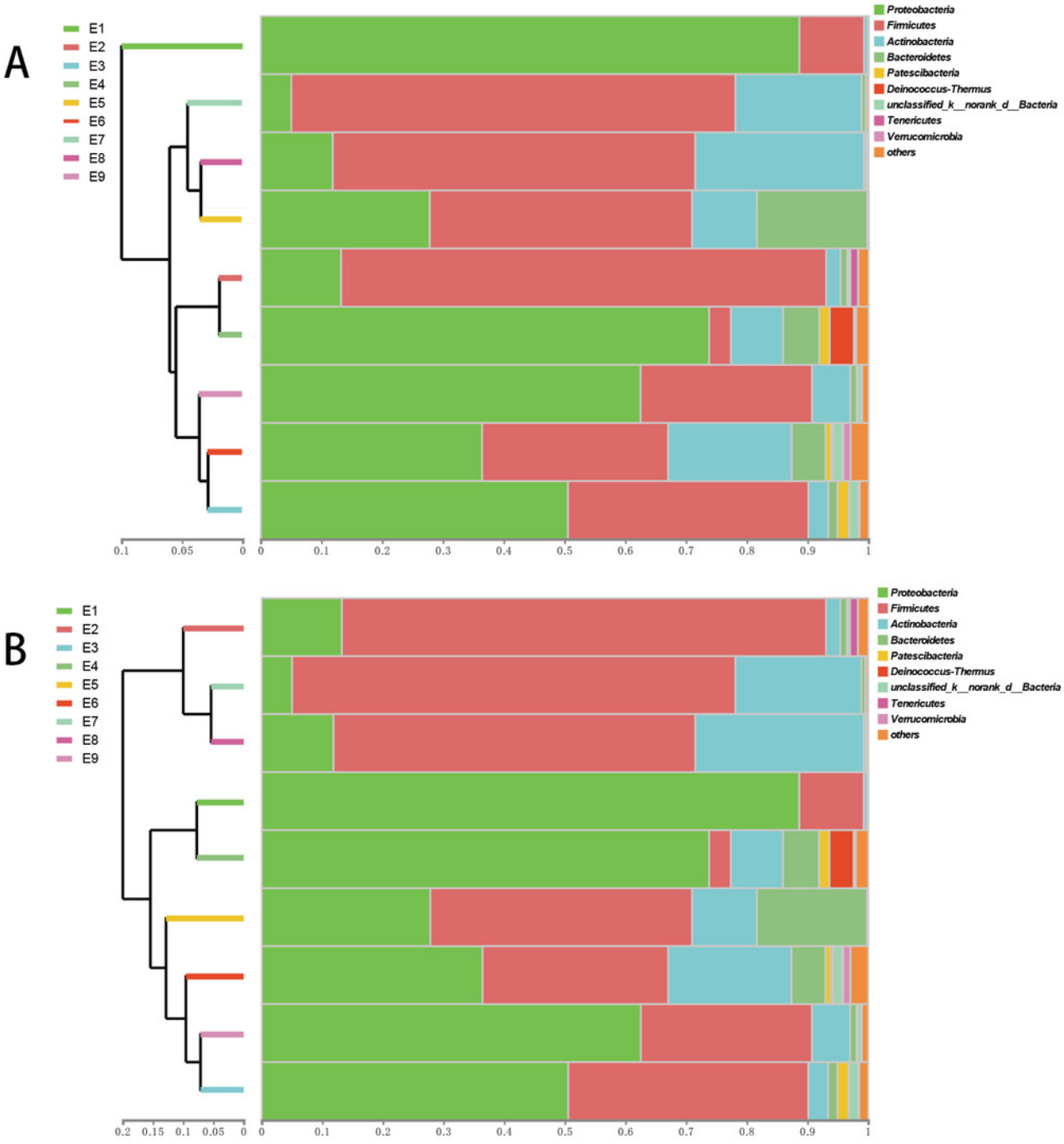
A
E1
E2
E3
E4
E5
E6
E7
E8
E9
0.1 0.05 0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Proteobacteria
Firmicutes
Actinobacteria
Bacteroidetes
Patescibacteria
Deinococcus-Thermus
unclassified_k__norank_d__Bacteria
Tenericutes
Verrucomicrobia
others
B
E1
E2
E3
E4
E5
E6
E7
E8
E9
0.2 0.15 0.1 0.05 0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Proteobacteria
Firmicutes
Actinobacteria
Bacteroidetes
Patescibacteria
Deinococcus-Thermus
unclassified_k__norank_d__Bacteria
Tenericutes
Verrucomicrobia
others

Figure 4

PCoA of the bacterial population structures.

The different shape with colors represented all samples of Common Kestrel respectively. For PCoA, (A) was generated with unweighted Unifrac distance while (B) used weighted Unifrac distance.

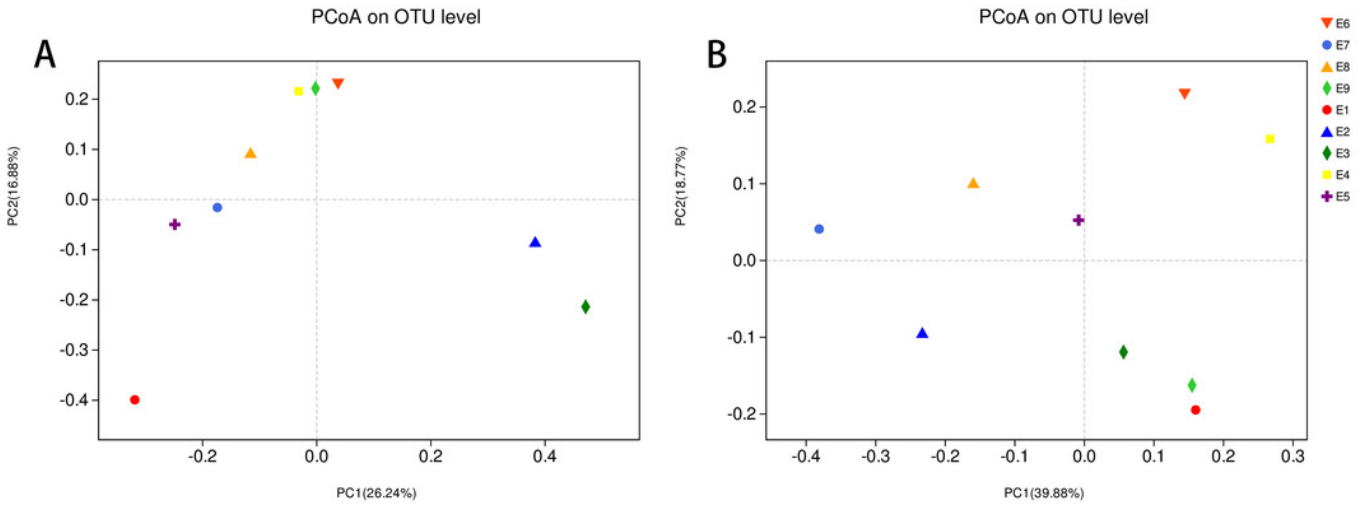

Table 1(on next page)

Alpha diversity of gut microbiota in Common Kestrel feces

Sample	Sobs	Shannon	Simpson	Ace	Chao	Coverage
E1	66	0.596	0.788	78.114	73.583	1.000
E2	649	2.780	0.204	674.412	672.193	0.998
E3	515	2.965	0.184	524.452	522.519	0.999
E4	578	4.233	0.053	594.498	594.050	0.999
E5	235	3.285	0.057	448.368	378.103	0.997
E6	476	4.802	0.020	479.110	480.091	1.000
E7	263	1.604	0.399	292.553	281.800	0.999
E8	317	2.706	0.143	364.651	359.519	0.998
E9	317	2.374	0.335	330.906	331.607	0.999

1

Table 2(on next page)

The relative abundance of species in gut microbiota of Common Kestrel at phylum level

The names of phyla in Table 2 represented *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Patescibacteria*, *Deinococcus-Thermus*, *unclassified_K_norank_d_Bacteria*, *Tenericutes*, *Verrucomicrobia* respectively.

1

Sample	*Pro*	*Fir*	*Act*	*Bac*	*Pat*	*Dei*	*unc*	*Ten*	*Ver*	others
E1	88.630%	10.634%	0.623%	0.006%	0.003%	0.000%	0.096%	0.000%	0.006%	0.003%
E2	13.211%	79.816%	2.376%	1.085%	0.085%	0.065%	0.361%	1.291%	0.017%	1.694%
E3	50.540%	39.567%	3.286%	1.502%	1.857%	0.087%	1.553%	0.121%	0.011%	1.474%
E4	73.770%	3.574%	8.602%	5.950%	1.719%	3.960%	0.220%	0.008%	0.158%	2.038%
E5	27.797%	43.152%	10.694%	18.166%	0.104%	0.042%	0.006%	0.000%	0.000%	0.039%
E6	36.410%	30.610%	20.330%	5.572%	0.944%	0.324%	1.511%	0.135%	1.223%	2.940%
E7	5.000%	73.097%	20.770%	0.676%	0.003%	0.000%	0.076%	0.000%	0.006%	0.372%
E8	11.832%	59.652%	27.752%	0.369%	0.073%	0.003%	0.031%	0.000%	0.031%	0.256%
E9	62.507%	28.205%	6.285%	1.065%	0.096%	0.056%	0.671%	0.045%	0.006%	1.063%
Mean	41.078%	40.923%	11.191%	3.821%	0.543%	0.504%	0.503%	0.178%	0.162%	1.098%

2

Table 3(on next page)

The relative abundance of species in gut microbiota of Common Kestrel at genus level

The names of phyla in Table 3 represented *Lactobacillus*, *Escherichia-Shigella*, *Acinetobacter*, *Bifidobacterium*, *Enterococcus*, *Clostridium_sensu_stricto_1*, *Paracoccus*, *Burkholderia-Caballeronia-Paraburkholderia*, *Glutamicibacter* respectively.

Sample	*Lac*	*Esc*	*Aci*	*Bif*	*Ent*	*Clo*	*Par*	*Bur*	*Glu*	others
E1	6.618%	88.610%	0.011%	0.037%	3.140%	0.023%	0.000%	0.000%	0.000%	1.561%
E2	68.336%	1.787%	0.581%	0.034%	2.528%	0.042%	0.017%	5.567%	0.042%	21.066%
E3	24.037%	0.862%	38.448%	0.093%	0.448%	0.707%	0.101%	5.544%	0.023%	29.736%
E4	0.392%	0.166%	2.153%	0.031%	0.118%	0.214%	19.488%	0.854%	0.860%	75.724%
E5	0.693%	7.962%	5.040%	0.034%	15.026%	0.011%	0.149%	0.014%	9.411%	61.659%
E6	1.356%	0.130%	5.823%	3.086%	0.510%	1.043%	6.023%	4.445%	6.113%	71.470%
E7	66.056%	0.054%	0.211%	20.356%	2.120%	0.192%	0.031%	2.841%	0.011%	8.129%
E8	9.589%	1.536%	0.536%	25.502%	8.988%	28.177%	0.054%	2.060%	0.099%	23.459%
E9	7.988%	57.183%	0.798%	1.446%	3.340%	1.866%	0.273%	0.347%	0.000%	26.759%
Mean	20.563%	17.588%	5.956%	5.624%	4.024%	3.586%	2.904%	2.408%	1.840%	35.507%

1