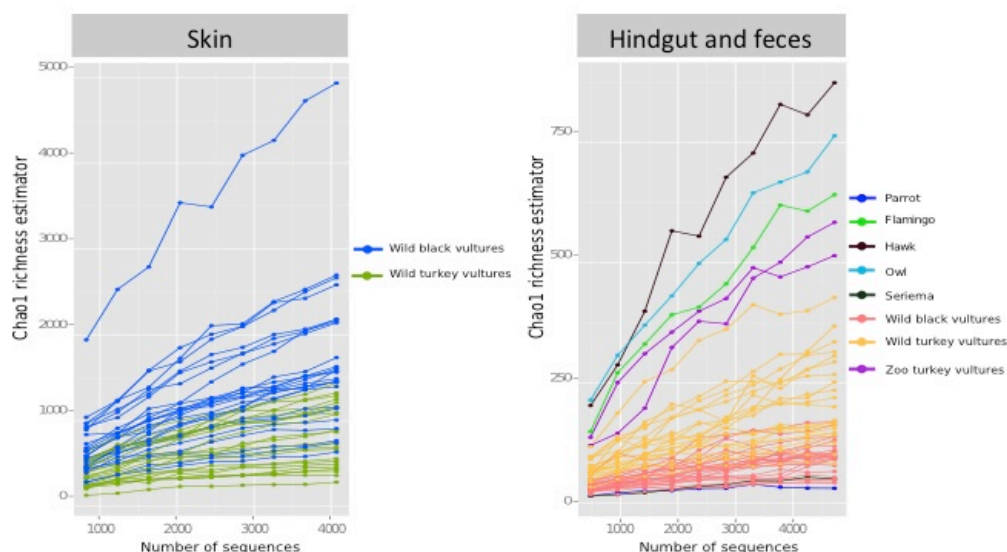


Supplementary Information



Supplementary Figure 1. Chao 1 richness estimator of microbial OTUs (16S rRNA gene survey) from skin and hindgut of wild vultures and from feces of zoo birds.

Supplementary Note 1. Skin microbiota. OTUs were assigned to 59 microbial classes. The microbial skin community was dominated by Clostridia, Gammaproteobacteria, Actinobacteria, Bacilli, Betaproteobacteria, Fusobacteria, Flavobacteria, Bacteroidia, Alphaproteobacteria, Thermomicrobia, Cyanobacteria, and Epsilonproteobacteria (Supplementary Table 2). Black vultures carried more Actinobacteria, Epsilonproteobacteria, and Thermomicrobia whereas the turkey vultures supported significantly more Betaproteobacteria and Clostridia (Wilcoxon-Rank-Sum test, $P < 0.05$). Archaea accounted to only 0.3% of the relative skin sequences. We were able to assign 60.2 % of facial skin sequences from black vultures and 63.8 % of sequences from turkey vultures to the generic level. *Acinetobacter*, *Arthrobacter*, *Clostridium*, *Flavobacterium*, *Herbaspirillum*,

Ignatzschineria, and *Psychrobacter* were the most prominent skin bacteria (Supplementary Table 3).

Supplementary Note 2. Hindgut microbiota.

The seven bacterial taxa illustrated in Figure 3a comprised 74% of sequences at the species level (97% sequences similarity) in black and turkey vultures (listed in Supplementary Table 8). *Fusobacterium* was more prevalent in turkey vulture. There was no difference between the species in the counts of the most frequently observed *Clostridium* OTUs. As indicated in the Figure 3a, *Catellibacter* and *Campylobacter* were more prevalent in black vultures, whereas *Peptostreptococcus* was mainly associated with turkey vultures (Wilcoxon, $P < 0.05$).

Supplementary Note 3. Fecal samples from zoo birds.

Hawk feces hosted the highest bacterial OTU richness among zoo birds, followed by owl, flamingo, and the captive turkey vultures. The parrot and seriema exhibited the least diverse microbial community (Supplementary Fig. 1). Feces from zoo vultures hosted a more diverse bacterial flora than those observed in wild turkey vultures. The Gammaproteobacteria was the most abundant bacterial class in zoo bird feces with the exception of the flamingo and the two captive turkey vultures (Supplementary Table 6). Clostridia were not observed in seriema feces, but accounted for more than 50% of the reads in captive vultures and the flamingo. Fusobacteria were not found in the parrot, seriema, hawk, or owl. Flamingo and the captive turkey vultures fecal samples contained similar levels of Clostridia and Fusobacteria. However, the major difference between the flamingo and vultures was described on genus level where the flamingo feces contained *Megamonas*, that were

relatively common in flamingo feces (18 %) but were rare in captive and wild vultures (< 0.1%).

Of the 12 microbial classes observed in the wild turkey vultures, only 10 were found in the captive turkey vultures. The microbial profile of the zoo vultures was highly similar to those of wild vultures with the exception of the Bacteroidia, which were rare in wild vultures. Observed patterns are most likely due to the dietary difference between wild and captive vultures.

Supplementary Table 1. Mammalian taxa detected on facial skin swabs and in the hindgut of *Cathartes aura* (turkey vulture) and *Coragyps atratus* (black vulture).

Numbers indicate the percentage of vultures in which taxa were detected.

Family	black vulture skin n=23	turkey vulture skin n=22	black vulture hindgut n=24	turkey vulture hindgut n=21
<i>Bovidae</i>	100	91	0	19
<i>Canidae</i>	30	27	0	0
<i>Cervidae</i>	100	100	0	0
<i>Didelphidae</i>	4	41	0	0
<i>Equidae</i>	9	9	0	0
<i>Felidae</i>	0	5	0	0
<i>Homo sapiens</i>	0	5	0	5
<i>Leporidae</i>	4	5	0	0
<i>Mephitidae</i>	0	9	0	0
<i>Procyonidae</i>	0	14	0	0
<i>Suidae</i>	26	18	0	0

Supplementary Table 2. Microbial class (mean) distribution on facial skin (relative abundance in %). Only taxa >1% shown. Significance: Wilcoxon $P < 0.05$.

Class	black vulture n=26 $\bar{x} \pm SD$ [min; max] in %	turkey vulture n=24 $\bar{x} \pm SD$ [min; max] in %	W; P -value
Actinobacteria	15.5 $\pm$ 8.6 [3.3; 33.9]	10.6 $\pm$ 10.5 [1.5; 40.6]	427; 9.75E-03
Alphaproteobacteria	2.3 $\pm$ 1.3 [0.8; 5.5]	3.7 $\pm$ 3.5 [0.2; 13.5]	253; 3.57E-01
Bacilli	9.6 $\pm$ 8.2 [1.1; 31.6]	13.1 $\pm$ 10.8 [0.9; 33]	245; 2.79E-01
Bacteroidia	5.9 $\pm$ 10 [0.1; 36.4]	2.3 $\pm$ 2.5 [0.1; 8.4]	300; 9.92E-01
Betaproteobacteria	5.4 $\pm$ 4.9 [0.7; 20.1]	11 $\pm$ 11 [0.6; 38.3]	191; 3.13E-02
Clostridia	24.4 $\pm$ 10.3 [7.5; 40.5]	26.2 $\pm$ 15.7 [7.9; 68.2]	351; 5.68E-01
Cyanobacteria	0.8 $\pm$ 1.3 [0; 6.3]	3.7 $\pm$ 8.2 [0; 36.6]	218; 1.07E-01
Epsilonproteobacteria	1.8 $\pm$ 3 [0; 12.8]	0.1 $\pm$ 0.2 [0; 0.8]	525; 6.26E-06
Flavobacteria	6.2 $\pm$ 5.3 [0.2; 20.2]	2.3 $\pm$ 2.2 [0; 9.1]	479; 3.23E-04
Fusobacterla	6.1 $\pm$ 9.0 [0; 31.5]	3.0 $\pm$ 5.3 [0.2; 23.6]	310.5; 8.E-01
Gammaproteobacteria	13.1 $\pm$ 8.1 [3.4; 47]	16.5 $\pm$ 7.7 [4.7; 32.9]	209; 7.28E-02
Thermomicrobia	3.9 $\pm$ 5.1 [0; 19.3]	0.5 $\pm$ 1.1 [0; 4.8]	511; 2.32E-05

Supplementary Table 3. Microbial genera (mean) composition for facial skin.

Relative abundance in % (taxa >1% shown). Significance: Wilcoxon, $P < 0.05$.

Class	black vulture n=25 $\bar{x} \pm \text{SD}$ [min; max] in %	turkey vulture n=24 $\bar{x} \pm \text{SD}$ [min; max] in %	W; P -value
<i>Acinetobacter</i>	1.6 $\pm$ 1.4 [0.4; 7.3]	3.7 $\pm$ 3.7 [0.1; 12.5]	199; 4.51E-02
<i>Arthrobacter</i>	6.1 $\pm$ 5 [1.1; 23.4]	4.7 $\pm$ 7.7 [0.2; 32]	429; 9.47E-03
<i>Brucella</i>	0.6 $\pm$ 0.6 [0; 2.1]	2 $\pm$ 2.7 [0; 10.3]	191; 3.12E-02
<i>Catellibacter</i>	1.7 $\pm$ 3.3 [0.1; 16]	0.6 $\pm$ 2.4 [0; 11.5]	522; 6.45E-06
<i>Clostridium</i>	6.2 $\pm$ 4.9 [0.9; 20.1]	5.6 $\pm$ 4.8 [0.6; 20.0]	328; 5.75E-01
<i>Flavobacterium</i>	2.3 $\pm$ 2.9 [0; 11]	0.6 $\pm$ 1.3 [0; 6.5]	469; 7.06E-04
<i>Herbaspirillum</i>	3.9 $\pm$ 4.3 [0; 17]	9.1 $\pm$ 9.8 [0.1; 33.8]	192; 3.29E-02
<i>Ignatzschineria</i>	1.8 $\pm$ 4.6 [0; 23.6]	2.8 $\pm$ 4.2 [0; 16.7]	256; 3.94E-01
<i>Peptostreptococcus</i>	1.1 $\pm$ 2.4 [0; 12.6]	4.1 $\pm$ 5.5 [0; 22.1]	201; 4.96E-02
<i>Prevotella</i>	4.2 $\pm$ 7.8 [0; 28.2]	0.4 $\pm$ 0.4 [0; 1.6]	364; 1.96E-01
<i>Pseudomonas</i>	1.1 $\pm$ 1 [0; 3.6]	3.6 $\pm$ 2.8 [0; 9.8]	109; 141E-04
<i>Psychrobacter</i>	5 $\pm$ 3.7 [1; 11.8]	2.6 $\pm$ 3.6 [0.2; 17.4]	451; 2.48E-03
<i>Tissierella</i>	1.4 $\pm$ 2.6 [0.2; 13.3]	1 $\pm$ 1.3 [0; 4.1]	374; 1.35E-01
<i>Vagococcus</i>	0.4 $\pm$ 0.5 [0; 2.3]	1.9 $\pm$ 2.6 [0; 11.7]	141; 1.60E-03

Supplementary Table 4. Unique bacterial species (OTUs identified at 96% sequence similarity) by body site and sex in black and turkey vultures. Values represent percentages. Significance: Wilcoxon $P < 0.05$.

Sample site	$\bar{x} \pm \text{SD}$ [min; max] in %	W; P -value
Black vulture; skin	20.6 ± 7.4 [11; 41.6]	257; 4.06E-01
Turkey vulture; skin	20.7 ± 7.8 [7.9; 37.9]	
Black vulture; hindgut	6.4 ± 6.3 [1.5; 26.6]	422; 5.05E-03
Turkey vulture; hindgut	10.9 ± 7.4 [3.5; 36.8]	
Black vulture; skin; female	23.4 ± 7.3 [11.8; 41.6]	36; 8.28E-02
Black vulture; skin; male	17 ± 6.7 [11; 31.2]	
Turkey vulture skin; female	20.2 ± 6.6 [7.9; 28.9]	74; 5.16E-01
Turkey vulture; skin; male	23.9 ± 9.5 [10.8; 37.9]	
Black vulture; hindgut; female	6.4 ± 6.1 [2.2; 26.6]	6.5; 9.53E-01
Black vulture; hindgut, male	8.3 ± 7.1 [1.5; 21.2]	
Turkey vulture; hindgut; female	11 ± 9.2 [3.5; 36.8]	54; 7.93E-01
Turkey vulture; hindgut; male	10.8 ± 4.1 [6.6; 18]	

Supplementary Table 5. Microbial class (mean) composition of the hindgut; relative abundance in %. Only taxa >1% shown. (Significance: Wilcoxon, $P < 0.05$)

Class	black vulture n=26 $\bar{x} \pm \text{SD}$ [min; max] in %	turkey vulture n=23 $\bar{x} \pm \text{SD}$ [min; max] in %	W-value; P -value
Actinobacteria	0.2 ± 0.2 [0.0; 0.8]	1.2 ± 2.0 [0.0; 7.6]	417; 7.06E-03
Alphaproteobacteria	0.3 ± 0.6 [0; 2.2]	0.1 ± 0.2 [0; 1]	370; 8.26E-02
Bacilli	16.6 ± 15.0 [0.1; 58.9]	5.2 ± 7.3 [0.2; 26.6]	436; 1.98E-03
Betaproteobacteria	1.3 ± 2.4 [0; 9.7]	1.3 ± 1.5 [0.1; 5.5]	246; 4.08E-01
Clostridia	50.5 ± 18.1 [26.1; 84.8]	55.6 ± 17.4 [25.5; 83.6]	240; 3.50E-01
Epsilonproteobacteria	6.0 ± 5.5 [0.5; 17.1]	1.1 ± 2.4 [0.0; 10.7]	514; 2.61E-06
Fusobacteria	21.2 ± 15.9 [0.2; 54]	31.1 ± 16.3 [1.7; 68.5]	191; 4.99E-02
Gammaproteobacteria	3.6 ± 5.1 [0; 21.3]	2.9 ± 2.9 [0.1; 10.7]	279; 8.85E-01

Supplementary Table 6. Microbial class observations from the feces of zoo birds.

Relative abundance in % (taxa >1% shown).

Class	parrot n=1	owl n=1	hawk n=1	seriema n=1	zoo turkey vulture n=2	flamingo n=1
Actinobacteria	0	13.6	23.3	0.1	0.3	2.8
Alphaproteobacteria	0.2	12.2	1.5	0.2	0	0.4
Bacilli	0.6	1	10.8	0	0.7	0.6
Bacteria;Other	0	0.3	6.7	0.1	0.2	1.9
Bacteroidia	0	0	0	0	16.8	2.4
Betaproteobacteria	0	6.9	0.3	0	1.5	0.2
Clostridia	7.3	4.9	20.5	0	51.6	55
Epsilonproteobacteria	0	0	0	0	0	0
Erysipelotrichi	0	0	0.2	0	0.8	1.8
Flavobacteria	0	2.1	5.9	0	0.1	2.8
Fusobacteria	0	0	0	0	26.2	13.3
Gammaproteobacteria	91.8	47.7	28.2	98.7	0.6	15
Sphingobacteria	0	3.5	0.6	0	0	0.1
Thermomicrobia	0	0	0	0	0	2.2
TM7_genera_incertae_sedis	0	1.2	0.2	0	0	0.2
Verrucomicrobiae	0	6.1	1.4	0	0	0.6

Supplementary Table 7. Virulence factors observed in a single wild turkey vulture shotgun metagenome. Shown are only genes with full read length. *Strain ID in the virulence factor database (VFDB)

Strain (VFDB ID*)	Gene description	Coverage of the gene in %	Depth (cover of each position in the gene)	Length of the gene	Bases covered
<i>Clostridium perfringens</i> (VF0378)	<i>plc-phospholipase C</i>	100	188	1197	1197
<i>Clostridium perfringens</i> (VF0388)	<i>colA-collagenase</i>	100	138	3315	3315
<i>Clostridium perfringens</i> (VF0382)	<i>pfoA-perfringolysin O</i>	100	134	1503	1503
<i>Clostridium perfringens</i> (VF0389)	<i>nagH-hyaluronidase</i>	100	133	4887	4887
<i>Clostridium perfringens</i> ATCC1312 (VF0391)	<i>nanH-sialidase</i>	100	106	1149	1149
<i>Clostridium perfringens</i> (VF0391)	<i>nanJ-exo-alpha-sialidase</i>	100	99	3522	3522
<i>Clostridium perfringens</i> (VF0390)	<i>cloSI-alpha-clostripain</i>	100	89	1575	1575
<i>Clostridium perfringens</i> (VF0389)	<i>nagI-hyaluronidase</i>	100	78	3894	3894
<i>Clostridium perfringens</i> (VF0389)	<i>nagJ-hyaluronidase</i>	100	76	3006	3006
<i>Clostridium perfringens</i> 13(VF0391)	<i>nanI-exo-alpha-sialidase</i>	100	75	2085	2085
<i>Clostridium perfringens</i> (VF0389)	<i>nagK-hyaluronidase</i>	100	74	3492	3492
<i>Clostridium perfringens</i> (VF0389)	<i>nagL-hyaluronidase</i>	100	68	3384	3384

Supplementary Table 8. Distribution of the microbial species (OTUs) most frequently observed in the hindgut (see Figure 3a). Wilcoxon, $P < 0.05$.

OTUs	black vulture n=25 $\pm$ SD [min; max] in %	turkey vulture n=24 $\pm$ SD [min; max] in %	W; P -value
<i>Campylobacter</i> _1	4.6 $\pm$ 4.7 [0.2; 14.1]	0.2 $\pm$ 0.6 [0; 2.4]	31; 1.12E-07
<i>Catelliboccus</i> _1	12.1 $\pm$ 11.5 [0; 34.8]	1.9 $\pm$ 4.6 [0; 16.4]	60; 2.82E-06
Unclassified Clostridiales_2	5.3 $\pm$ 4.7 [0; 14.2]	6.4 $\pm$ 6.2 [0; 23.7]	306; 6.89E-01
<i>Clostridium</i> _5	16.9 $\pm$ 17.6 [0.2; 53.8]	14.1 $\pm$ 12.8 [0.5; 44.9]	305; 7.05E-01
<i>Fusobacterium</i> _1	19.8 $\pm$ 14.4 [0.2; 49.4]	30.7 $\pm$ 15.6 [2.2; 63.6]	405; 1.30E-02
Unclassified <i>Peptostreptococcaceae</i> _1	17.1 $\pm$ 15.8 [0.1; 49.6]	7.1 $\pm$ 8.7 [0.3; 38.4]	144; 2.82E-03
<i>Peptostreptococcus</i> _1	2 $\pm$ 4.1 [0; 16.7]	7.4 $\pm$ 10.8 [0; 43.9]	452; 5.86E-04

Supplementary Table 9. Pairwise interactions between the bacterial OTUs within the vulture gut shown in Figure 3b&c. Comparisons shown here are based on Spearman Rank correlations (cutoff=0.6, permutation test, $p < 0.05$).

Interaction types	black vulture in % (n=26)	turkey vulture in % (n=23)
Number of total interactions	100 (133)	100 (167)
Negative correlation (total)	8 (10)	10 (17)
Positive correlations (total)	92 (123)	90 (150)
Negative correlations within the same microbial classes	2 (3)	5 (9)
Positive correlations within the same microbial classes	86 (114)	81 (135)
Negative correlations between microbial classes	5 (7)	5 (8)
Positive correlations between microbial classes	7 (9)	9 (15)
Negative correlations between Fusobacteria and Clostridia	3 (4)	2 (3)
Positive correlations between Fusobacteria and Clostridia	0 (0)	5 (9)