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**Supplemental Information for:**

# Using DNA metabarcoding for simultaneous inference of common vampire bat diet and population structure

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## Supporting Information S1: Metabarcoding of common vampire bat blood meal and faecal samples

**Table S1a.** Overview of the common vampire bat blood meal and faecal samples analysed. Blood meal samples were collected from individual bats, while faecal samples were either collected from individual bats or collected non-invasively underneath bat roosts.

| | | | | | No. samples | | |
|---|---|---|---|---|---|---|---|
| **Ecoregion** | **Area** | **Province** | **District** | **Site** | **Blood meals** | **Individual faecal droppings** | **~5 pooled faecal droppings** |
| **Amazon** | Amazonas | Rodriguez de Mendoza | Chirimoto | AMA1 | 3 | | |
| | Madre de Dios | Manu | Huepetuhe | MDD130 | 13 | | |
| | Madre de Dios | Manu | Mazuko | MDD134 | 9 | | |
| | | | No. samples | | 25 | | |
| **Andes** | Apurimac | Chincheros | Rio Blanco | API1 | 10 | | |
| | Apurimac | Abancay | Abancay | API13 | 2 | | |
| | Apurimac | Andahuaylas | Pacucha | API9 | 2 | | |
| | Cajamarca | Cutervo | Rodeopampa | CAJ2 | 15 | | |
| | Cajamarca | Cutervo | Ambulco | CAJ3 | 10 | | |
| | Cajamarca | Jaen | San Ingacio | CAJ4 | 2 | | |
| | Huanuco | Tingo Maria | Tingo Maria | HUA1 | | 1 | 1 |
| | Huanuco | Ambo | Cayna | HUA2 | | 2 | 1 |
| | Huanuco | Huanuco | Molinopampa | HUA3 | | 2 | 1 |
| | | | No. samples | | 41 | 5 | 3 |
| **Coast** | Lima | Huaral | Chancay | LMA10 | 8 | | |
| | Lima | Canete | Mala | LMA4 | 15 | | |
| | Lima | Huara | Huacho | LMA6 | 21 | | |
| | | | No. samples | | 44 | | |
| | | | **Total no. samples** | | **110** | **5** | **3** |

**Table S1b.** Samples with common vampire bat and vertebrate prey taxa assignments from metabarcoding analyses using the 16s (Taylor 1996) and COI (Geller *et al.* 2013; Leray *et al.* 2013) primer sets.

| | | **Blood meal** | **Individual faecal samples** | **Pooled faecal samples** | **All samples** |
|---|---|---|---|---|---|
| Total samples | | 110 | 5 | 3 | 118 |
| No. samples with vampire bat id | 16s | 108 (99.2%) | 5 (100%) | 3 (100%) | 116 (98.3%) |
| | COI | 110 (100%) | 5 (100%) | 2 (66.7%) | 117 (99.2%) |
| No. samples with vertebrate prey id | 16s | 100 (90.1%) | 4 (80%) | 3 (100%) | 107 (90.7%) |
| | COI | 91 (82.7%) | 4 (80%) | 2 (66.7%) | 97 (82.2%) |

**Table S1c.** Number of prey taxa identified within the common vampire bat samples that had vertebrate prey taxa assignments in metabarcoding analyses using 16s and COI primer sets.

| | | **Blood meal** | **Individual faecal samples** | **Pooled faecal samples** |
|---|---|---|---|---|
| Min. - max. (average) no. vertebrate prey taxa per sample where prey is detected | 16s | 1-2 (1.03) | 1 (1) | 2-3 (2.33) |
| | COI | 1-2 (1.02) | 1 (1) | 3 (3) |

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**Table S1d.** Vertebrate prey availability (expected counts, calculated using livestock densities) and prey eaten (observed counts, detected as prey through metabarcoding) in the six areas included in this study, and for all sites combined. All p-values show significant differences between observed and expected prey counts ($p<0.05$). However, for all individual areas and all areas combined, there are expected counts for species under 5, and the results must be interpreted with care.

| **Area** | | **Cow** | **Chicken** | **Pig** | **Sheep** | **Pearson's $\chi^2$ statistic** | **p-value** |
|---|---|---|---|---|---|---|---|
| AMA | Observed | 3 | 0 | 0 | 0 | 127.93 | 1.16 x $10^{-29}$ |
| | Expected | 0.069 | 2.828 | 0.088 | 0.015 | | |
| API | Observed | 11 | 0 | 1 | 0 | 31.05 | 2.52 x $10^{-8}$ |
| | Expected | 2.861 | 4.736 | 1.336 | 3.068 | | |
| CAJ | Observed | 18 | 0 | 8 | 0 | 43.46 | 4.33 x $10^{-11}$ |
| | Expected | 7.700 | 13.578 | 2.337 | 2.385 | | |
| HUA | Observed | 5 | 0 | 4 | 3 | 29.66 | 5.15 x $10^{-8}$ |
| | Expected | 0.935 | 7.194 | 1.422 | 2.449 | | |
| LMA | Observed | 24 | 0 | 8 | 3 | 1121.46 | 7.16 x $10^{-246}$ |
| | Expected | 0.613 | 33.799 | 0.361 | 0.226 | | |
| MDD | Observed | 13 | 2 | 1 | 2 | 15.80 | 7.03 x $10^{-5}$ |
| | Expected | 5.338 | 6.625 | 1.545 | 4.492 | | |
| All areas | Observed | 74 | 2 | 22 | 8 | 740.54 | 4.57 x $10^{-163}$ |
| | Expected | 7.471 | 86.910 | 4.639 | 6.985 | | |

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**Table S1e.** Mammalian prey availability (expected counts, calculated using livestock densities) and prey eaten (observed counts, detected as prey through metabarcoding) in the six areas included in this study, and for all sites combined. All p-values show significant differences between observed and expected prey counts ($p<0.05$). However, for some areas, there are expected counts less than 5, and for these the results must be interpreted with care.

| **Area** | | **Cow** | **Pig** | **Sheep** | **Pearson's $\chi^2$ statistic** | **p-value** |
|---|---|---|---|---|---|---|
| AMA | Observed | 3 | 0 | 0 | 4.49 | 0.03 |
| | Expected | 1.201 | 1.530 | 0.269 | | |
| API | Observed | 11 | 1 | 0 | 14.06 | $1.77 \times 10^{-4}$ |
| | Expected | 4.725 | 2.207 | 5.068 | | |
| CAJ | Observed | 18 | 8 | 0 | 7.19 | $7.35 \times 10^{-3}$ |
| | Expected | 16.117 | 4.892 | 4.991 | | |
| HUA | Observed | 5 | 4 | 3 | 4.69 | 0.03 |
| | Expected | 2.335 | 3.551 | 6.114 | | |
| LMA | Observed | 24 | 8 | 3 | 4.67 | 0.03 |
| | Expected | 17.876 | 10.521 | 6.603 | | |
| MDD | Observed | 13 | 1 | 2 | 7.60 | $5.82 \times 10^{-3}$ |
| | Expected | 7.508 | 2.174 | 6.319 | | |
| All areas | Observed | 74 | 22 | 8 | 51.42 | $7.47 \times 10^{-13}$ |
| | Expected | 40.690 | 25.268 | 38.042 | | |

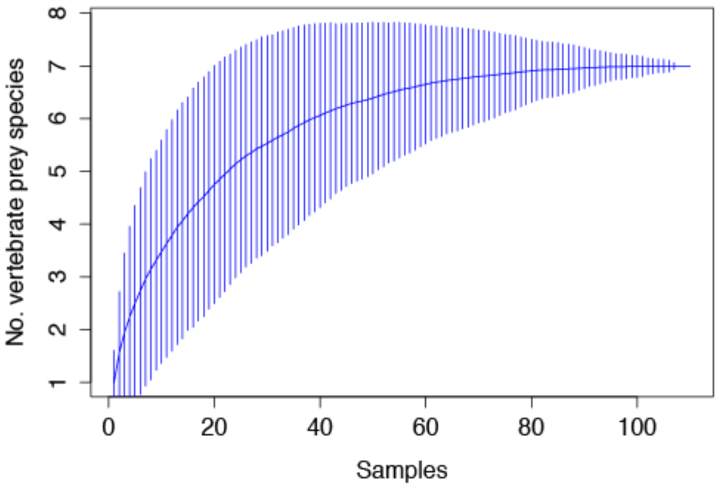

**Figure. S1a.** Species accumulation curve for vertebrate prey detected in common vampire bat through metabarcoding of blood meal and faecal samples with 16s and/or COI primer sets. Vertical bars indicate the bootstrap estimate of the standard error in the number of prey species detected.

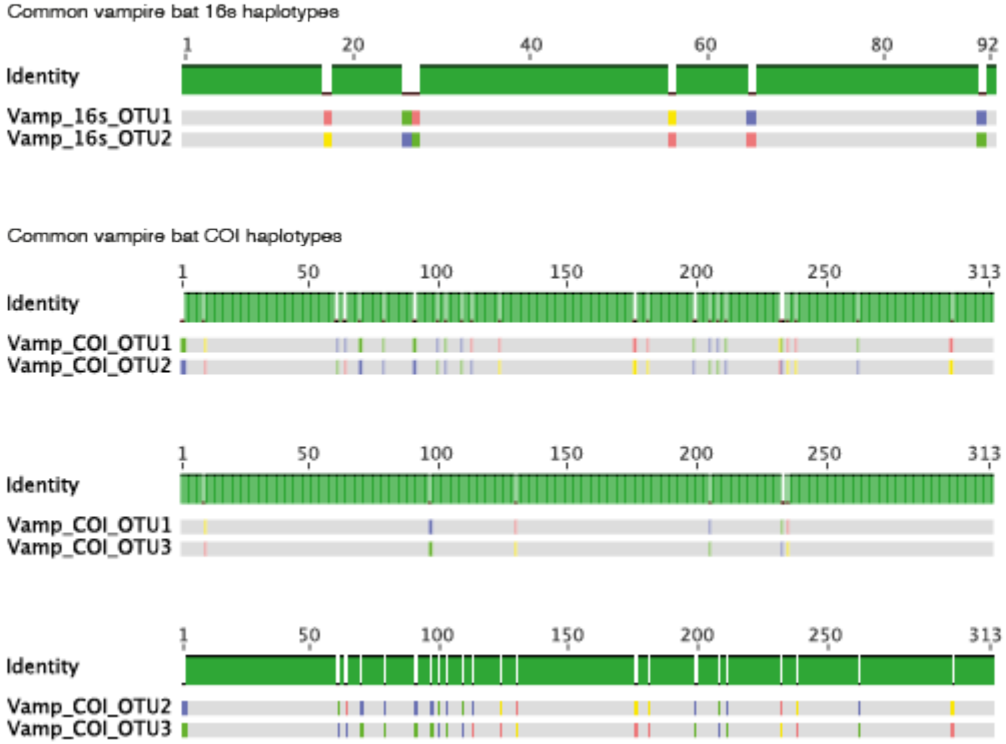

**Figure S1b.** Pairwise alignment of 16s and COI common vampire bat haplotype sequences. Image: Geneious version 6.1 created by Biomatters, http://www.geneious.com.

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## Supporting Information S2: Metabarcoding of blood meals from hairy-legged vampire bats

Three hairy-legged vampire bat (*Diphylla ecaudata*) blood meal samples collected in the MDD134 site in the Amazon were metabarcoded alongside the common vampire bat (*Desmodus rotundus)* samples with the mammal 16s (Taylor 1996) and metazoan COI (Geller *et al.* 2013; Leray *et al.* 2013) primer sets. Blood meal samples were collected from captured and morphologically identified hairy-legged vampire bats. One hairy-legged vampire bat OTU was identified by each of the markers. The 16s OTU only had a 96% match to *Diphylla ecaudata* in ncbi genbank, while the COI hairy-legged vampire bat OTU had matches to *Diphylla ecaudata* spanning from 92.88-99.35% indicating mitochondrial intraspecific diversity in the hairy-legged vampire bat. As only bird prey was detected, only the metazoan COI primer set could be used to identify prey. With the COI primer set, four vertebrate prey OTUs were identified of which three were found in one sample and two samples contained the same OTU (Table S2). The BOLD database (boldsystems.org) was used to identify the OTUs. Criteria for taxonomic assignments were as follows: i) Prey species assignment: 100% match to only one species. ii) Prey genus assignment: 100% matches to more than one species within the same genus. As the remaining OTUs had poor reference database coverage, comparisons were made against all barcode records in BOLD. iii) Prey family assignment: identity from 96.12 to 88.03 within the same family. In the sample where Tinamous sp. (Tinamoformes) were identified (Table S2), an OTU with amount of sequences and low identity matches to the order Tinamiformes were also found. Therefore, this OTU was assumed to be an artefact. Nematode taxa assignment followed that of the common vampire bat.

Hairy-legged vampire bat has been reported to rely solely on birds as prey (Greenhall *et al.* 1984) (although potential predation on humans have been reported (Ito *et al.* 2016)). In agreement with this, we only detected birds in blood meal samples from three individual hairy-legged vampire bats. Two of the bats had preyed on chicken (*Gallus* sp.), while the third bat had preyed on two wild birds, spix's guan (*Penelope jacquacu*) and tinamous (*Tinamus sp.*) (Table S2). To our knowledge, no other study has identified other birds than chicken in any vampire bat species' blood meal or faeces. The spix's guan is a large, fairly common arboreal bird weighing up to ca 1.4 kg and known to occur in rainforest in the area where the samples were collected (IUCN 2016; Del Hoyo *et al.* 2017). Species in the tinamous genus are larger bird species weighing up to ca. 1.9 kg, they roost in trees, and the genus is known to occur in the area where the hairy-legged were caught (Khanna 2005; Del Hoyo *et al.* 2017). Although based on just one sample from one individual, this gives the first evidence that the hairy-legged vampire bats can prey on spix's guan and tinamous and that these wild birds might offer them a reliable and accessible food source in the MDD134 site.

**Table S2.** Overview of the taxa identified through metabarcoding with the COI primer set (Geller *et al.* 2013; Leray *et al.* 2013) in three blood meal samples from hairy-legged vampire bat (*Diphylla ecaudata*) collected in the MDD134 site in the Amazon ecoregion. Vertebrate prey are listed as English name, order, family, genus and species.

| Area, province, district | Site | No. blood meal samples | Vertebrate prey | Vertebrate prey | Additional detections |
|---|---|---|---|---|---|
| Madre de Dios, Manu, Mazuko | MDD134 | 1 | **Spix's guan**<br>Aves, Galliformes, Cracidae, *Penelope jacquacu* | **Tinamous**<br>Aves, Tinamiformes, Tinamidae, *Tinamus* sp | |
| | | 1 | **Chicken**<br>Aves, Galliformes, Phasianidae, *Gallus* sp. | | **Nematode**<br>Nematoda |
| | | 1 | **Chicken**<br>Aves, Galliformes, Phasianidae, *Gallus* sp. | | |

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