

Measuring Protected-Area Effectiveness using Vertebrate Distributions from Leech iDNA

Yinqiu Ji*, Christopher CM Baker*, Viorel D Popescu, Jiaxin Wang, Chunying Wu, Zhengyang Wang, Yuanheng Li, Lin Wang, Chaolang Hua, Zhongxing Yang, Chunyan Yang, Charles CY Xu, Alex Diana, Qingzhong Wen, Naomi E Pierce, and Douglas W Yu

*These authors contributed equally to this work.

Contents

1 Supplementary Methods: Laboratory Processing	1
2 Supplementary Methods: Bioinformatics Pipeline	2
3 Supplementary Methods: Site-occupancy Modelling	4
4 Supplementary Figures	9
5 Supplementary Note: Chinese Language Main Text	20

1 Supplementary Methods: Laboratory Processing

DNA extraction. We extracted DNA from each replicate sample following the protocol in [1]. Leeches were transferred to a new tube to remove the preservative, soaked in a volume of digestion buffer (10 mM Tris-HCl, 10 mM NaCl, 2% SDS, 5 mM CaCl₂, 2.5 mM EDTA, 40 mM dithiothreitol, and 0.2 mg/mL Proteinase K) equal to 5 times the volume of each sample's leeches, and incubated at 55 °C (rotating) until all the leeches were dissolved. Following this incubation, we aliquoted 0.6 mL of digestion buffer from each sample for purification with the QIAquick PCR purification kit (Qiagen, Hilden, Germany). To detect any DNA cross-contamination, negative controls were created in both steps, digestion and purification.

PCR amplification. We PCR-amplified two mitochondrial markers: one from the 16S rRNA gene (*MT-RNR2*; primers *16Smam1*: 5'-CGGTTGGGTGACCTCGGA-3' and *16Smam2*: 5'-GCTGTTATCCCTAGGGTAAC-3' [2]), and the other from the 12S rRNA gene (*MT-RNR1*; primers forward: 5'-ACTGGGATTAGATAACCC-3' and reverse: 5'-YRGAACAGGCTCCTAG-3' modified from [3]). Target fragments were 81 to 117 bp and 82 to 150 bp respectively, excluding primers. Hereafter, and throughout the manuscript, we refer to these two markers as LSU (16S) and SSU (12S), referring to the ribosomal large subunit and small subunit that these genes code for. (We do this in part to avoid confusion with the widely used bacterial 16S gene, which is homologous to our 12S marker, rather than our 16S.) The LSU primers are designed to target mammals, and the SSU primers to amplify all vertebrates. A third primer pair targeting the standard cytochrome *c* oxidase I marker [4] was tested but not adopted

in this study as it co-amplified leech DNA and consequently returned few vertebrate reads. We also tried using human blocking primers, to help avoid wasting read depth on human amplicons. However, in our initial trials, we found that over 70% of our samples failed in PCR, and even successful amplifications had low yields. We did not pursue this approach further, and instead chose to compensate for the presence of human reads by increasing sequencing depth.

Primers were ordered with sample-identifying tag sequences. We used 8-bp tags with a minimum difference of 3 nucleotides. The file 8bp_Tags_leeches.txt lists all tag sequences used in this study and is available at https://gitub.com/jiyanqiu/ai_laoshan_leeches_method_code. To identify (and remove) ‘tag jumping’ errors [5], we used a ‘twin-tagging strategy,’ meaning that both forward and reverse primers used the same tag sequence for a sample (e.g. F1/R1, F2/R2, F3/R3). Thus, if a library contained tag combinations F1/R1, F2/R2, and F3/R3, an F1 tag-jump would produce F1/R2 or F1/R3, which could be detected and removed, since these combinations were not used in this library. We used the DAMe protocol [6] to remove these tag-jumped Illumina reads and to identify and remove reads containing PCR and/or sequencing errors. The DAMe protocol PCR-amplifies each sample three times per marker, each time with a different twin-tag pair, which allows the PCRs to be individually identified after sequencing. Reads containing errors are more likely to show up in only one PCR and at low copy numbers, which allows them to be filtered out bioinformatically (see below). Different libraries sent for sequencing at the same time (see below for details of library construction), and thus potentially sequenced in the same lane, used different sets of tag pairs so that we could also identify and remove any mis-assigned samples due to index hopping [7].

We used the same PCR conditions for both markers. The 20 μ L PCR reactions consisted of 1X buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μ M per primer (synthesized by Invitrogen, Shanghai, China), 5% DMSO (Amresco, Solon, Ohio, USA), 0.6 U ExTaq HotStart DNA polymerase (TAKARA Biosystems, Dalian, China), and 1 μ L of template DNA, with a thermal cycling profile of 95 °C for 5 min, then 40 cycles of 95 °C for 30 s, 59 °C for 30 s, and 72 °C for 45 s, with a final extension time of 7 min at 72 °C. PCR products were visualized on 2.5% agarose gels, and samples that failed to produce a band at the expected size were reattempted at least three times. The successfully amplified samples were quantified using the Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, New York, USA), and equal masses pooled into a total of 13 LSU and 14 SSU libraries. The number of twin-tagged replicates pooled into each library ranged from 100 to 245. For most samples, all PCR replicates were pooled into the same libraries. The main exceptions were reattempted PCRs, since some reattempts happened after the successful PCR replicates had been sent out for sequencing. LSU and SSU amplicons were never pooled into the same library. Libraries were purified with QIAquick gel extraction kit (QIAGEN, Hilden, Germany), and sent to Novogene (Beijing, China) for library construction using the PCR-free NEBNext Ultra II DNA Library Prep Kit (Ipswich, MA, USA), and 150 bp paired-end sequencing on an Illumina Hiseq X.

2 Supplementary Methods: Bioinformatics Pipeline

Preprocessing. Sequencing of the 27 libraries yielded a total of 1.354×10^9 paired-end reads. We used AdapterRemoval v2.1.7 [8] to remove adapter sequences from reads and Sickle v1.33 [9] to trim reads of low quality nucleotides. We then used BFC v181 (parameters: -s 3g -k 25) [10] to de-noise the reads, and we merged the read pairs with Pandaseq v2.11 [11]. Except for BFC, we used default parameters.

Demultiplexing and DAMe quality filtering. To filter out tag-jumping events and to remove artifactual reads arising from PCR or sequencing errors, we used the DAMe pipeline [6]. DAMe’s `sort.py` function was used to remove reads with unused tag combinations, and the `filter.py` function was used to keep only the haplotypes that appeared in 2 PCRs, with 9 (LSU) or 20 (SSU) copies per PCR, using the logic that sequences which appear in multiple, independent PCRs and in multiple copies per PCR are more likely to be true sequences (`filter.py` parameters for 12S: `-x 3 -y 2 -p 14 -t 20 -l 81`; for 16S: `-x 3 -y 2 -p 13 -t 9 -l 82`). Filtering parameters were chosen after inspection of the control samples. After DAMe filtering, each PCR replicate yielded about 105,000 sequences.

De novo chimera removal. DAMe filtering also removes the chimeric sequences that can result from incomplete PCR extension, but we also used the *de novo* chimera detection function `uchime_denovo` in VSEARCH v2.9.0 [12] to remove any remaining chimeras after dereplicating with the `derep_fulllength` function.

Clustering into preliminary operational taxonomic units. We used Swarm v2.0 [13] to cluster the filtered sequences into preliminary OTUs (‘pre-OTUs’) and then used the R package LULU v0.1.0 [14] to merge Swarm pre-OTUs that shared high similarity and distribution across samples (i.e. over-split OTUs) and output a representative sequence for each pre-OTU. For both, we used default values.

Assigning taxonomy to preliminary operational taxonomic units. One of the more crucial steps in the iDNA bioinformatic pipeline is taxonomic assignment. With vertebrates, exact species identity can have important management consequences because some species, but not their close relatives, are given high conservation value [15]. Existing taxonomic assignment programs are typically biased toward assigning sequences to species that happen to be in a reference database, even though we know that some of our leech-derived sequences are likely from known species that have never been sequenced, or more rarely, that are undescribed. We thus used PROTAX for taxonomic assignment of the pre-OTU sequences [16, 17]. PROTAX provides an unbiased, estimated probability of assignment at each rank, where unbiased means, for example, that 70% of all assignments given a 70% probability of accuracy are indeed correct. Thus, a PROTAX assignment of a pre-OTU to Carnivora (probability = 0.999)/Canidae (0.996)/*Nyctereutes* (0.821)/*Nyctereutes procyonoides* (0.557) means that this pre-OTU is very likely to be in the genus *Nyctereutes*, but there is a $(1 - 0.557) = 44.3\%$ probability that the species is not *N. procyonoides*. PROTAX can also estimate the probability that a pre-OTU sequence is ‘unknown,’ i.e. not in the reference database. Thus, PROTAX helps prevent mistaken assignments of sequences to species, potentially avoiding wasted management effort directed towards species that are not actually present.

We refer the reader to Somervuo *et al.* [16, 17] for in-depth discussions of PROTAX and to Axtner *et al.* [15] for details of the bioinformatic pipeline used to create the LSU and SSU reference databases and to train and assess the PROTAX models. We built the reference databases starting from the Midori Unique_20180221_lrRNA and Unique_20180221_srRNA databases [18], supplemented with mitogenomes from [19]. We used the R package `taxize` [20] to build a taxonomy database of all Tetrapoda and to harmonize species names between the Tetrapoda taxonomies and the sequences in the Midori + Salleh reference database, and we used SATIVA [21] to identify reference sequences mislabelled at family level and above, which we removed. With the curated reference database, we then trained PROTAX models for both LSU and SSU, setting a 90% prior probability for the set of Tetrapoda species known from Ailaoshan, thereby reducing false-positive assignments [22]. Raw similarities between each query and all reference sequences were calculated with LAST v.982 [23], after which the trained PRO-

TAX models were used to assign probabilities of assignment for pre-OTUs at class, order, family, genus, and species ranks. The bioinformatic scripts, reference datasets, trained models, and bias-accuracy plots are available for download from GitHub [24].

Using pairwise correlations between LSU and SSU OTUs to reconcile taxonomies. Different marker genes have different levels of taxonomic coverage and discrimination power [16, 17], and as a result, the same species can be assigned to different taxonomies by SSU and LSU. For instance, as described above, the SSU dataset confidently detected *Nyctereutes procyonoides*, but the LSU dataset did not, although it did assign one OTU to Carnivora (probability = 0.999)/Canidae (0.999)/*Canis* (0.475)/*Vulpes*, unknown species (0.231). Given the confident assignment to Canidae, this LSU OTU might also have derived from *Nyctereutes*. To combine taxonomic information across the two markers, we therefore calculated pairwise correlations of SSU and LSU pre-OTUs across the 619 replicates for which both markers had amplified and visualized the correlations as a network (Supplementary Fig. 2). If an SSU and an LSU pre-OTU occur in the same subset of replicates and are assigned the same higher-level taxonomies, the two pre-OTUs are likely to have been amplified from the same set of leeches feeding on the same species. We manually inspected the network diagram and assigned such correlated pre-OTU pairs the same taxonomy.

Final operational taxonomic units and dataset filtering. After using PROTAX and then searching for network correlations to assign taxonomies to pre-OTUs, we verified that the positive and negative control samples were free of any substantive contaminants before removing them from the dataset, along with one sample that had neither ranger nor patrol area information. We eliminated any pre-OTUs to which we were unable to assign a taxonomy; these pre-OTUs only accounted for 0.9% and 0.2% of reads in the LSU and SSU datasets respectively, and most likely represent erroneous sequences rather than novel taxa. Within the LSU and SSU datasets, we merged pre-OTUs that had been assigned the same taxonomies, thus generating a final set of OTUs for each dataset. Finally, we removed the OTU identified as *Homo sapiens* from both datasets prior to analysis. As expected, since the leeches were collected with bare hands and might have in some cases been feeding on the rangers themselves, human DNA was obtained from the majority of samples in both datasets.

Our final OTUs are intended to be interpreted as species-level groups, even though some could not be assigned taxonomic names to species level. We therefore refer to our final OTUs as species throughout the main text. After excluding humans, the final LSU and SSU datasets comprised 18,502,593 and 84,951,011 reads respectively. These reads were assigned to a total of 72 species across 740 replicates and 127 patrol areas in the SSU dataset, and 59 species across 653 replicates and 126 patrol areas in the LSU dataset. We attached IUCN data for individual species by using the R package `rredlist` v0.6.0 [25] to search for scientific names assigned by PROTAX (or synonyms where we were aware of nomenclature changes). For mammals, we used the PanTHERIA database [26] to obtain data on adult body mass for each species; where species-level information was not available, we used the median adult body mass from the database for the lowest taxonomic group possible.

3 Supplementary Methods: Site-occupancy Modelling

Overview. We used hierarchical multispecies site-occupancy models to analyze our data, using parameter-expanded data augmentation [27, 28], an extension of the single-season occupancy model in [29]. We estimated separate models for the LSU and SSU

data.

These models assume that the $n_{LSU} = 59$ and $n_{SSU} = 72$ species observed in each dataset are, respectively, subsets of larger communities of size N_{LSU} and N_{SSU} species that are present in the vicinity of Ailaoshan and vulnerable to capture (e.g. fed on by leeches and amplified by the LSU and SSU primers). Although N_{LSU} and N_{SSU} are unknown, these communities can be modelled by embedding them in a larger ‘supercommunity’ of fixed size M . We wanted to choose a value of M that was as small as possible to minimize computational effort, but large enough that it did not materially constrain model estimates. We therefore estimated models with values of M ranging from 100 to 474 (the latter being the total species richness for mammals, birds, non-avian reptiles and amphibians in the 1984–85 survey of Ailaoshan [30], which might be regarded as a reasonable upper bound on true species richness). Estimates of N_{LSU} and N_{SSU} were similar for $M = 150$, and we chose to set $M = 200$ for our final models.

For each species in the supercommunity, our models explicitly capture (i) a ‘community process’ governing whether the species is in the Ailaoshan community or not; (ii) an ‘ecological process’ governing the presence or absence of the species in each patrol area, given that it is in the community; and (iii) an ‘observation process’ governing whether we detect the species’ DNA in each of our replicate samples, given that it is present in the patrol area. The community-, ecological- and observation processes for individual species are linked by imposing community-level parameters and priors as described here.

In addition to the detailed model description provided here, the data and code to produce our model results are available on GitHub at [31].

Community process. Each species i was assumed to be either a member of the Ailaoshan community or not. We denote this unobserved state with w_i , which was assumed to be a Bernoulli random variable governed by the community membership parameter Ω_{g_i} , i.e. the probability that species i was in the Ailaoshan community:

$$w_i \sim \text{Bernoulli}(\Omega_{g_i}): \quad (1)$$

For the community process, we separated the species into two natural groupings – homeothermic mammals and birds, and poikilothermic amphibians and squamates – and allowed them to have different probabilities of being in the Ailaoshan community. This is denoted by the subscript on the Ω_{g_i} parameter, in which g_i represents which of these two groupings species i belongs to. This approach reflected our expectation that these groupings would differ systematically in their community probabilities, and we employed the same grouping for parameters governing the ecological and detection processes (see *Community model* below for further discussion). We assigned unobserved species to these two groups such that the assumed size of each group in the supercommunity varied linearly with M between the observed values from the LSU dataset in our study (i.e. 36 mammals + birds, and 23 amphibians + squamates) when $M = n_{LSU} = 59$, and the observed values in the 1984–85 survey of Ailaoshan (i.e. 409 mammals + birds, and 65 amphibians + squamates [30]) when $M = 474$, which was the total observed richness in the 1984–85 survey. (We used the LSU data to anchor the group sizes for both datasets in our analysis so that the assumed supercommunity for any M was the same for both datasets.)

Ecological process. Each species i was assumed to be either present or absent in each patrol area j . We used z_{ij} to denote this unobserved ecological state, with values of 1 and 0 corresponding to presence and absence respectively. We assumed that the z_{ij} are constant across all replicates taken from patrol area j – sometimes referred to as the ‘closure’ assumption – consistent with all the leech samples for any patrol area

being collected at essentially the same point in time. Any species present were assumed to be members of the Ailaoshan community (i.e. $w_i = 1$), so we modelled z_{ij} as a Bernoulli random variable governed by both w_i and an occupancy parameter π_{ij} , i.e. the probability that a species i in the community was present in patrol area j :

$$z_{ij} | w_i \sim \text{Bernoulli}(w_i \pi_{ij}); \quad (2)$$

We allowed the occupancy probability π_{ij} to vary among species as well as among patrol areas, to capture e.g. preferences of different species for particular habitat types. In particular, we modelled π_{ij} as a function of environmental covariates that varied over the patrol areas, scaled by species-specific coefficients:

$$\text{logit}(\pi_{ij}) = \alpha_0 + \alpha_1 \text{elevation}_j + \alpha_2 \text{TPI}_j + \alpha_3 \text{road}_j + \alpha_4 \text{stream}_j + \alpha_5 \text{reserve}_j \quad (3)$$

where elevation_j , TPI_j , road_j , stream_j and reserve_j are, respectively, the median values of elevation, topographic position index, distance to nearest road, distance to nearest stream, and the distance from centroid to nature reserve boundary for patrol area j , and the α_i are the usual logit-scale slope coefficients. All occupancy covariates were normalized to a mean of 0 and a standard deviation of 1 prior to modelling.

We began by estimating the full model in (3), but ultimately reduced the set of occupancy covariates to elevation + reserve for the LSU dataset, and elevation for the SSU dataset. See *Model selection* below for details.

Observation process. Although we cannot directly observe the true ecological state z_{ij} , we do know whether we detected DNA from species i in each replicate k from patrol area j . But this is an imperfect proxy for the true ecological state. For replicate k from patrol area j , we assumed that we detected DNA from species i with probability p_{ijk} when i was truly present in patrol area j , and with probability 0 when i was absent:

$$y_{ijk} | z_{ij} \sim \text{Bernoulli}(z_{ij} p_{ijk}); \quad (4)$$

where the y_{ijk} are the observed data (i.e. detection or non-detection of species i 's DNA in each replicate). Our model therefore assumes that false positives do not occur, i.e. that we never falsely detect species i 's DNA through lab contamination or through incorrectly assigned sequence reads. On the other hand, since p_{ijk} may be less than one, it allows for the possibility of false negatives, i.e. that we failed to detect species i 's DNA when species i was actually present. Although false positives probably do occur, we focused mainly on lab procedures (e.g. use of negative controls) and the taxonomic assignment pipeline (e.g. use of DADA2 [32] to filter out OTUs not observed in 2 technical replicates) to address these, and we expect false negatives to far outstrip false positives in our final datasets.

We modelled the conditional detection probability p_{ijk} as a function of the conditional detection probability for species i per 100 leeches, r_i , and the number of leeches in the replicate, leeches_{jk} :

$$p_{ijk} = 1 - (1 - r_i)^{\text{leeches}_{jk}=100} \quad (5)$$

$$\text{logit}(r_i) = \alpha_0 + \alpha_1 \text{leeches}_{jk} \quad (6)$$

We allowed r_i (and its logit-scale equivalent, α_0) to vary among species, to capture e.g. variation in leech feeding preferences for different taxa. We used $\text{leeches}_{jk}=100$ rather than leeches_{jk} to avoid computational problems arising from rounding that prevented fitting the model.

Community model. Equations (1) through (6) define a site-occupancy model for each species i . We united these species-specific models with community models for both ecological and observation processes, by assuming that the species-level and parameters come from community-level distributions:

$$m_i \sim N(\mu_m; \sigma_m^2) \quad m = 1, 2, 3, 4, 5 \quad (7)$$

$$(o_i; \phi_i) \sim MVN([o_{g_i}; o_{\phi_i}]; \begin{matrix} \sigma_{o_{g_i}}^2 & \sigma_{o_{g_i}} \sigma_{\phi_i} \\ \sigma_{\phi_i} \sigma_{o_{g_i}} & \sigma_{\phi_i}^2 \end{matrix}) \quad (8)$$

where $N(\cdot)$ and $MVN(\cdot)$ denote normal and multivariate normal distributions respectively. These distributions were characterized by community-level hyperparameters and σ , with separate distributions for each parameter as denoted by the first subscript. We used a multivariate normal prior for $(o_i; \phi_i)$ to allow non-zero covariance between species' occupancy and detection probabilities, as we might expect if, for example, variation in abundance affects both probabilities [27].

These community models allow rare species effectively to borrow information from more common ones, producing a better overall ensemble of parameter estimates [27, 33, 34]. As for the community process described above, we separated the species into two groups – homeothermic mammals and birds, and poikilothermic amphibians and squamates – and allowed them to have different community distributions. This is denoted by the subscripts on the μ and σ community hyperparameters for the occupancy and detection intercepts, in which g_i represents which of these two groupings species i belongs to. This approach reflected our expectation that these groupings would differ systematically in occupancy probabilities (e.g. due to different habitat preferences) and in detection probabilities (e.g. due to different encounter rates with leeches, or leech feeding preferences).

Missing data. Incompletely labelled data points (i.e. sequence data without records of which patrol areas they came from) were retained in the model by including these data points without accompanying environmental covariates. Since the identity of the collecting ranger was known and could be used to identify replicates that came from the same unknown location, this allowed these data to contribute to both detection and occupancy estimates. At the same time, we generated occupancy estimates for patrol areas without accompanying data by augmenting the data matrix with rows of missing values and including their environmental covariates.

Choice of priors. For the Ω_{g_i} parameters in (1), our initial exploration with broad priors (e.g. uniform [0,1]) and different values of M revealed that N_{LSU} and N_{SSU} were likely to be in the order of 100 to 200 species. We thereafter focused on models estimated with $M = 100, 150$ and 200 . To facilitate comparisons between these different values of M , we switched from using uniform [0,1] priors on Ω_{g_i} to using a beta($5, b$) distribution where $b = 0.6$ when $M = 100$, $b = 3.3$ when $M = 150$, and $b = 6.1$ when $M = 200$. This choice of distributions had the effect of keeping the expected species richness at around 90 species for all three values of M without constraining species richness unduly.

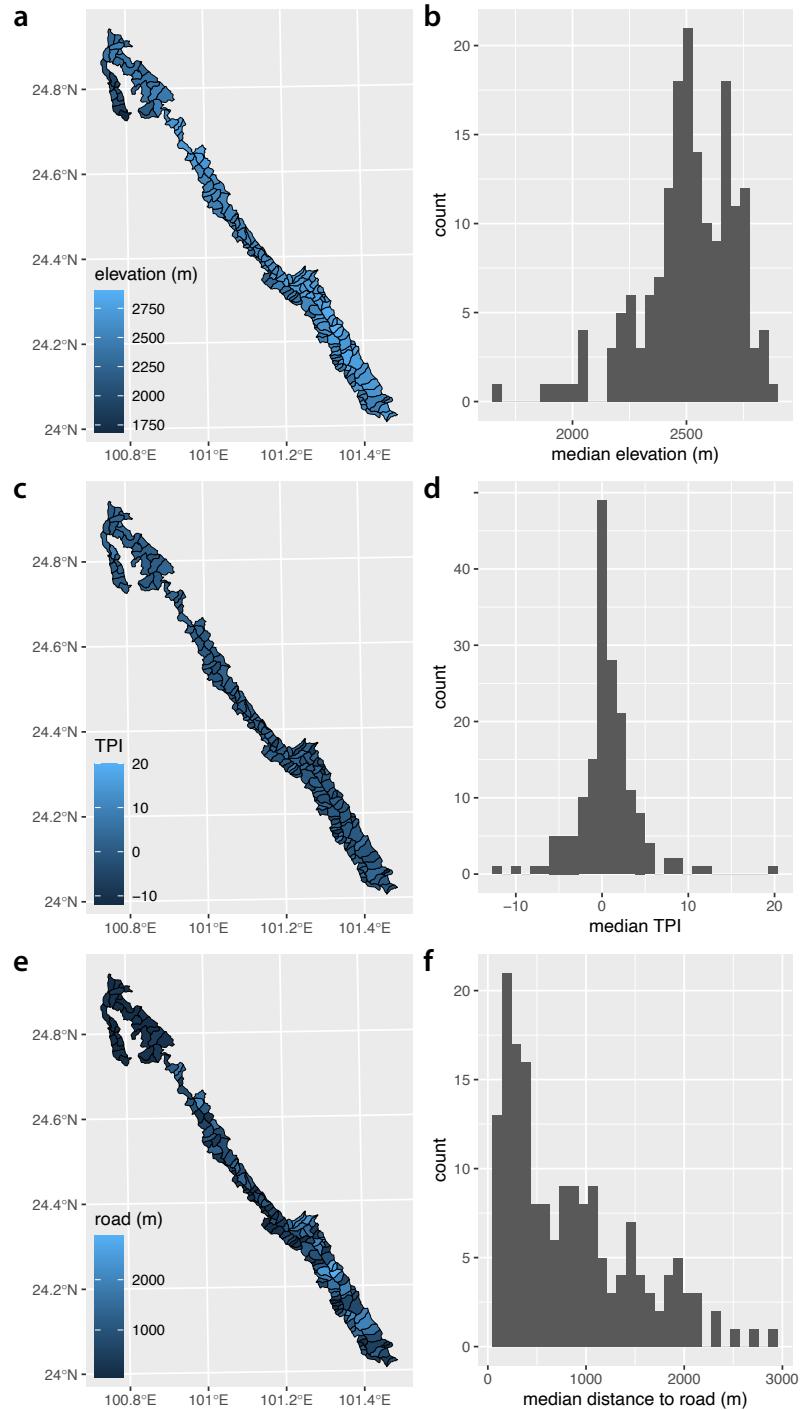
For the μ and σ hyperparameters in (7) and (8), our intention was to use priors that would be uninformative on the probability scale. We chose the t -distribution with $\nu = 1.566267$ and $\sigma = 7.763179$ proposed in [28] for each of the μ_{g_i} and σ_{g_i} ($b = 1, \dots, 5$) hyperparameters; the half-Cauchy $\sigma = 1$ distribution proposed by Gelman [35] for each of the μ_{ϕ_i} , σ_{ϕ_i} and σ_b ($b = 1, \dots, 5$) hyperparameters; and a uniform [-1,1] distribution for ϕ_i .

Model selection. Our final models, as reported in the main text of this paper, used a reduced set of occupancy covariates: elevation + reserve for the LSU dataset, and ele-

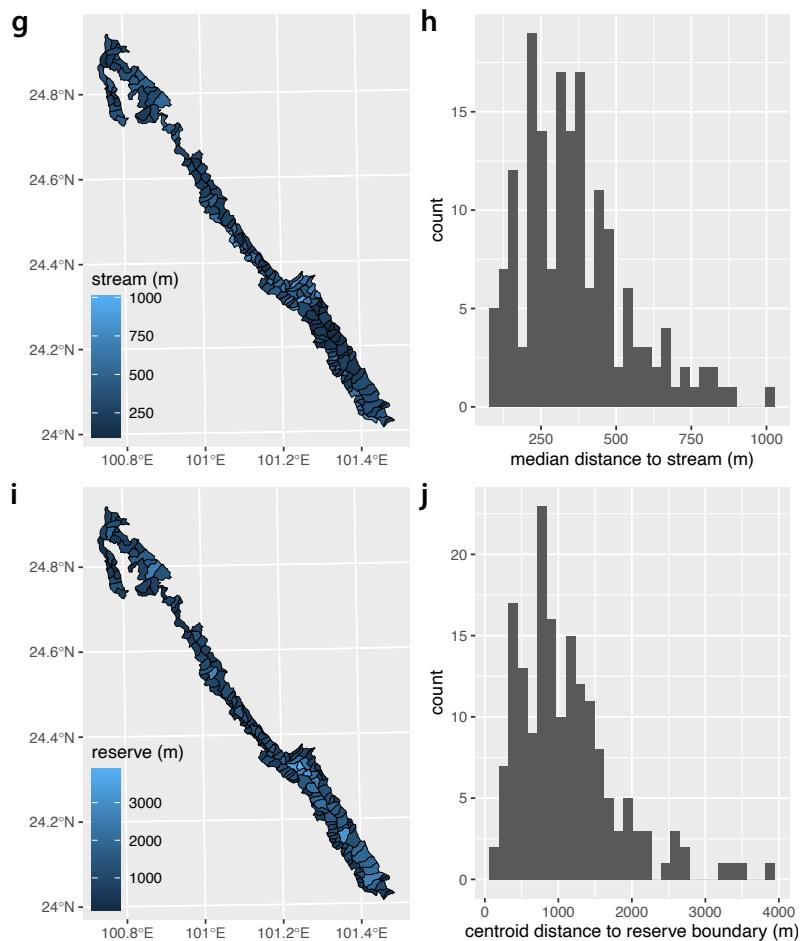
vation for the SSU dataset. To arrive at these model selections, we began by estimating the full model in (3), and examined the posterior distributions for the slope parameters. We retained in our final model those covariates for which the 95% Bayesian confidence interval excluded zero.

Model estimation. We estimated our models using a Bayesian framework with JAGS v4.3.0 [36] in R v3.5.1 [37] via `rjags` v4.8 [38] and `jagsUI` v.1.5.1 [39]. We ran 5 Markov chains of 100,000 generations, including burn-in of 50,000. We retained all rounds (i.e. without thinning) for the posterior sample, except for where we needed to save the Z matrix for beta diversity or cluster occupancy calculations; memory limitations prevented us from retaining all posterior samples for the Z matrix, and we thinned tenfold in order to make these calculations feasible. We assessed convergence and MCMC mixing by inspecting trace plots, and confirmed convergence by ensuring that the \hat{R} statistics were close to 1 [40, 41].

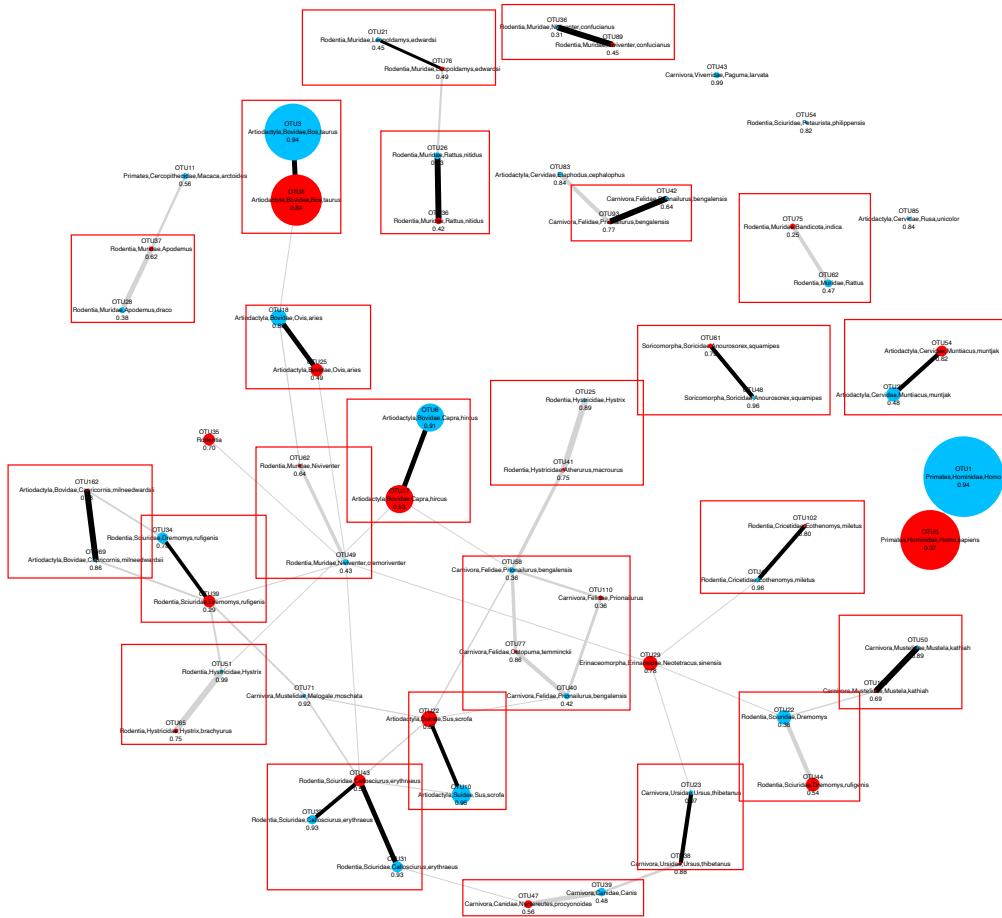
4 Supplementary Figures



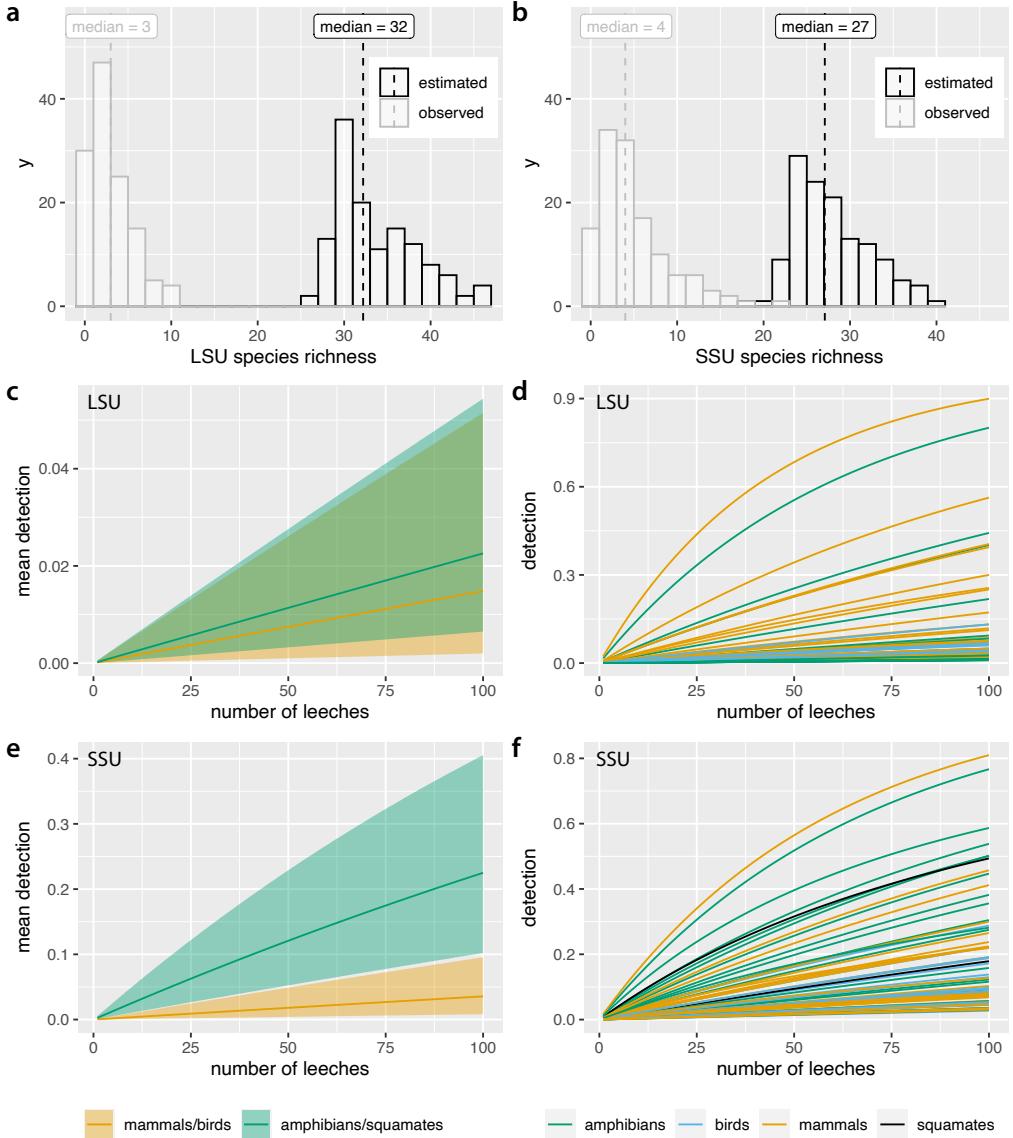
Supplementary Figure 1: Environmental covariates. Maps and histograms for environmental covariates used in occupancy modelling. (a,b) Median elevation. (c,d) Median topographic position index (TPI). (e,f) Median distance to nearest road.



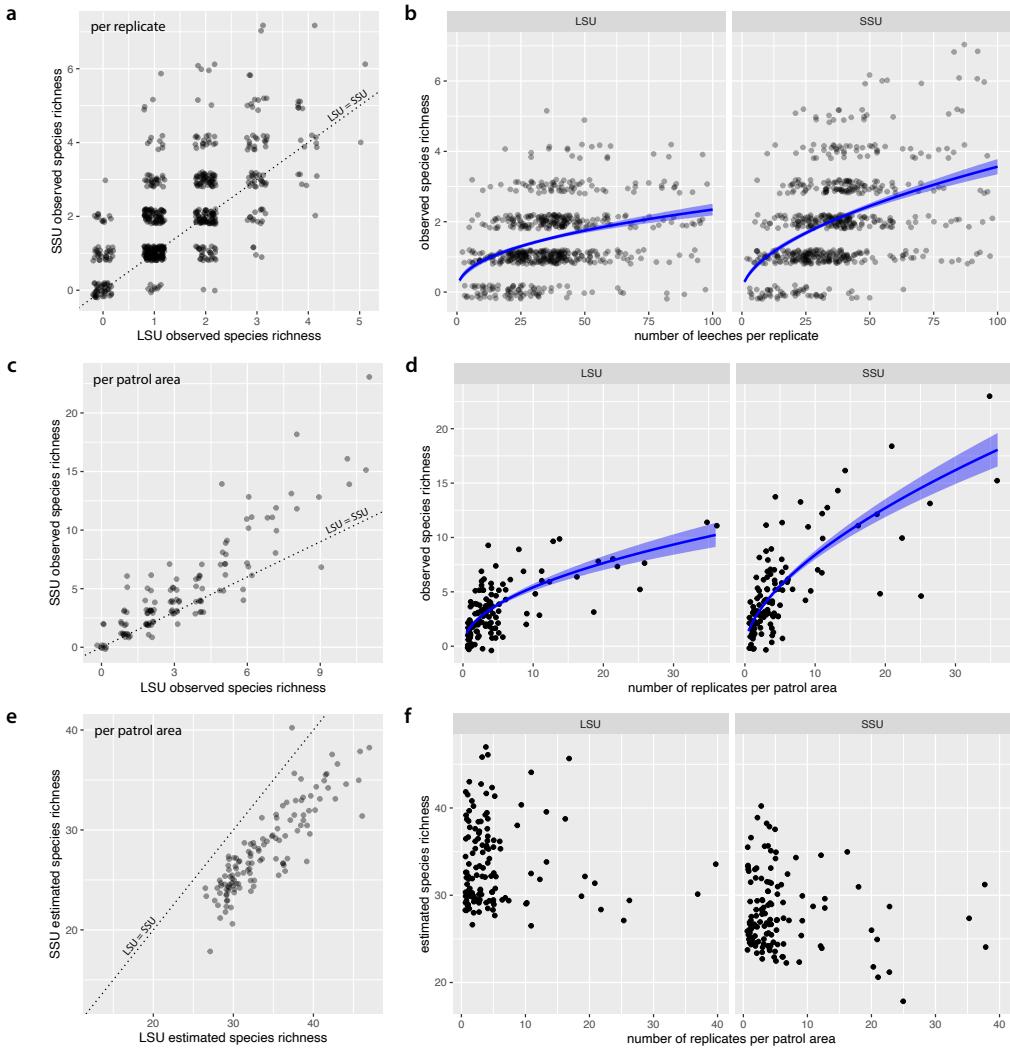
Supplementary Figure 1: Environmental covariates (continued). Maps and histograms for environmental covariates used in occupancy modelling. (g,h) Median distance to nearest stream. (i,j) Distance from patrol area centroid to nearest reserve edge.



Supplementary Figure 2: Pre-OTU correlations between datasets. Bipartite network visualization of pairwise Spearman correlations between mammal LSU and SSU pre-OTUs across lab replicates. Blue and red nodes represent pre-OTUs from the LSU and SSU datasets respectively. The size of each node is proportional to the square-root transformed occupancy of the pre-OTU calculated across lab replicates (i.e. the fraction of replicates in which the pre-OTU was detected). Each node is labelled with the lowest taxonomic assignment that was not missing or unknown, as well as the PROTAX probability for that assignment. For every pair of LSU and SSU pre-OTUs, we calculated the Spearman correlation of read counts across lab replicates. We discarded any correlations that were < 0.1 , or that were not significant at $\alpha = 0.5$ after false discovery rate correction. We drew a bipartite graph using the package `iGraph` [42] with the remaining correlations as edge weights connecting nodes representing the pre-OTUs. Thicker edges thus indicate higher correlation coefficients. Edges are shown in black where they join nodes with the same lowest taxonomic assignment, and are otherwise shown in grey. Red boxes show manually assigned groupings of pre-OTUs that were deemed to be the same taxon. For example, at the bottom of the figure, pre-OTU38 (SSU) and pre-OTU23 (LSU) were both assigned to the Asiatic black bear, *Ursus thibetanus*, and the thick line indicates that these OTUs were found in (nearly) the same subset of replicates, as expected if the two OTUs were amplified from the same bloodmeals and thus from the same individual mammals. Also at the bottom of the figure, pre-OTU47 (SSU) was assigned to Canidae, *Nyctereutes procyonoides*, but pre-OTU39 (LSU) was assigned to Canidae, *Canis*. Given that these OTUs were also found in nearly the same subset of replicates, we conclude that pre-OTU39 is also *N. procyonoides*.

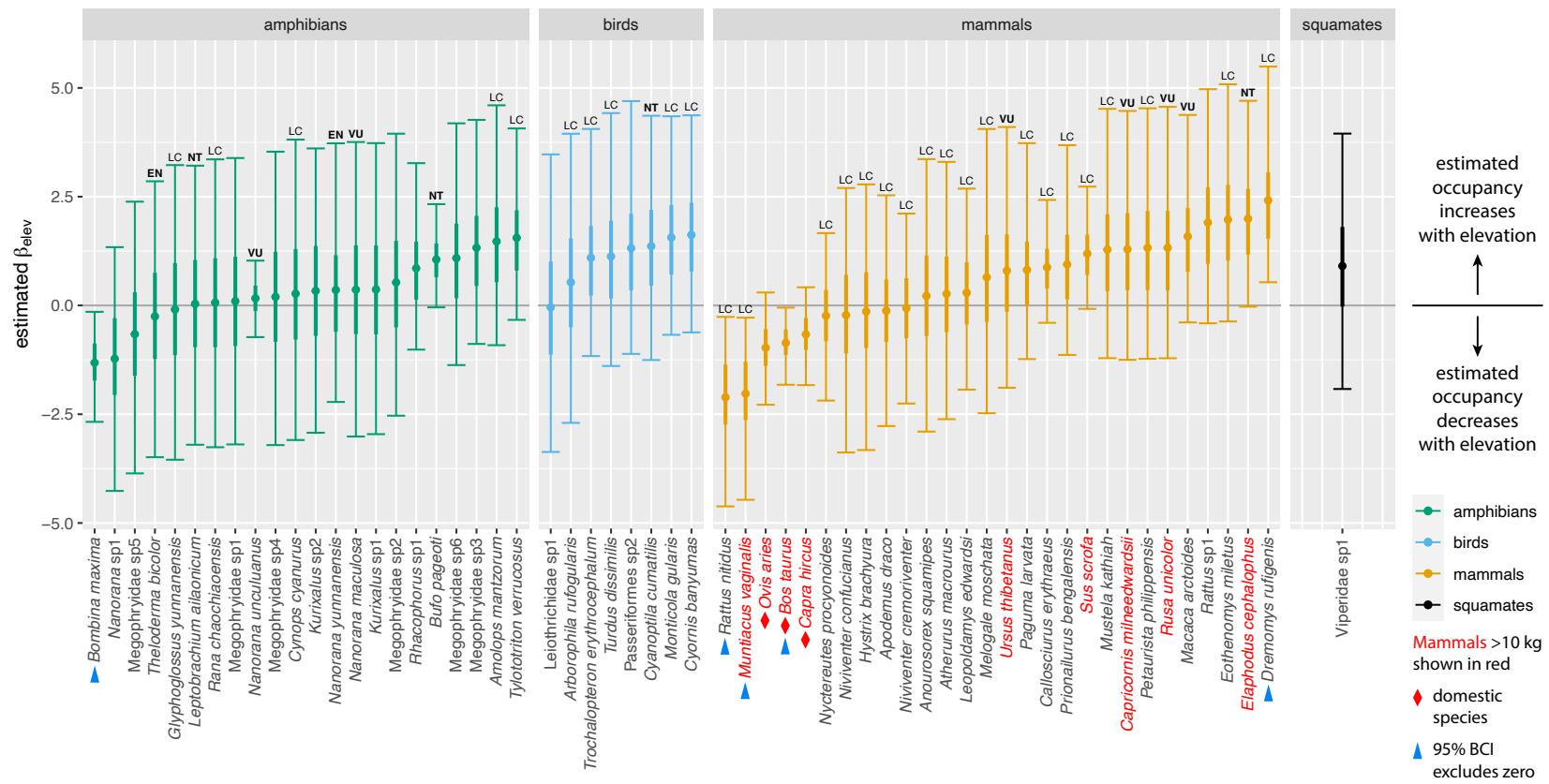


Supplementary Figure 3: Species richness and detection estimates. Histograms of observed and estimated species richness per patrol area in (a) the LSU and (b) the SSU datasets respectively. Dashed lines in panels (a) and (b) show median values. (c) Community mean detection estimates and (d) detection estimates for each species as a function of number of leeches per replicate in the LSU dataset. (e) Community mean detection estimates and (f) detection estimates for each species as a function of number of leeches per replicate in the SSU dataset. Lines in panels (c) through (f) show posterior means. Shaded areas in panels (c) and (e) show 95% Bayesian confidence intervals from models based on $n = 893$ replicate samples.



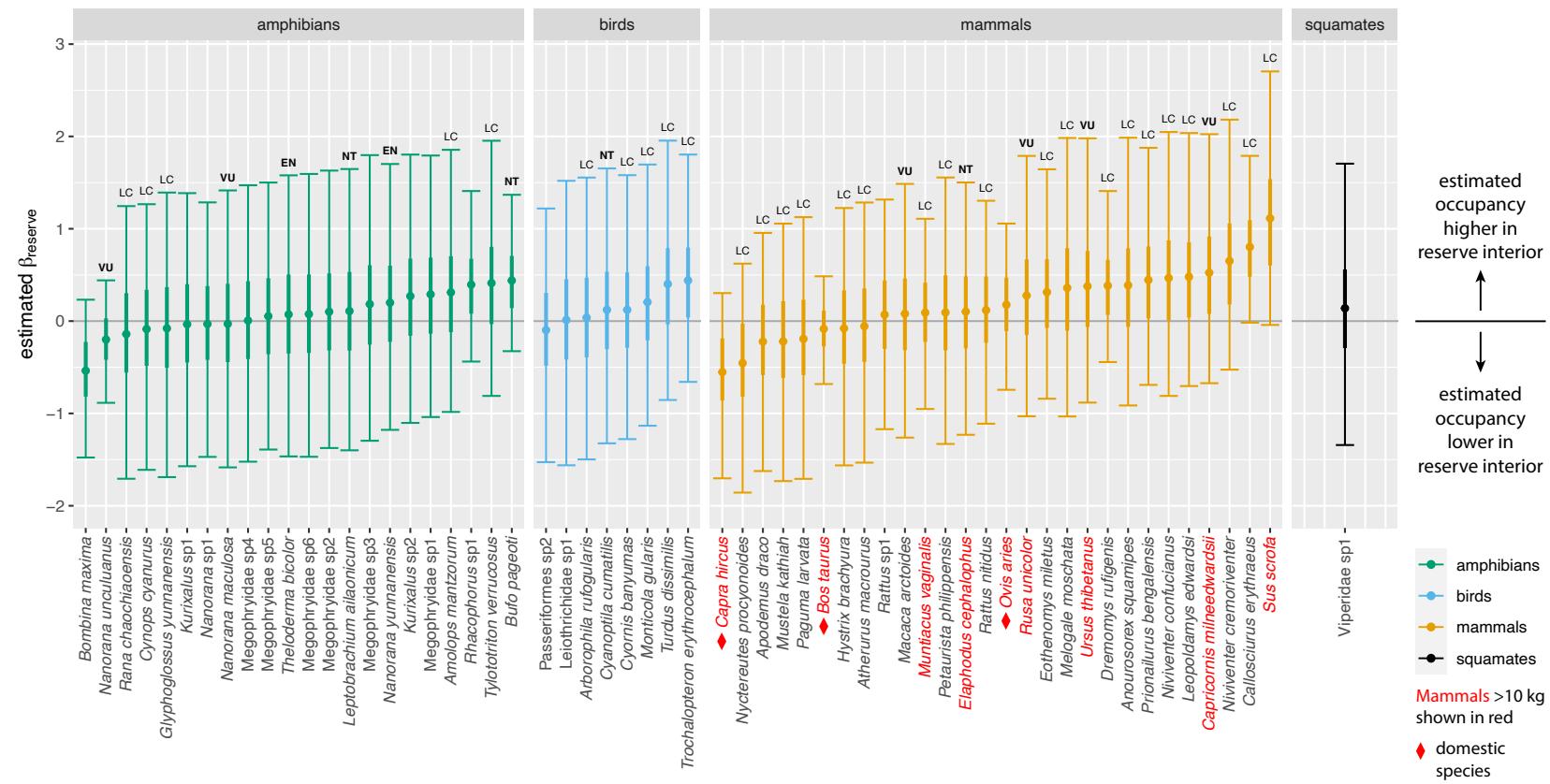
Supplementary Figure 4: Species richness correlations and relationships to sampling intensity. (a) Observed species richness per replicate was positively correlated between LSU and SSU datasets (Pearson's $r = 0.65$; $t_{616} = 21.2$, one-tailed $p < 0.001$). (b) More species were detected in replicates with more leeches. Blue curves show predicted values from Poisson GLMs of species richness against log-transformed number of leeches per replicate (LSU: slope z ratio = 6.9, one-tailed $p < 0.001$; SSU: slope z ratio = 10.0, one-tailed $p < 0.001$); shaded areas show standard error. (c) Observed species richness per patrol area was positively correlated between LSU and SSU datasets (Pearson's $r = 0.89$; $t_{120} = 20.8$, one-tailed $p < 0.001$). (d) More species were detected in patrol areas with more replicates. Blue curves show predicted values from Poisson GLMs of species richness against log-transformed number of replicates per patrol area (LSU: slope z ratio = 10.2, one-tailed $p < 0.001$; SSU: z ratio = 14.9, one-tailed $p < 0.001$); shaded areas show standard error. (e) Estimated species richness per patrol area was generally higher in the LSU dataset than the SSU dataset, and positively correlated between the two datasets (Pearson's $r = 0.87$; $t_{120} = 19.5$, one-tailed $p < 0.001$). (f) In contrast to observed species richness, estimated species richness did not increase with number of replicates per patrol area, as the occupancy model corrects for variation in sampling effort. Slope coefficients for least-squares regressions of estimated species richness against log-transformed number of replicates per patrol area were non-significant (LSU: $F_{1,124} = 0.006$, $p = 0.94$; SSU: $F_{1,125} = 1.5$, $p = 0.22$). Points in all plots are jittered to allow overlapping points to be visualized. p -values are not adjusted for multiple comparisons.

LSU dataset

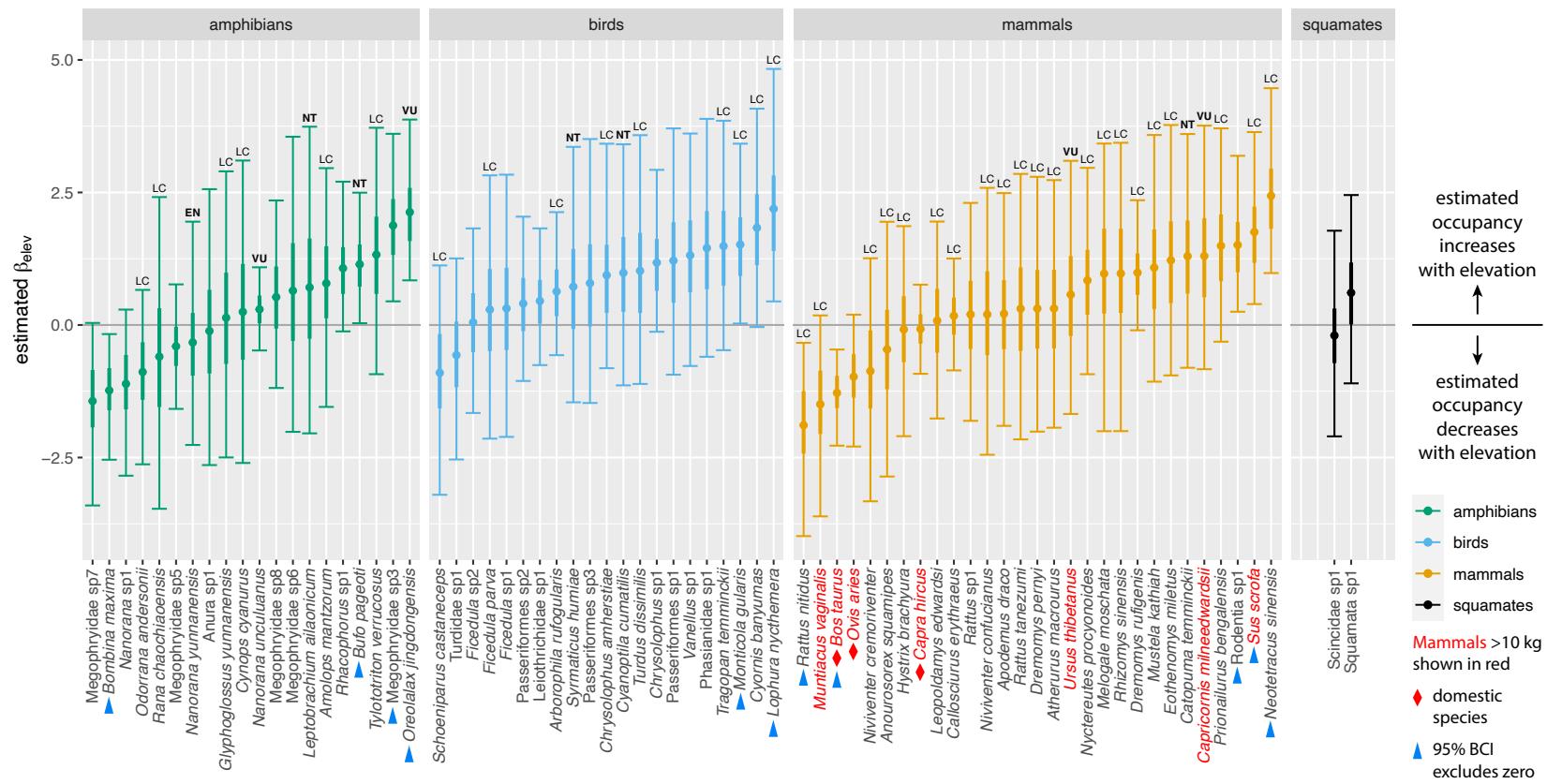


Supplementary Figure 5: LSU occupancy slope estimates (elevation) by species. Estimated occupancy slope coefficients on elevation from the LSU model. For each species, plot shows posterior mean (dot), interquartile range (thick line) and 95% Bayesian confidence interval (BCI; thin line with crossbars) from model based on $n = 893$ replicate samples. Slope coefficients are shown on the logit scale, so positive coefficients correspond to occupancy increasing with elevation. Within taxonomic groups, species are ordered by slope coefficient. Blue triangles mark species whose 95% BCI excludes zero. Annotations above bars denote IUCN categories: LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered. Categories NT and above are shown in bold. Taxa without annotations have not been assigned a category by the IUCN. Species names for mammals over 10 kg adult body mass are shown in red. Domestic species are denoted with red diamonds.

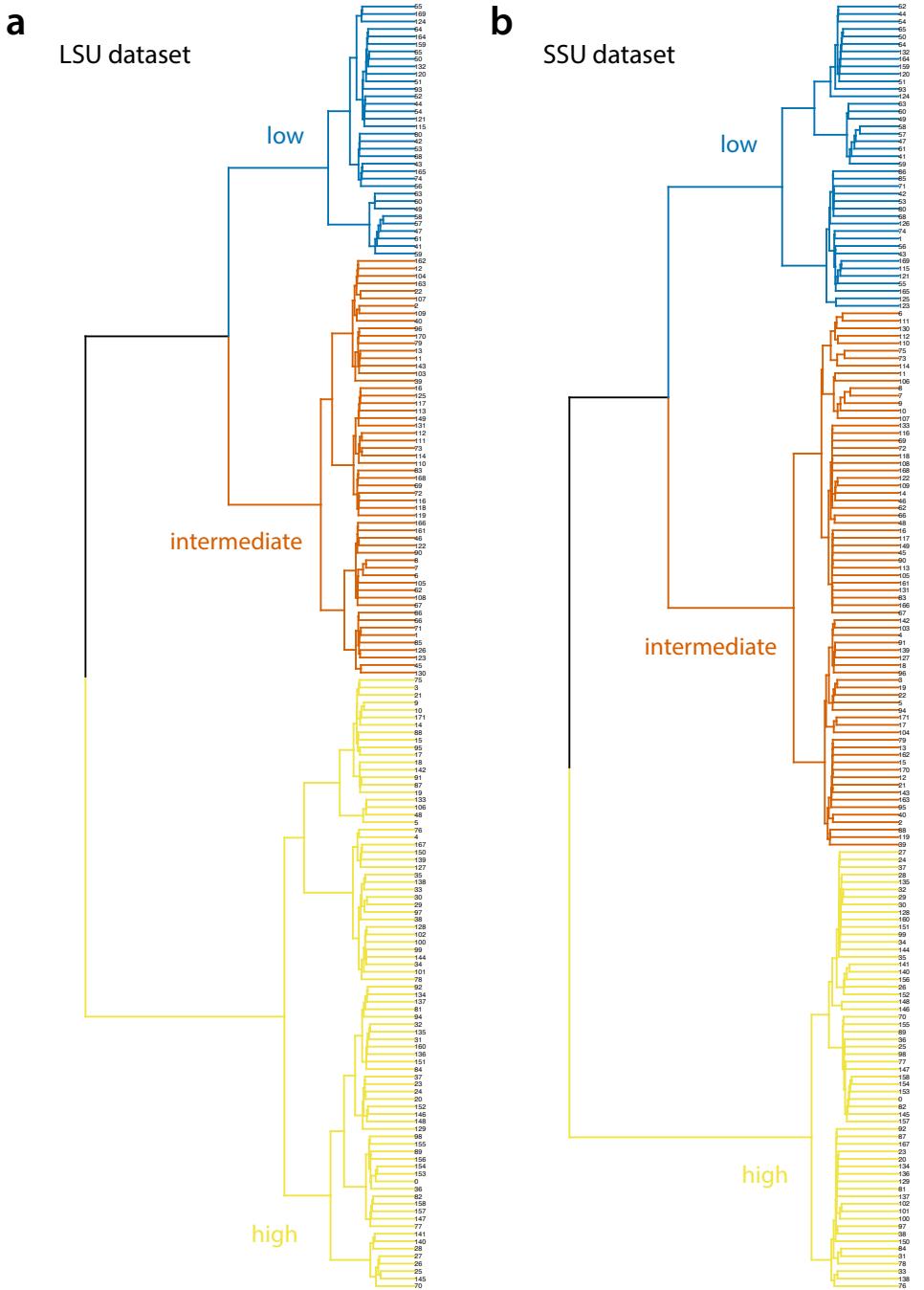
LSU dataset



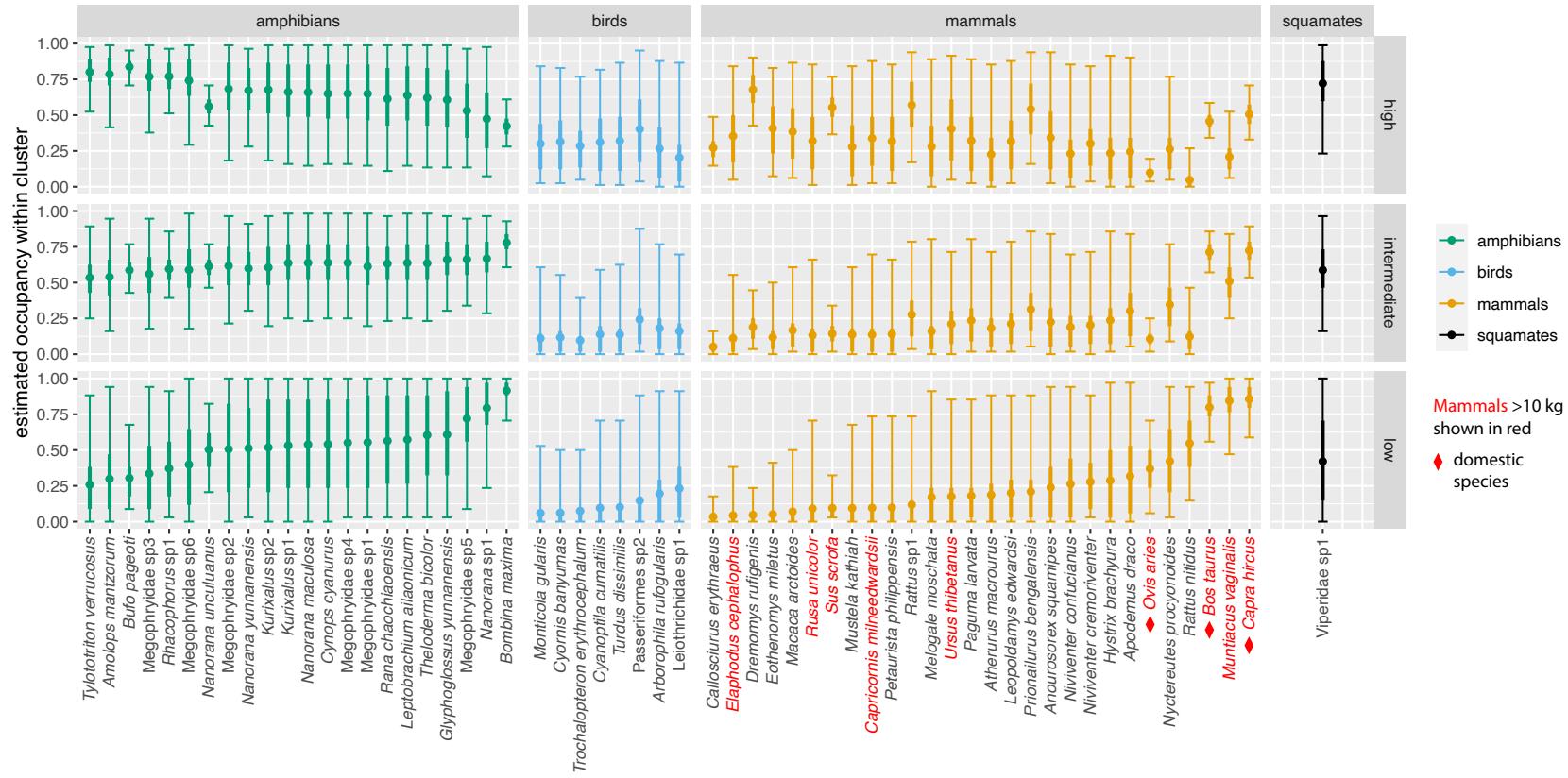
Supplementary Figure 6: LSU occupancy slope estimates (reserve edge distance) by species. Estimated occupancy slope coefficients on distance to reserve edge from the LSU model. For each species, plot shows posterior mean (dot), interquartile range (thick line) and 95% Bayesian confidence interval (BCI; thin line with crossbars) from model based on $n = 893$ replicate samples. Slope coefficients are shown on the logit scale, so positive coefficients correspond to occupancy increasing with distance to reserve edge. Within taxonomic groups, species are ordered by slope coefficient. No species had a 95% BCI that excluded zero. Annotations above bars denote IUCN categories: LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered. Categories NT and above are shown in bold. Taxa without annotations have not been assigned a category by the IUCN. Species names for mammals over 10 kg adult body mass are shown in red. Domestic species are denoted with red diamonds.

SSU dataset

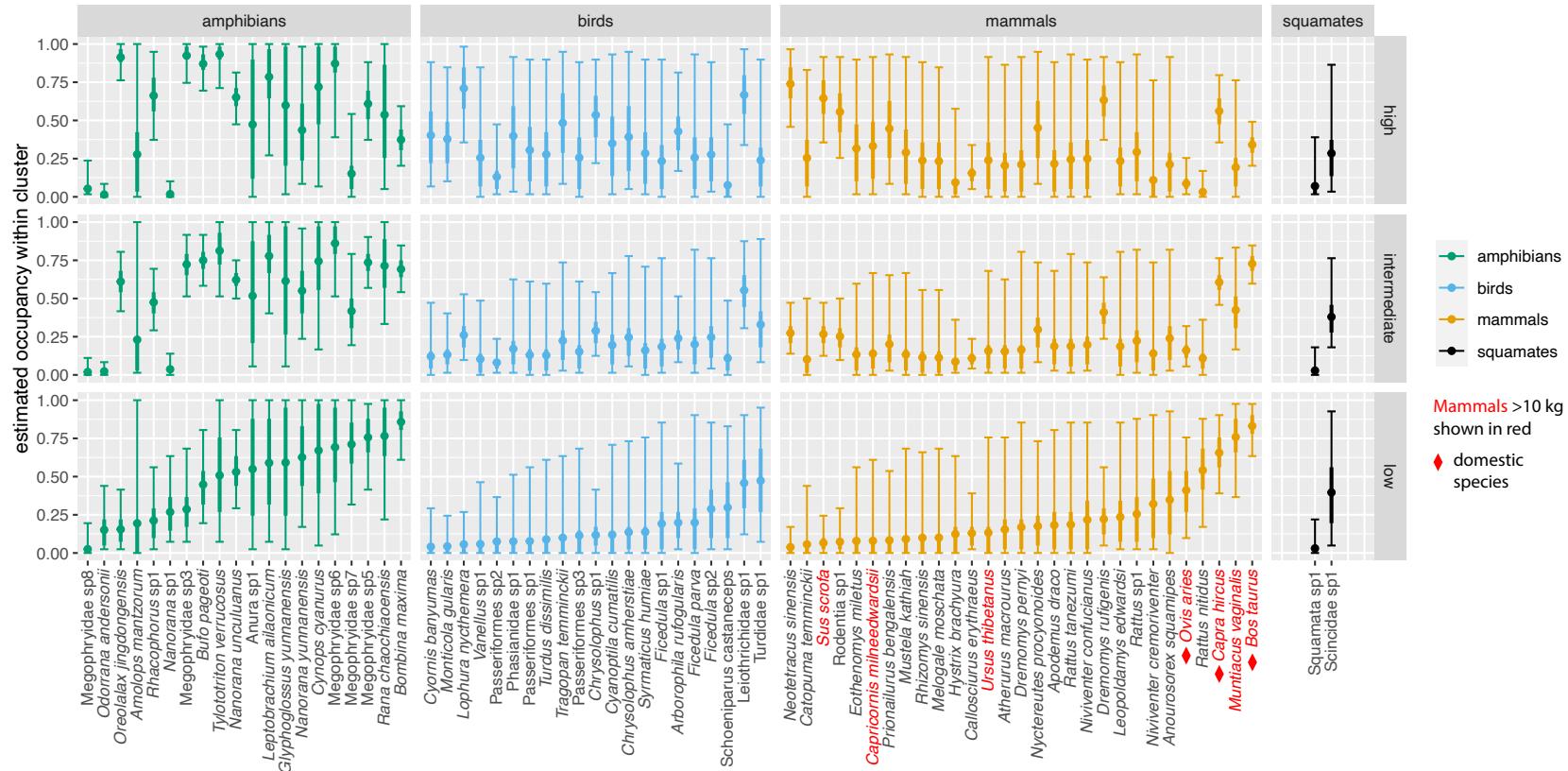
Supplementary Figure 7: SSU occupancy slope estimates (elevation) by species. Estimated occupancy slope coefficients on elevation from the SSU model. For each species, plot shows posterior mean (dot), interquartile range (thick line) and 95% Bayesian confidence interval (BCI; thin line with crossbars) from model based on $n = 893$ replicate samples. Slope coefficients are shown on the logit scale, so positive coefficients correspond to occupancy increasing with elevation. Within taxonomic groups, species are ordered by slope coefficient. Blue triangles mark species whose 95% BCI excludes zero. Annotations above bars denote IUCN categories: LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered. Categories NT and above are shown in bold. Taxa without annotations have not been assigned a category by the IUCN. Species names for mammals over 10 kg adult body mass are shown in red. Domestic species are denoted with red diamonds.



Supplementary Figure 8: Clustering of sites by community composition. Dendrogram of patrol areas in (a) the LSU dataset and (b) the SSU dataset based on posterior mean Jaccard distances clustered using Ward's criterion. Splitting the patrol areas into three groups, as shown here, produces clusters containing low-, intermediate- and high-elevation sites (see also Fig. 5 in the main text). Each branch represents a single patrol area, labelled with the same patrol area IDs used to identify sites in Supplementary Data 6.

LSU dataset

Supplementary Figure 9: LSU occupancy estimates by species. Estimated occupancy in high-, intermediate- and low-elevation patrol areas for species in the LSU dataset. For each species, plot shows posterior mean (dot), interquartile range (thick line) and 95% Bayesian confidence interval (BCI; thin line with crossbars) from model based on $n = 893$ replicate samples. Patrol areas were divided into high-, intermediate- and low-elevation by clustering based on Jaccard distances as shown in Fig. 5a,c and Supplementary Fig. 8a. Within taxonomic groups, species are ordered by occupancy in low-elevation sites. Species names for mammals over 10 kg adult body mass are shown in red. Domestic species are denoted with red diamonds.

SSU dataset

Supplementary Figure 10: SSU occupancy estimates by species. Estimated occupancy in high-, intermediate- and low-elevation patrol areas for species in the SSU dataset. For each species, plot shows posterior mean (dot), interquartile range (thick line) and 95% Bayesian confidence interval (BCI; thin line with crossbars) from model based on $n = 893$ replicate samples. Patrol areas were divided into high-, intermediate- and low-elevation by clustering based on Jaccard distances as shown in Fig. 5b,d and Supplementary Fig. 8b. Within taxonomic groups, species are ordered by occupancy in low-elevation sites. Species names for mammals over 10 kg adult body mass are shown in red. Domestic species are denoted with red diamonds.

5 Supplementary Note: Chinese Language Main Text

è , - ‡ H, Å + c ‡ , X• M Øœ .. ° 1 Öå ÈpnŒ , ii .. "

‡ Wè vYè ÷ ö¥å ñ‡H

1. X•

úEê6Ý¤: /ž° i 7' Y¤, 8Åª ½ 6 , UÄOvÝ¤H‡Í 6
 / * ¾~ : tā³ Ù ¾~ i - !) (, W8B@² - „ DNAÜL† ! ' Ä! „ Ö ù` O677s¹¹¹ „ MŽ - y• Wè' W „ Äbqý¶é6Ý¤: Ü L† * h@ „ i 7' , ÄO (, v- åY¤: « 172* á; : , 1163M¤-X(áÆC - ÇÆ†; q30,468ê, W, (Ü› , W, K• pn- i tš-O86* " i i í i \$ { ús{ { E, L{ „ i „ i i „ n ! < • Øœ>: (¤=4s i i OI | • @wÖ, GØåÈ Y¤: 1 Y» „ É• ž ;(i i 4s Y' p' < i ús" i (, 4• NŠ i èš N,)(fØwÖ ` NÝ¤: - . , O: ô * ¶{ „ i i i ([öS qS)E * i „ i i i (d,) (wØfN, ` NÝ¤: 1 , O: ô , v „ Øœ>: úZ• t, WeDNAŒ i i „ n! < , €/i á: ÄOê6Ý¤: ü i „ Y¤H‡ ú * ö¥„ ØH, i i „ Z«' ¥x á, v i á « i „ Øœ á i á (ZÄOY¤: ü i „ i 7' , Y¤H‡ i D ØÝ¤: üz° h i 7' i „ ! .

2. M

2010t i 7' I | ~r y Ç† 2011-2020t , 1å i 7' i [43] v- 1åi 11%È† i 7' , Y¤ • B* Ø2020t Ø • 17%, FØŒ...F4B oO³ eY¤: üB , y¶l i E v Öé6Ý¤: v åü B" S(w åh' ø' KôX(o} , sT' åÈ @l sŒ H, i 9n2018t Lé6Ý¤TBN „ ¥J>: h FObi „ 15%Ø « ³ eY¤ : ô Ü* pnöI ¥N1åi 11 „ • B[44, 45, 46] v- - y\ : I | ~r yK vY¤: , bii é(2008t 1öI ` OF Oy bii „ 15% | 143 s¹¹ I [47, 48]

: tž° 1åi 11 - yU: tøS' „ L" > EY... : „ Y> < , (- y• èO: ê6Ý¤: àNí å¾OyE4s µÖ i t; • „ : i 7' Y¤, H: BåÈ i) 6 « { < [49] v úZ e pn, v Øœ>: - y, ê6Ý¤: öI Y; bti — , å åN[50] 6 (- y Wè, ê6Ý¤ : v åh' øùfî [51] , Y¤: / " » „ [49] v sZÙ, Y¤: üSO ¤, qí _@å ô: i „ / i åSÙ, Y¤: / &y HOY¤ ; (v- , i i

6 , UÄOê6Ý¤: „ Hí 6X(è' Ü* /h „ s^ ¾ö¥ÄOúZ: B, Y¤å\ / &Ö-ti „ i 7' œ åóZ NùY¤ : „ i å¤: v Hœ: " * å " [46] i Mêyç(„ ö¥¹ Ö < , ° XMnŒ, — E³ „ ÄO " • eÄO" [46] aØŒ° { < | i 7' Å, ÄO " ÄI ÄO" [46] (Y¤: : B \$v/ E> i å) (e | Ø i €/ „ O¹ Ü LÝ¤: H, ÄO[44, 52, 53, 46] 6 s• H° t i • e, èrŒ/ öå Å, i Y¤S t i „ i 7' Øœ[46] Ü, ö¥¹ Ö_v yÄKÝ¤: („ i i { øKô/ &X(„ Y¤Hœ _àÖÆ+ „ Å

ad (, v- i „ a³ „ i ~ / i / &y) (- fDNA eDNA eù „ i i 7' ÜL' Ä! „ KI v K\: è6Ý¤: Y¤H‡, ö¥å i AE i sè „ i i ús{ { \$ { E, L{ „ Yà / - y „ i { øb4 „ i „ ÁK 1/Ci „ N[54] • (e 1 Övà ÖAKO ÜI Å àdy + ¾å ÄO öÅµ i 7' ÄO" ¾OfØ, z öŒi i

{ .. ♫ vA, (' ô... Aô° åçý è ÅKŒSMÎ .. i í ø, ¶
 ØØ ï .. - i ý „ Yà ÇŒeQa ½ vùeQa ½, H' ÜLKÖ v
 ! " åý 1i È,) Èøs œ- È, , 1 Ô, Øb ùÄOØœ, ž'
 œ H' ÜL%<ŒÁ v ÄO" å/ó¥, —súži í ÅK /vÖö¥¹
 Ø—Ùuž%oí „ å³åÈ üŒÀ± Hi ý/Å• „ i 7' „ Ä
 O" /ØH ž «³V œI á, ï ©ŽS@?V, i í ' ÑUŒi
 Ø, „ Ø' [55, 56, 57]

Ñte• @¢ ø: œ i ðf | €/, ÜeŒ• Ü" (. - i i å(f' Ä
 ! U i 7' ÑKå\ 6 - p èrœô¤d{ ¾ „ , i 6P6†f
 i ý ÑK, zô ô <, Beaudrotl ° [58]) (ø: €/ (15* i & i -Y ø:
 ùO ús{ œ { „ 511* ø = ÜL† • ¾3O8t „ ÑKå\ 6 = i Øi „ ø
 : èr ø(i *Y ø: t Ö†140O320s 111, bř FÙÅ` vpnÆ- ' Y
 ø: „ 1-2% " vvYà; • /à: i M, ø: €/ (i " i ÑK, " (i U¹ b
 X(> P6 - H1/ - pø: „ , fØ : t t Ö! Ä! zô: | „ Y ø: •
 - p, ø: „ , €€/å~ : UM v! 1/dtH ø: QÜ, èr ø •
 š PØ7¹ ùø: ÜLôø E> Ob B³/åμ³/ 7O : ôøå\ & e •
 „ Ø³/ • 1/ (t *ÑKÇ - ø: „ Ñ Á_Œ" 1 Ü7 Å " i „
 - 1 ö: %í „ /qí 4Oí pn, ÇÆ[59, 60] d ø: €/Œ i ðf
 ¹ Øi ý ÇøS' è „ " i i í 7' <, \$ { „ L{ „ i œ,
 { ý «ø: UI O „ , ús{ \$ { œ, L{ (i ðf ÑK- i ý
 «W

c à: , d eDNA€/ (eEø: œ i ðf €/ ¹ b >: útè' „ \
 > [61] i å ö• M(ÑKÇ - èrø¤¾ „ Ø³/ ¾ „ _ 1 åÈpn
 • - i í { Øi l í i ~ („ v- i C(„ /, WiDNA \: ° t „
 { eDNA7, [62] iDNA« • ÜD(Ži í , ÅKŒ i 7' „ ÑKå\ iDNA/
 ÇÇÆà „ i — i @8' Ä k „ , W ŠP «G 11 œå å:
 B „ i „ , i G NÓi I — e · — " i DNA, i 1 Ø[63, 64, 65]
 • @ŠeŠ „ , vå\ ÇiDNAù „ i i í Èv ¾Å „ ôÜL
 å [66, 67, 68, 69, 70, 71] ¢ ø: Èv Ö¹ Ø, pn ÜL Øf [72, 22, 73]
 åÈøsžŒŒ • A „ , Ñå\ [15, 74] iDNA€/ (- „ 9ÜŒ Y

i („ v- Ç(„ WiDNAùMŽ - ý• Wè' W ` Obi | 677s 1 |
 „ Åbqý¶Sé6Y ø: þ1 „ " i ÜL† ! ' Ä!, ÄO å
 å Y ø: úÉŽ 1981t 1984Ø 1985t „ ål tåY ø: ... 86í ús{ 323í
 { 39í „ L{ œ26í \$ { „ i „ i í /ši í U[30] d v° XÜL†
 ! ' „ i å [75, 76, 77]œU* i í „ v[78, 79, 80, 81, 82] å Y ø: ÑteÜL
 „ yúž¢ ø: „ åå\ [83]ÅKO10í ús „ i œ10í { Fåå\ h
 b Å åtY ø: „ 2*å; : ; q 172*å; : àÖu „ i i 7'
 ÜLnåÄO àd í 6: O „ i i 7' „ h@ å Y ø: ... „
 i i í „ ° ¶œí øØ <çú, ž * å ¶

i „ vKÖ†(c „ Y ø: j - f- Ç(iDNA å „ i L' úž, W
 „ iDNA¹ Ø\ : i „ ' Ä! ÑK€/w å à¹ å - H ÇÆ, W „
 ° X „ • èù- ÅbqY ø: : 172*“å; : ” i *å; : i ý i » Ñ
 Q, Çc „ ø-XÜLáÆ Üø ø-Xi å(ècá; öÇÆ, W7, àd i
 ý åøùfn „ , ùt*Y ø: ÜL7, ÇÆ v! „ , WiDNApní å: i
 D> i H, ¹ œ ci í ÅKÇ - S „ > i Ü; • i G4' s*
 ýÅKOžEX(„ i í œG3' ssDi í žE X(ötÅKOvDNA
 <, S *i í (D*O¹ i „ «, W8O@ö 1 ú° G4' åi í
 „ DNAj „ YOi ž cno { _ S G4' G3' „ e• i ý i 7
 ¹ Kô „ , WÁü ÇÆç žœø- „ 7ÅaÓ K• i áo - S „
 i |

B_j ! < ī å(eā³ Ù> G4' ḠG3' , ī ÅKī ~ (, yī - ī •
 (BM¹ ` n! < [29, 27] • t iDNA, K• Øœ å! < ī å" + ī * M¹ ÅK
 O, i ī / & ž X(v _ī å" K—O(DM¹ i Ô¥ÅBO, i ī / &ī ý
 X(M¹ ` n! < , ú, Ý / Ç + O—ī * M¹ X(Di ī „, ‡åÅåi ī
 X(öý « ÅKO, , ‡e" - ī * i ī cX(„ Mn[29, 84] • O—Ù>
 ‡; • • VZí ½7¾j • (³ ¥Ñ, z ÔŒÖÖ ô...ÜLí Ç7 Ü7
 . —, pñMýí " i ī „, ž ¶µ (7, ÇÆM i ī * ø—XÑ>
 * ÅE < ī * < P- >n * 7, ÇÆj • B(ööŒO¹ ÇÆ, , W •
 >n(„, i PŒ< P- [65] ÇÙ*¹ i ī å· —í Ç7, 7,
 , WiDNA, , * ž (Ž i ý· —ô: • Ü, i ī { ø à: i öř å
 S, W, B' ô^ • f 8B i < œ' < ús{ { , L{ œ\$ {
 i " i || " i „ @² àd ø: œ i Òf{ 1 ÖøÖ ,
 WiDNAř å: i Ð> * ôô• „ i ī { ø pñ[85, 69, 70] ö DNA• ī
 å. © i : > ÅEÉ ¾å" + „ i ī [72] } 6iDNA• (Ð> { ø _X(
 @i ī „, ð¾ v>: , W8B, @² DNA(vS...ÝX• ¾4*
 K í 6ý ÇPCri ž ÅKO[1] ī } „, 1 beô Ü• — i ī å Çž ,
 W, ÇÆř e. —v HMà * 8B@², áo ī ÐØiDNA, ÅKH‡ æ 1
 b , WiDNA, Ü y' MN „ i ÅK, öz " ‡ à: (« ÇÆU. K
 M Ü>, Wå Èfì à* M@8B@², ž; ī ý (Ç7: BKÖÑ • Ý» Á
 Ü[65]

(, å\ - i Ç(Ø ī ab €/ metabarcoding [86] eÅKī , W@
 8B, " i ž; { ø v(` n! < eÅOÙ> " i (- ý' W Åbqé6
 Ýø: „ t * Åµ (• - i Ú enšt Ü> øs, - fà
 v " i , Øœ>: Ç, WiDNApñ å· —ô½• " i { ø, !
 v- i t £ø ^ ¾« ø: œ i Òf¹ ÖÅKO, i ī àd Ç, v i
 , Ø° / Ç, WiDNAř å· — " i i ī „ z ô œ- føsáo : i
 Ð> * ař ê6Ýø: H' , ÅO ī . © i Ýø: ..., i VeÈ
 øs^a ½

3. Øœ

3.1 7, ÇÆŒØ ī ab

Åb q ý ¶ S é 6Ýø: b ¶í • v q ž p 1 • Wö 8
 | 125l | 24.9°N 100.8°E 24.0°N 101.5°E s G½; Å6l | ½ wÖØ;
 ī 422Ö3,157s t M4l : 1,000Ö1,860ës wSÖ³ ž wÖØ; [87] þ1Œe
 EP™- „ þ1a, b åÝø: „ ; • « : ší &8ý ö— v \$S: øe (O
 MŽ * 1 „ NwÖœa Ýø: „ L5l | ö... 261* Q, [88] Oi °
 å ...Ç20,000

, v - „ 7, + t 30,468ê, W + 1163Mø—Xž 2016t „ èc 7
 ö9 (t * Ýø: „ 172* á; : ÇÆ e Ü>, W È« + t 893ýí 7
 , wS 1 Ý ÷ Á 1 Öè

i ùl ýí 7, ÜL t DNAÐÖŒ\$ * ž' S ú à G µ , PCri
 ž 16S rRNAúà (MT-RNR2)Œ12S rRNAúà (MT-RNR1) i (‡ - +
 åLSU 8ÖS' šú œSSU 8ÖS šú eð| Ü\$* úà i Ü7Z; •
 / : t • MŒi M® i v • Ü• („ 16Súà ø÷Æ à: Ål „ 16Súà / œ
 i @ („ 12S ^ 16S úà • (ùK• pñÜL t i áo i • (Åpi
 U, pñi E parameter-expanded data augmentation ÜLSUœSSU pñÆ
 „ i ī M¹ ` n! < multispecies site-occupancy models [27, 28] + ÜL Oi
 å' di ī ÅKÇ - § „ G4' ḠG3' i ī t ŠÆ+ i pñ- X(„
 ž !

3.2 三 一 一

i (LSUCESU_p n - ; q Å K O86Í ^ ° { „ ” i i í v
 - LSU 59Í SSU 72Í } 6LSU i / è : ús „ i ¾ i „ F
 / LSUCESU, i ýí žú\$ { { ús{ E, L{ „ DNA SSU i \
 : („ ” i i i ž-Otô „ { i í þ2a 45* i í (\$*
 úà, pn- öAKO v- i tE> Ç(í 7, - „ tš-O, i í
 eEP™þS2 ê(LSU- AKO, i í 14* SSU 27* (Ù@ 86* i
 í - 58* i áÙOI í 4s, { LSU 45* SSU 50* h1Eh2 g!
 < O-ú, ` ‡ útř * úàpn-, M20* i í

i (ÈH, ` n! < - • („ ...S ø=Ä! : M = 200 (LSUpn- „ —
 ú, Åbq, ; i í OI | : 119Í (SSUpn- : 113Í þ2b ¾nM =
 150S tøÑ, Øœ ¾nM = 100 > ùi í OI | , O-S P6

(„ pn- ¶{ „ i i í ` t^ „ Øí eEpñ1 Ù i (ž E
 B- ÅBO, Yø: ...Ù> ¶{ „ i „ >gÅµø; (\$* úàpn- ¶[
 Bos taurus ý/Ñ° ‡ Ø, i í àN(Jp, á; : - ýýAKO ¶{
 qS Capra hircus _ ^ 8A (Ñ K „ á; : «AKO ¶{ oS Ovis
 aries (; 6%, á; : « Ñ° oS, Ñ° AE- (Yø: „ Wé ¶^ž° s
 ž c} óORÓŠÁÍ „ ; • A-: —óOGø»

(„ i AKO, i „ i i í - > « IUCN : x Á ¥Ñ Á, {
 ø h3 (ús „ i - 4* i í ^ ž IUCN qI S š2ÑS Ursus thi-
 betanus - N#S Capricornis milneedwardsii 4• Rusa unicolor Eí >
 4 Macaca arctoides (\$ „ i - ' WØÙ Nanorana yunnanensis Eí r
 ñ® Ù Theloderma bicolor « : Øqi í ±ØÙ Nanorana maculosa
 ØÙ Nanorana unculuanus Eo • þ Oreolax jingdongensis ž q¶
 Ü „ i - „ > i í y+/ \$ { (Åbq • Ü h3EeEpñ1
 • útåYø: (YøÜ i í „ i „

i iDNApn „ i v@øs ¶D> „ i í h eEpñ2 ÜL
 Øf Ñ°, WiDNA(ÅKÅbq, ús{ E\$ { 1 bO { E, L{ Ô: Y
 (ús{ - Åbq, 127* i í 34* «AKO v- S< f „ i - G | Jp «
 AKO vDí 8/11 B‰í 7/18 E^° u• { 1/4 (S< f „ i
 - i AKO t41* n• i í - „ 14Í i 2* j * i í >j * Atherurus
 macrourus Eí ej * Hystrix brachyura (24* Ø: i Eulipotyphla i í -
 AKO2Í ÈvøÑí F / i AKOY` 0/25 TP 0/1 • q2
 0/1 E 0/1 i ØAKO” å On• i - „ 2* * } i í (\$
 { 1 b Øåà>i Anura (ÅbqX(25* i í v- 12Í « i AKO
 ÅbqX(„ ~ ^ Ñ, 2* i í _ ý «AKO ddK i ØAKO ^ ž à
 >i „ 13* i í 9n° Å pnàÖtšÙúi í v- 2* ^ ž Ü ^
 Kurixalus Ü^, i í vi (KM, Åbq å - Ñ° F / å ^ • Ü
 ž' W eEpñ3 (, L{ - i éÑ° t3í * } i í Åbqøå
 „ , L“ i 39Í i „ i í - * ^ ž Pí Squamata v f „ + ^
 ZÓ™PÑ Scincidae EepçÑ Viperidae ({ - Åbqøå {
 462* i í i éAKO12* • 10* éy tšO^ ØØ { B, i í
 E, / („ Ñ° „ ý tšOí í 4s, 12* i í - 5* ^ ž ObB', {
 E F „ ÉÑ E{ EevøÑí æ 7* ^ ž | ObE— B', „ { 9n
 å€ü å, ¥Je w Üí y • { (Åbq q-1 Ñ° t14Í tž
 i , LSUCESU i úus „ i E { ýw Øi ž Y‡Bc æA¹ Ö-, PžE
 è i e i í { øKöAK‡, i Ràž, W sfí ; • / 1 ø-
 X(ObçE, àd ^ ObÅB, { øO , vçE, W8BOÙ{ @² „
 , ‡ ØØ

ÅK' ≠ Ø, i í (LSUCESSUpn-, O-` ≠ + | : 0.6€0.8 h1€
 h2 6 ' p i í ÅBO, à ≠ f N ÅBO, á; : - Mp
 (LSUCESSUpn- + / 2€3* á; : Ú(, i í - í : N` ≠ €N
 ÅK ≠ þ2c M¹ @` Ö₁, - Mp LSUCESSU +: 0.33€0.24 ī 100ē
 , W-, ÅK, ≠ - Mp (LSUCESSU +: 0.02€0.08

3.3 i í Oí |

9nLSUpnO-, ī * á; : „ i í Oí | - <: 32 SSU: 27 øÖK
 ī * á; : ÅBO, i í Oí | - < (\$* úàpn- +: 3€4f eEP™
 þ3a b ī * í 7, - ÅBO, i í Oí | - < (LSUpn- : 1 (SSU-
 : 2 ī * á; : „ í 7, p- < (\$* úàpn- +: 3€4
 (Ù\$* úàpn- ī * á; : „ i í Oí | , ÅB<€O-<KôX(@è'
 ī Y Üø>+ù " i í ÑKÇ - S , ÅK ī ī Y ù i 7' ÄOS
 š | , Oí } 6O-, ÅK, ≠ (i í KôX(^ i F/ ' pi
 i , ÅK, ≠ y^ N y+/ (E₁, W* Spf „ í 7, - þ3c-f Üø Ö
 œö t(• (iDNAÜL i 7' åö cG4' ī , í • '

à NJ p, á; : i øs, i í Åkpñ • H/à: i ÇÆO, W • H
 /à: ÇÆPe, 7, i Dø E „ Mnáo þ3a,b ÷è } 6dþ >: à
 Mnáo, 7, F/ i , ! < ī 6Ç(+ù 7, „ pn i , ` n! < O
 -t: 1pn àdà° i ÅB< yí åù@ á; : „ i í Oí | ÜLO-
 þ3c,d \$* úàpn, Öœy>: t ÅbqYø: „ Wè | K : Bw
 Ø, i í Oí |

(x=4s i í öi yú° (wÖfØ (f | Ü» Yø: 1
 „ Oí Üi ål \$* 1 b úe - H \$* úà, pný>: t Yø: „ O-
 i í Oí | • @wÖ, GØ ž Å LSUpn>: O-, i í Oí | • @ Y
 ø: 1 Y», ž ž þ3e,f v! \$* pn-, x=sG` ≠ 1
 11€12 • @wÖ, ž ž þ4a,e (LSUpn- (wÖØ| Y Ø
 „ Åµ x=sG` ≠ • @OYø: 1 Y», ž ú° * >, GØ< ž
 þ4c

i í Oí | (LSUCESSUpnKô>: t ^ } „ ö' \$* úàpn- ÅBO,
 i í Oí | (ī * í 7, eEP™þ4a €i * á; : eEP™þ4c GHcø
 s àd(„ tmK- \$* pnKô, O-i í Oí | _H' Ä, cøs eEP™
 þ4e Ç7H‡i åž i í ÅK ≠ , Wpř Š „ í 7, - €€ +ô „
 i í eEP™þ4b „ í 7, pŠ „ á; : „ 7 eEP™þ4d 6
 c, „ £7 O-, i í Oí | vi • @Ç7H‡ ž à: i , ! < (,
 Wpř €í 7, „ př Ø Z+e•í c eEP™þ4f

(i í 4s wÖØ| \$* úàpn €OYø: 1 Y» ÅLSUpn „
 qí (1 €: | y @ þ4b,d,f (Sí ...Ç10l ø, ús{ „ i - ¶
 [B. taurus ¶{ øS O. aries ¶{ qS C. hircus ¶d, Muntiacus
 vaginalis „ ≠ • wÖGØ MN eEP™þ5€7 NwÖO: €€ö` ÑY
 ø: 1 6 1 ø=sG` ≠ Y» Yø: 1 „ i Èqí ^ eEP™
 þ6 øÖK , Ü • Elaphodus cephalophus 4• R. unicolor - N
 #š C. milneedwardsii š 2ÑS U. thibetanus åÈ† * Sus scrofa I i
 I , EO, ≠ • @wÖ, GØ ž àdöi yú° (Yø: - ÅwÖfØ, ī --
 eEP™þ5€7

' è S< (10l øå „ ús{ „ i i í i ÇŠ• ; ~ Dremomys rufi-
 genis €, Neotracus sinensis (Yø: - ÅwÖôØ, ī -- w ôØ, `
 ≠ eEP™þ5€7 { _ 7 „ > s(wÖfØ, Oí fØ, EO‡
 æ 1 b > < ús{ „ i i í , ' 3 Rattus nitidus (Yø: 1 w

Ôf N, i -- f \$ { h° ú CØ > i í , 8ª þ Bufo pa-
geoti IUCNÑq Æo • þ O. jingdongensis IUCN q ô8ÁŽ wØf Ø
¤ „ O: Fv Öi í , ' | Äþ Bombina maxima ô8ÁŽ Y ã: 1
„ NwÖi --

3.4 ã = Ä

(\$ * úàpn - +ù@ 7, ÜLB! Z{ • Óœ>: @ 7,
gv@(á; : „ wØØ! N - ØwØM¹ Ñ †Z{ þ5a bÆ
eEP™þ8 ÜÍ Z{ (\$Äúàpn - w Ø! „ Õ' Cramer's
V = 0.79 95%ná: ô0.73-0.85 wØf Ø, : B€€MŽ Y ã: ...è \$v
/(Wè NwÖO: øÖ +ö °ùUö, i — eEP™þ1a,i wØ
Ø! „ M25%* á; : OÝ ã: 1 „ sGÝ» ± Æi i : 1540 m 850 m
25%: 830 m 390 m

NwÖá; : „ ã = å ¶ [B. taurus q Š C. hircus d, M. vaga-
nalis Æ' | Äþ B. maxima : ï i í þ6 Ù> i í X(Ž' pNw
ÓO: Æ O J „ ØwÖO: øÖK 8ª þ B. pageoti Æo • þ
O. jingdongensis h° úøí „ ! sfì (' pNwÖO: ý X(
F(' pØwÖá; : ýX(<ž ofŽ NwÖO: „ \$ { Æ { (
ØwÖO: „ † öØ eEP™þ9Æ10 çØ > i í , Ø> Ù N.
unculuanus (NwÖ - wØÆØwÖO: h° úøÑ, † þ6

ÇÔfM¹ ã = Ä (\$ * úàpnKô, Ø i Ñ° \$ÄpnôX(>W
„ O ' RVü p[89] 0.77 p 0.001 Üh \$ * pn- X(' i , q « á ÷
\$ * pn, ø<ü p—JaccardY» _H° Ø! „ Øs' PearsonØs r = 0.94,
p = 0.001

4. .. o

Ç, v i Á t úž, WiDNA, Ø i ab €/i åù " i i
7' ÜL' Ä! Øzô " † „ ÄO å i „ v/ÄE: b• (iDNAÜL
" zô " i Ø Ä! • „ i 7' å , WÇÆ1*Üù- „ ã-X(:
2-3* - ÜL È1i å -t * bi : 677S¹ i l , è6Y ã: „ ús{ Æ\$
{ „ áo v Øi å | . i { Æ, L{ „ áo þ1 i „ v
Óœh Åbqé6Y ã: ; • (v8Ä: B : w ØY ã ÷ < „ i i
í Ø> t Y ã z ã v ÓœØU: t Y ã: 1ž° { ; „ , œ \gÆw
Ñ „ 1' þ3Æþ5 , v: ÅbqY ã: Ø> t úžiDNA,
i i 7' úž * e, iDNA åi åúžÙ*úž KÖ * i í „ Ø
\: ÄOY ã: , H‡ [58] ; , eö i „ v/sž(ž i - f - •
(iDNAÑK, ÜU¥J v: È „ Øså\Ø> t 9Ü, ú@

4.1 Åbq „ i i 7'

i „ iDNA å Ñ° t 86i ^° " i i ús{ \$ { { Æ, L
{ v- „ /8Ä, i „ i i í ¶{ i í , [i „ pn- Ø i t
* 8Ä, i í , eö , œ/i „ ù' „ B å Ü> 8Ä, i í àÖ
« Ñ° v- i 15i « IUCNãš: x Á ¥Ñx Á, i í h3

„ n! < „ Óœ>: ÅbqØwÖO: „ " i i í Øl | Ø i , Óœ
i yí t Y ã: ...wØfN f ¥Ñ, : B X(f' „ °: r p , é ¶\³/4
Å ÓÙi " i å E oO, 9Ø Üi yüö, NwÖi " i i í (SO
mY öw Åuý, i í i yöi fì „ oOi å Mœ", NwÖO:
I uO * „ ØwÖO: å • ° { „ eH[61]

wØØ! ÆOY ã: 1 „ Y» / " i ã = Øl | ÆÄ „ í • „ KàP
þ3e,fÆþ5a,b üU* i í „ v h „ , i í y + / { Æ < ú

s{ (fØwØŒÝ¤: 8Ã: ß,, ` ≠ fØ Ù> i í ì t à í (IUCN-
 : x Å ¥Ñx Å,, i í í > 4 Macaca arctoides Ù • E. cephalo-
 phus 4 • R. unicolor - N#š C. milneedwardsii Œš 2Ñš Ursus
 thibetanus Ù> i í - „ > @ i í ùÝ¤: 1 „ oOØ w l ° {
 ; „ f: O v (Ý¤: h¹ O: \$v/ ' Tr \ „ OB ^ 1 Ñ °
 { ŒÍ „ i Kô, Ø' 2 • , š 2Ñš ÇøÔK — > ï i í ,
 d, M. vaginalis < N(Ý¤: 1 O: w ôØ, EO‡

4.2 • (iDNAÜL i 7' ÑK

Ç(, WiDNAÜL å 2* s.. „ ð a ý å ØvÖ¹ Øô: • Ù „
 „ i i í {¤ b i å ÇÇc' l ° X ê • T Ç€Uù- s i Ù
 L' l „ 7, ÇÆå\ Ù7i å, ööŒ , • -š „ ' Ä! i 7'
 åô i L 6 Ù> ð à: å > à « è Oµ^ žŒ¤è'
 „ å\l i å Q Çé „ l \sðä³ Ù* i „ 7, ÇÆå±³/4i „
 wSÁ ‡ iDNAy „ ½7i i ŒOî å È i áo ŒBj ú! ø
 s, å\l i „ • 12° * e i p, W ĐÖDNAŒÐLPCR K • I ø • ø
 * e, ú‡ " ŒÜLK• , v- àDNAÐÖ PCRŒK• § „ „
 , | : 210,000C° 30,000ZC ÙØj — i „ • , | 80,000C°
 12,000ZC Ù> žŒ , ³ å© i ÜLà! Ùí Ä!, 7, ÇÆ

7, ÇÆå± „ ³/4i

< ž Å 1¤—XÜLÇ7/(t * ê 6Ý¤: 6Ä! l , W, i L¹ Õ¤—
 X, / l Ý¤: » ÑQ, - Ç(, Öi v / Ø¥Œ i ù¥ (l AE } 7,
 ÇÆ, P 1— Ä b, \° XDM 2016t 6 07 Ù> P ØY¤
 : „ * Ù¹¤—XÜSO¤—X å 4Ñúøs : v(9 ÇÆÓ_ ÖP7
 , : t 1 ð¤—Xí \å È „ l 7, i : l * á; : +Æ t 1*
 p l * p - > n *Æ <Œ 7, ÇÆ< yô „ f ö l *Æ < - Ä
 * ÇÆi ... 7, ÝX² RNALater å È1 áoÇÆaG bòl ³/4i }
 • 1 ÇÆ° k™ÇÆ7, , øsáo i ÇÆ° Ø « ý Á÷ Ç7å Ç7
 O¹ GPSáol ÇÙ* 1 ^ ê 6, • B¤—XÜL, W, i Ç7 „ •
 Zä È[65] Ù1 • —(U* öö¹ 6Ä! * á; : „ , Wí 7, : i ý ^ d
 t i l ` f áo • @ööØ Ø l üö öö¹ ÇÆ, 7, w o}
 l „ , i „ [72] Ç1Ž ä Ýà l „ á; : ÜL, 7, ÇÆ; q ± t 3*
 „ öö

6 l ù7, áopn, 6Ä * Ý à: „ 7, } 6 sž ÇÆ°¤—
 X, áo F i sžá; : ß,, áo * e • Q Đ> 7, áoÙyÙi \: Ø
 >, a ö è¤—X ; • / Dtfñ,¤—X „ å ¥x 7, áopn 6Ä! b
 „ ù- * ö• „ i ý / i å(¤—X, K: %oÅ * / GPS, „ (•
 (ž 6ÄÇ7O¹ „ P æ 1 b i „ ` n! < ú! F¶ý ^ } O Sp
 l „ : 1pn v i ù6 ZGáo, „ : • ÜL(N <, i „ ³ Š
 , Wp l @• („ ÇÆi p l Ø9 à: Üi ý Ä•¤—X(Ý¤: å „ : ß
 6Ä, W i Ñ° Ç(ú, l ³ ö ÇÆO ê, W, VÑ Ù7, Hœ1
 ^ } i öl (v Ö, 7, ÇÆå\ - • (t Ül 1 „ i „ i È (7, ÇÆ¹
 b • SÖz ° ål ³

iDNA, 7, i i ŒOî

(„ v- à * i ý „ i e • „ * /, W ! ÜBŒ i Ö7
 Kô, ööö i ý • ³/4a * [1] } 6DNAXYöö• Œž l ù „ i „ Ñ° FÙ
 – s@AKO, DNA š l ÇÆ7, ö, i í Åµ „ i ð; i ý («
 8B@² „ i ÇÆO, WKM» å Ç7á; : „ oe: &, W, ð; „ i å
 • ÅL „ { „ • Ü c[90] Üi ý ! ÈAKOœ, öz „ f Ç i
 „ p n >: „ i ÇÆ, , W; • å i ý \ Y(„ * á; : ... (Ø »: ßKô

ū", ē; : β <, RÙ àd i sŽAbqī ḥ{ i ī ; S Óœ
 β3Eβ5 * xÙÍ Oí %l qí d å * : öö—ā(* MnÇ
 Å@ ī 7, Ü71P6t, W ē; • Ÿ» Áú, ī y à: i ī • Ÿ» Áú
 Ÿí ` n! <, MÐG¾ si ī (ī 7, - " SÝ Ø "í øí "G
 ¾[91, 65] Ç_à: , d Ç7Öö, ö• 1P6t, WiDNAÅK * (Ž^
 8ë Ø , Åµ <, (à * ...Ñ , < ö[65]

ī , ḥ* e• ī y /, Wø=(á; : Kô, úBî åÈ, Wi ī Kô
 " nBØ} <, , œ, Wi ī • @wØ Ñ Ø i i v\:
 ÅKOØt ØwØ, Wi ī ô> Ž8BÙ@ ^[@ Ù • Ù> i ī Å
 KO, • @wØÑ Ø i 7, - , WpI Ç Ù• — i àÖùt
 è, WÜL Uì tš i Ç7, O MnE, W, B b @ ^Žqí ^
Haemadipsa, W Ø[70] å^, i ī tš, 1X(Ø¾ Øåqí å ī
 " i : β[70, 69] Ü^ Ÿ/1Žfì , ' ³š à: fì /: ; I YPI ...
 " Å k v h fì Kô, nBî ^8 P[70, 72, 66] t Žd i
 ét", Wd", ¹H / Øp , W, * Sy• EñBØ} Q(... i ø:
 , WnB, i ŸyäÈ i v- , ÚU; • Øœ

, * Ÿy, Ÿe• /PCR i Ew °, é Üi y D> i ī }
 6vDNA(X(Ž7, - F/à: PCR6μàÖ« i žt àÖ« i ÅKO (,
 å\ - i Ç(\$* úà °, pnè Oä³ tÙ* i ~ \$* úà ° yÅK
 O Jå, i ī v \$* úapnKô, i ī Oì ; Eø=Ä X(>W, O
 Øs' Ü_1/ô s• i è étv- * úà sži l ; S Óœ_
 xO%l qí β3Eβ5 æ ¹b i (i žyši l DNA, y>
 ú° > i <, i (* , á; : , LSUpn-ÅKOtí >4 M.
 arctoides ù" • p +: 2,700 170,066E245,477a ØÖK (SSU, Ø"
 pn- t j Ñ° i å MÙ^° u• i " • (ô , i ô , úà
 ° Ÿy ÅKOô , i ī { ø[92] F/• ž , žE/K• , ^ ŸyE@—O
 P¥ cÖ (* e • (8xΦ^ E/ • úà ÅK• [93] • (l CRISPR-
 Cas13ÅK, CARMEN° ¹ Ö[94] Ÿy ÖäPCR <, Ü> ¹ ÖyA, i •
 (ŸE (, i ab úà—ÆPr C' vi COI vÅ pn" ö: h
 b[95] öØi å vfw °(ŸCOI Ÿ^): , i ī { ø

, WiDNA ê6' dv@8B@² Ÿ; - i ^ } åh' , i ī { ø
 v h \$ ø: øÖ , i ī , iDNAÅK H‡fN = i iDNA< Nô
 ÅKS< f , ús{ i ī [1, 22, 73, 96, 61] i , v h \$ " i ^ Ÿd{
 Åµ S6, œ ³ , 7, ÅK‡N, i ī 6i å « Ñ° fì , NÅK‡
 i ~ i å (M¹ ` n! < eä³ * « ÅKO, i ī 6i å (ŸBj ú! <, •
 (pn i E[27, 28] Ffì >6å Ö: ! < D> pn S, WÇ7×OP6ö <
 , 1 v° XÜL7, ÇÆÖ Abramsl ° [72]ú®i å • (, WiDNAeùΦ ø:
 ÑKpnÜLeE <, Tilkerl ° [61] Ñ(ŠWØ " * Ÿø: ..., 139* M
 1 17393 ø: p*) p ÜL† ! ø: å å ô: 900 s¹ l l v•
 (eêv- 93* M¹ , 2,043é, W, iDNAÜLpnE ø: ø: pnÅKO23l F
 ús" i v- ~ E' < n• " i / y ÅBO, i ī { ø Ü> { ø
 8 S ö , ÅKpn (åå\ - , WiDNAÅKotø: pn- i Ñ°
 ' + *Pardofelis marmorata* v • E&ø *Chrotogale owstoni* Eš2ÑS U.
thibetanus , ÅK‡DØt Ñ æ ¹b • ÅKO *: B- X(, T
 * i ī i 6y Å+—O• ' " ! <, Gogartenl ° Ñ° ø: pn
 EúŽí G, iDNApn@ÅKO, ø=ú, i à (@ 7¹ - \$í ¹ ÖÑ°
 , q« i ī : 6% 43% [96] F Ü\$ { pnýù oOÜL†ø<, Z{

i ī M¹ ` n! <

M¹ ` n! < i å (Ÿnš(U* i ī 4s ÅK‡E` ‡Kô, Øs' 6
 ' pi ī ÅK‡^ N (Uì • öfì üÅK‡E` ‡y D> , áo^ à

: ^ ¾: NÀK‡ s•?` ØEN` ‡ s ' 6 Ç Ù> NÀK
 ‡ „ i í pnt O` nŒÀK, ☉=! < - q« i í Øá; : ßKÔ, áo EH
 t*pn1ý : i Ð> *ô: Øt „ Ábq " i 7' „ , Ø ÙÍ ú! ¹
 ^ } O †: 1pn Á †(ö- F¶- ` n! < (Ø, pn1b,
 H' 6 æ ¹ b " (Ž' pn1 „ pni U! < 1 s@ù pn, i —:
 sØ fØ, I ö• B v • ± 9f• „ ØLööåEH ú! ö ÝB ! < @
 „ ' Í KÖ
 (, v- i ú! í ÁÆ- (cG4' i S6• cG3' i
 _/í y „ < , 1žžŒøaÓ { tš i „ G3' } 6(i „ pn
 - G4' i y ÖG3' ô%í FG3' i y üô i 7' ÄOÑ %í „ O
 ↑ [97] Ÿ B! < _i (Ž cG3' i F(žö- (i sŽG3
 ' ÁK „ . áo „ Áµ ^ ¾ùv HœÜLÄO[98] } 6 N v>:
 ÜLG3' „ ÁK < , [99] FÙ> ¹ ØØ (Ž i í Ø Tab p
 n

- @iDNA¹ ŸSeŠ O(Ž' Ä! „ i 7' å (å\ UKM *
- Q, í • ž Ø¾i i —s, U v, W7, QO, W *S Ÿ
- X • (O' i „ ÇÆj Øøs P àd v7, i åi ÇÆ, ØÇÆüj „ B', W í OÝX² - àd6ÆO ÇÆj - „ , W y \: i
- É, í 7, „ , ø g* SÜL • 1 • E: U*, W• (Uì „ ÇÆ
- i v7, 7Øi åi „ žÆ, Øå\i Çi ae ¹ be • (
- ^ v7, „ pnö ` n! < Hœ ô} , øUì „ , W i åùv Ü
- L DNAab tš i . —, Wi í nßO} , áo åáoi å(` nû! - (
- \AKOØi Ñé „ Ø i „ ž Ø¹ H, [94] ©žä³ ž Øå\i „
- † „ • —({ < i Ú7, ' 7, s>30,000* * S å\ - i åù, W, *S
- + ÜL (7, ÇÆ6µ_í å Q * ~ w¹ H 1/ ÇÆ Ð> Ô, v-
- @(ô „ ÇÆj < , 2ëG å MNi „ , Wpí F šy ¾OU*, W „ 4s

4.3 iDNA * \>, i 7' ÑKåw

S i AE (* 2020t å „ ° F¶Øä1å i 7' i ö | • í
 Ä>(, U Ñ• (i 7' „ , i í „ ‡ O| Øí øØ < ð eö
 ¥ÄOÝ ☉: „ H [100, 46, 101] 6 „ , Y ☉: D• ³ ° > ³ [44]
 i 7' „ ÑKå\ ^ ¾« H Q[46] (, v- i U: †• (úŽiDNA,
 Ø Tab €/\: i í N H, ¹ ØeÄOt * è6Ý ☉: ... w Øz ö
 „ ‡ † Øö i í { ☉: „ i i 7' „ i L' àd i „ å\ Á
 †iDNAï å\: * \>, i 7' ÑKåw ö¥ùÝ ☉: „ Y ☉ HÜLÄ
 O

dt—OÄKi í „ þ1 „ i „ å\ Øi å ü* e „ ÑKå\ ånš
 - fØ ° { ; „ qí åÈ t*í „ i ☉=“ Ø „ i y Yà i
 ú®• (i „ Øee ü(ÁbqÜLvÖ¹ „ , i 7' åå\ i ùí
 i í „ ÇÆ \$ ø: Ø i ðf ÑK å „ å\ ¹ H¾i l \$í ¹ Øy
 i åi Èù i „ ØœÜLKÖØA v Çq y ÁKO, i í „ ús{ O
 { e ðØi í „ ‡ ØÄK‡, O—Æn' [72] öÙ> ÑK¹ Õ_i å Ç
 • (` n! < F¶eO—Ð> i í „ i ☉A! Øí øØ < ð [102, 103, 104] d
 i ØÙ e ðÚ iDNAï å(Ž å i 7' „ vÖ¹ b , ° \qFÅ Ñ
 „ vh i å) (, W7, i i Ö7 . Öi ÅØÈv „ i ð; „ os
 áo [105, 71] 2020t „ h ° ' ALØ>t ètäi „ i ÅØ“ - ° \qFÅ
 „ ' è' — • @h Ø—Ø O („ i Ø Ü Bï y Ø—ô %
 è [106]

5. ¹ Ø

, , ; • / Ú¹ Œ, , Œ eEP™- D> †, WÇÆ žŒœ i áof
• A œM¹ ` n! < ú! „ æÆÔ i áof • á Á[107]Œ[24] B_j ú
! Œ • á Á[31]

5.1. W7, „ ÇÆ

, v- „ 7, 1Abqê6Ýœ: „ œ-XŽ2016t 7 ó9 „ ècÇÆ t *
ê6Ýœ: « : 172* àí à, á; : Ù> : B, bï ̄ 0.5s¹ l̄ O12.5s¹
l̄ l̄ s G3.9 sd 2.5s¹ l̄ l̄ bï „ (Dí | | tÙ> á; : í µ
¾, ¾ | 8f „ : Bô - ¾åµ ¾ Ù> á; : (, v KM1 ðl̄
nŒ v(Žå8Ýœ: „ j „ Yœ: 9nL?: „ : 6* è 16* ê» „
WN ZÄ o GÜ l̄ l̄ °s i 9n» N | : vj - ô..., Q,
Má; : Q, Eä\Ä (á; : ... Uâ\ àd l̄ * œ-XI yl̄ tý
ôb Fá; : BŒ #á; „ Q, / úš,

i l̄ * œ-XD> *Æ < l̄ *Æ < yÅ * 7, ÇÆj v- Å
DNA/RNAÝXB œ-X« • B á; ö O, W , l̄ Ob c >e
ÇÆj - i á(l̄ *ÇÆj - >e a, W FÇÆj : ø P Ü7 8 • œ-
X• (* i PÜL, W, ÇÆ

, ! å\ - ; q 163 œ-X(@ 172* á; : ... Ž3* - 6Æ† 30,468ê
, W S *Æ < - „ , W; p Ž100êö i v- @ ÇÆj „ , WÆ- w
e \: y7, l̄ l̄ žŒœ „ å\l̄ Œ , S *Æ < l̄ Å@, W
„ p̄ ≥100êö i v- „ , W gÇÆj vwe 5y, Wp̄ ' ôøl̄
l̄ 7, á • MÛU7, - + Ç „ , W (, ! å\ - 81%, Æ <-
+ < 100ê, W 78%, á; : ÅX(NŽ <, Æ < * á; : 8ÖP
* + < 100ê, W, Æ < (l̄ Ç7, „ v K l̄ y7, „ , WsGp̄
: 34ê ô: 1O98ê ; q 893y7,

5.2 - f y •

i • (ArcGIS Desktop 9.3 Esri Redlands CA œR v3.4.0[37]e j -i * á
; : B, y • i : wÖØ! ObMn p sï * i v h Ö l̄ Kô, l̄
< [108] O NSi „ Y» åÈO N³A, Y» ú† 30s „ < pB 6
i i -i * á; : B, <<- < \: Bj ú! - „ , K < h4ŒeEP™p1
i Øj -t i * á; : (ÄO Nê6Ýœ: 1 „ Y»

5.3 PžŒ

i l̄ l̄ l̄ y 7 , - D ÖDNA 6 (PCRi ž † \$ *
z ' S ú à „ . * e ê16S rRNA MT-RNR2 ú à
i 16Smam1 5'-CGGTTGGGGTGACCTCGGA-3'Œ16Smam2
5'-GCTGTTATCCATCCAGGTAACT-3'[2] æ * e ê12S rRNA MT-RNR1
ú à i 5'-ACTGGATTAGATACCCC-3'Œ5'-YRGAACAGGCTCCTCTAG-3'
1 [3]† 9 - ‡ - i Ù\$ * ° + ö : LSU 16S 82-150bp
ŒSSU 12S 81-117bp Ù\$ * ° + „ / Ù> ú à „ 8ÖS „ š ú
Œ š ú i Ù7Z / : † • M • Ù• („ Æl 16Sú à ÷ Æ á ú à
i „ 12S ° i • / i „ 16S ° i • i _ùÆPr C
v Iú à COX1 [4]ÜL t KÖ F /, ‡ - i Ç(à: á ú à „ i i á i ž,
WDNA à dÖP „ i pn •

LSU i / è ^ ú ú s „ i ¾j „ SSU i / ^ ú @ „ i ¾j „
i D(ecoPCR v0.5 [109] å MIDORIPn „ [18]- „ Ù³ „ i Tetrapoda
„ • : Ä p n (A , 3* M, Äµ O—LSUŒSSU i „ „ i ž Ÿ
‡ Bc< Bc< / (i á o • - i á « i ž „ i l̄ (Ä p n - @ „ Ö<
C, „ „ E 7 LSU i ú ú s { 99.3% œ { 96.2% „ Bc< f Ø Ù\$
{ 79% „ Bc< - ú MIDORIPn „ „ L { „ i l̄ Ä | l̄ + v

.. i + Ö .. i + Y Ö .. 39.9% „ Bc< f N SSU i ü ús { \$ { E { „ Bc< f Ø > 98% ü „ L { „ Bc< - 79.8% àd i „ i ' è @ Åbq „ ús { { E \$ " i y i å Ç * \$ * i ÜL i ž Ç E % 41 „ i „ Y f N

i (\$ - i ö 7, A+ ~ 3/4 i (i „ 5 i Ç (i ~ V e • (DAMe [6] e A+ E ^ d " ~ ö Ä " S „ i [5] (893 y 7, - i (LSU i Y i ž t 661 y 7, • (SSU i Y i ž t 745 y 7, Y PCR i ž S i « ONovogene - y ~ ÜL PCR-free " , ú v (Illumina HiSeq X Ten Ü L PE150 bpK •

i ÄPCR G i 4' ùg Ä , oe < ÖY ÖÄ K > : 4' ùg Ä - X (a Ö i È > ä ÄPCR S i v i Øu 4' ùg ÜL t K • à : Y öv y ÄK O ^ 8N4S „ a Ö (i „ 4' ùg S i - ú ° t ° [x q S * ! E > i i „ • F PCR i ž H f • N - O „ • p _ ^ i • (Ü > 4' ùg , p n (i áo f • Á , E e E P TM - : @ 7 , „ p n (DAMe- 3/4 n Çä Ä p (ž d p n - i y X („ a Ö • LSU ESSU „ Ä pyG 3/4 : 2 s (3! PCR - ö 2! PCR y ú ° „ • M < YY LSU „ Ä p t 3/4 : 9 SSU 3/4 : 20 t / YY • (i ! PCR - Ä { ú ° „ ÷ p i Øi ž E K • t > 3' ùg S i v ! • 1 \$ í n „ i „ DNA Ä Ü \$ * i i / Myodes glareolus E Apodemus flavigollis f i Ä (' 2 E • š (i „ , W p n - v i Ä K O

5.4 i áo f • A

i „ i áo f • A * s . y • - H / DAMe 1 H [6] ä 1 H Ç (i ~ E 3 ! i È „ PCR i ž e A+ E » d " ~ ö Ä " E v f i y à S „ i • v ! / i • (\$ * i È „ úa ù Ä K O , i i { ÜL i i Ä ä e E P TM p 2 1 / i Ç (PROTAX 1 Ö [16, 17] e ÜL i i { , n š ä 1 Ö i ä i (Ä p n " * E , ö t s i i { ö ^ 1 Ñ „ Ç | é ä O i (, ä \ - ' | J „ ö ä Ä b q i i X (ž Ä p n " - e E P TM p n 2 v - ús { E \$ { „ ä h ' ö u f } (LSUp n " - 73 % „ ús { E 83 % „ \$ { i i X (Ä • (SSUp n " - ús { E \$ { „ X (‡ + : 70 % E 67 % { E , L { i i „ ä h ' f i LSUp n " - Ä 42 % „ { E 53 % „ , L { X (SSUp n " - 35 % E 34 % „ { E , L { ü Z i 9 M O Ä p n , OTU • PROTAX v M O ö Ø I S „ { U M , „ Ñ i i v v i i ° : " * ä " • i i ä 9 n v Ö ä o Ö , K M D E , ä p n æ * ú a p n , Ø " ä o Ü > OTU M O i y „ b i i

(DAMe Çä i • (VSEARCH v2.9.0 [12] » d < Y „ PCR i Ä • • (Swarm v2.0 [13] • Z { e „ i i \ { UC " pre-OTUs " 6 • (Ro Ö - „ LULU v0.1.0 [14] Ü > w Ø ! Ø < ' E Ø 7 , „ " pre-OTUs " ÜL v 6 i • (PROTAX v , OTU s h • ÜL i i { 9 M [15, 16, 17] v - i @ • („ Ä p n / (MIDORI p n " , ú @ ž t Ñ N h „ W š ús " i z ' S ú à Ä • [19] @ A , ÷ æ Ä e E f ö : t q « LSU ESSU p n Ä , i i { ä o i i - t 619 y 7 , (Ü > 7 , - LSU ESSU ú à y - Ö t Y , i ž E K • - \$ * ú à " pre-OTUs " K Ö , \$ \$ ö s ü p v v i Ä : * QÜp e E P TM p 2 , oe * LSU E * SSU „ " pre-OTUs " ú ° (y 7 , - v 9 M O { i i { UC - i i : B „ , W - i ž ú e „ i K " Ä ä t QÜp v Ü > Ø s „ " pre-OTUs " ä h • ü M Ü * i i {

i d t @ à Ö 9 M O ü U i i ä E ö Ø S + { U M „ " pre-OTUs " Ü > " pre-OTUs " Ä + ` LSU ESSU p n Ä „ 0.9 % E 0.2 % v • i y / K • S „ i • ^ ° „ i i • (LSU ESSU p n Ä - i v t w Ø i i

{ t š „ “pre-OTUs” ī : ī * pnÆ È „ ī ī \’ { UCÆ OTUs
 (B̄j ú! • KM ī ī \$* úà , pnÆ- d† « t š: ° (Homo sapiens) „ OTUs } 6Ù> ° „ OTUs ā: Ø6t * Ý¤: „ ° { D> áo F
 ī „ i ' è „ ° DNA/eê¤—X, « / (Ý¤: „ vÖ°

} 6à: Ā pn“ „ œ, üô > OTUsà Ö« t š Oi ī 4s F/ ī
 ī 6> Ž È· — „ OTUs(ī ī 4s ÜLà È àd ī Ç(spe āi
 ī „ LSUpnÆ- „ 2* Ù „ OTUs—Kurixalus sp1œKurixalus sp2—” ā È
 : \$í „ Kurixalus ī 7 „ LSUŒSSUpnÆ- „ ÜOTU—Megophryidae
 sp—” « ā È: Megophryidae- „ D * i ī àd (, v¥ e „ • -
 ī È „ OTUs9M { ÓœGð: ī ī

(d t š : ° „ • ÈLSUŒSSUp n - +
 + 18,502,593œ84,951,011a • Ü> • āh†LSUpnÆ(126* á; : „ 653y 7
 , - „ 59* i ī ā ÈSSUpnÆ(127* á; : „ 740y 7, - „ 72* i ī : t ÄO
 ī „ iDNA¹ Öý ÅKO „ i 7' „ • | ī i iDNAÅKO „ i ī
 h „ i v@vÖ v° X „ * N̄h „ Åbq āi ī hÜL†Öf

ī Ø(i ī h- D t • „ pn ī Ç• (RoÖ - „ rredlist
 v0.6.0 [25] " PROTAXnš „ ī ī f D t ý E ê 6Ý¤Tß IUCN sŽ
 Ü> i ī „ pn : d 9n N „ vœý E ê 6Ý¤Tß IUCN „ ° Ä
 O[110, 111] ī Capricornis milneedwardsiiÆ: Capricornis sumatraensis „ |
 ī ūžúš{ ī • (PanTHERIApn“ [26]. Öl * i ī „ tSí pn
 (ī ī 4sáo ī („ Åµ ī Ç(tåOTU(pn“ - „ N { UM „
 ^ N̄I - @ i ī „ tSí „ sG<

5.5 M¹ ` n! <

i • (Åpi U „ pni E parameter-expanded data augmentation e
 + Oi LSUŒSSUpnÆ „ ī ī M¹ ` n! < multispecies site-occupancy models [27, 28] Ü> ! < G¾ LSUpn- ÅKO „ 59* i ī (nLSU = 59)ŒSSU- Å
 KO „ 72* i ī (nSSU = 72) + / Åbqi ī œ= - ý Ç, WiDNAU | O „
 PÆ = i NLSU œNSSU / * å „ F/ ī ī å Ç fì LeO * úš „
 : M „ ... S œ= ”e ÜLú! ī (È! < - œ= ' ¾: 200(M = 200)
 ī M = 150OM = 474, < / 1984Ö 1985t Åbq å å \[30]- - „ ú
 s{ „ L{ œ\$ { „ ; i ī Oi | —ú† NLSU œNSSU „ N < Oi
 <

ūž...S œ= - „ ī * i ī ī „ ! < nOUI Oå 1 b „ áo
 i “œ= Ç ” community process „ áo s nš Ði ī / &X(Ž Åbq œ= -
 (ii) “ Ç ” ecological process „ áo s G¾Ði ī X(Ž Åbq œ= -
 - EHf(ī * á; : - / &X(á È iii “ÅBÇ ” observation process
 „ áo s Gš Ði ī X(Ž Ðá; : ... EH i ý&(åá; : „ 7, - ÅKOå
 i ī „ DNA ÇÇ(œ= S Åp EHœ< ī * i ī „ œ= “œÅKÇ 1
 báoTü(w wSe œ, @ð

(œ= Ç - G¾ï * i ī i (Åbq œ= - • HX(• H (ī (w_i
 e h: Üí * nš „ ¶ w_i « Gš: « œ= X Åp Ω_{g_i} @S6 „ / ^a) • : Øi
 Bernoulli random variable si ī i (Åbq œ= - X(„ , ‡

$$w_i \sim \text{Bernoulli}(\Omega_{g_i}): \quad (9)$$

(œ= Ç - ī i ī : \$* ê 6Ä—R „ ús{ œ { å È Ø), „ \$
 { œ, L{ — Ü71i å A, fì (Åbq œ= - w „ X(, ‡ Ü1 1 Ω_{g_i}
 Åp „ h: v - g_i h: i ī i ^ Z Ü\${ - „ ê { Ü* 1 „ • Öœ>:
 t ī „ „ s Ü\$* ê 6Ä(œ= - X(„ , ‡ nž ī àd (¥ e Ü

L $\text{CEAKC}_{ij} = \cdot - \ln(\text{C}(t\phi))$ $\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}$ $\tilde{U} e^{..} \circ A$

$\tilde{U} \tilde{I} * n\tilde{S}_{ij} = \ln(\text{C}(t\phi))$ $\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}$ $\tilde{U} e^{..} \circ A$

$$z_{ij}/w_i \sim \text{Bernoulli}(w_i)$$
 (10)

(LSUpn- $\ln(\text{C}(t\phi)) = \tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}$ $\tilde{U} e^{..} \circ A$

$$\text{logit}(y_{ij}) = \beta_0 + \beta_1 \text{elevation}_j + \beta_2 \text{reserve}_j$$
 (11)

(SSUpnA- $\ln(\text{C}(t\phi)) = \tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}$ $\tilde{U} e^{..} \circ A$

$$\text{logit}(y_{ij}) = \beta_0 + \beta_1 \text{elevation}_j$$
 (12)

$\tilde{U} = \text{elevation}_j / \tilde{A}$ $\tilde{A} = \text{reserve}_j / \tilde{A}$ $\tilde{U} = (\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}) / \tilde{A}$

$\tilde{A} = \text{elevation}_j / \tilde{A}$ $\tilde{A} = \text{reserve}_j / \tilde{A}$ $\tilde{A} = (\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}) / \tilde{A}$

$$y_{ijk} | z_{ij} \sim \text{Bernoulli}(z_{ij} p_{ijk})$$
 (13)

$y_{ijk} / \tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = (\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}) / \tilde{A}$

$\tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = (\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}) / \tilde{A}$

$$p_{ijk} = 1 - (1 - r_i)^{\text{leeches}_{jk}=100}$$
 (14)

$$\text{logit}(r_i) = \beta_0$$
 (15)

$\tilde{A} = r_i \tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = (\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}) / \tilde{A}$

$\tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = \text{leeches}_{jk} / \tilde{A}$ $\tilde{A} = (\tilde{A}\tilde{O}e^{\frac{1}{4}n\tilde{A}p}) / \tilde{A}$

! < \mathbb{E} } 6¹ 9O15ÅŠI t i ſ i „ M¹ ` n! < F i Ü> i ſ y „
 ! < \mathbb{E} ÈAKÇ „ $\alpha =$! < O we

$$1i \quad N(\cdot_1; \cdot_1) \quad (16)$$

$$2i \quad N(\cdot_2; \cdot_2) \quad (\text{for the LSU model only}) \quad (17)$$

$$(o_i; o_i) \quad MVN([\cdot_{og_i}; \cdot_{og_i}]; \begin{matrix} \cdot_{og_i}^2 & \cdot_{og_i} \cdot_{og_i} \\ \cdot_{og_i} \cdot_{og_i} & \cdot_{og_i}^2 \end{matrix}) \quad (18)$$

v - N() \mathbb{E} MVN() + h: c \mathbb{E} Cc \mathbb{U} å \mathbb{E} : $\alpha = \dots$
 p i * Åp „ 1, * h: i • (Cc H \mathbb{E} (o_i; o_i) e A, i ſ „
 n \mathbb{E} AK, \pm KÖ, ^öO¹ i <, i ſ å, KOi ſ O! „ Ø / & q ſ O
 Ü\$ ſ , \pm [27]

Ü> $\alpha =$! < i ſ A, i ſ HO¹ 8Åi ſ (åo i ſ S ſ o }, t S
 ÅpÅO< Çā ÷ / * SÅp „) i [34, 33, 27] öZ(ð, $\alpha =$ Ç • -
 i i ſ : \$Ä—R „ ús{ \mathbb{E} { åÈØ) „ \$ { \mathbb{E} , L{ —vA,
 f i w „ $\alpha =$ Ü¹ 1` \pm ÈAK \pm * Y— \mathbb{E} $\alpha = \dots$ Åp „
 h: v - gih: i ſ i ^ Z Ü\$Ä- „ è Ä Üí 1 Öi t i „ s Ü> α S(` n, \pm <, 1Ž „ oOO} ÈAK, \pm <, 1Ž „ W „ ¥
 æä \pm / 1Ž „ W „ nBÖ} 1b üB „ æ ſ { _i å ſ
 i f „ Öi ÜL° Ä <, 9n „ ús{ / F „ { / „ ú
 s{ \mathbb{E} { { \mathbb{E} , L{ Ä (w å ſ) Öi üBÑ²sü Ü> \ddot{y} ä Ä(i „ pn- h° s à: ' p { \mathbb{E} , L{ „ ÄB \pm *N àÖ: Ü
 { ÄD> E „ áo F* e <-ùÜí { Ö „ ú! ſ ° ÜL v

i • (JAGS v4.3.0 [36], ö- F¶ Åp 5 chains of 100,000 generations,
 including a burn-in of 50,000 ù i „ ! < ÜL t O— d t • Y Xzé 5å Ü
 L beta 7' \mathbb{E} Z { ` \pm i — Åµ i : \mathbb{E} 7, YYt@ np Ä b „
 Bi f • 1Ž...X „ P6 i àÖ: zé 5YY@ \mathbb{E} 7, : t • Ü> i —i L i 7, ÈtA e EP™- D> t (Ž! < Åp „ H \mathbb{E} , æÆ
 åo 9n! < Öœ i : @ %oÈ „ ! < Åp i —t \mathbb{E} G< \mathbb{E} Mp v O—t
 ſ á; : „ i ſ Oi | \mathbb{E} ſ * i ſ „ n „ M¹ p

5.6 Bi f •

i ſ Oi |

ùi * úàpn i Çùw/B \mathbb{E} ö¥† ! < - - - t Åbq@ i ſ Oi | „
 „ Oi < : t ÄOùM, é i Öf t M = 100 150 \mathbb{E} 200, @ i ſ Oi | „
 O<

(Åå t i ſ „ \pm ÈAK \pm O< i • (ö¹ þe i Ä ſ * á; :
 O-i ſ Oi | „ ÇùzjB \mathbb{E} -Oi * á; : „ j i - t ſ * á; : „
 „ O-i ſ Oi | - Mp å \mathbb{E} i * á; : \mathbb{E} i * ſ 7, - ÄBÖ „ i ſ Oi | - M
 p ÜLÖf i Ø6 t choropleths S@r þåi Ä t * è6Y α : ...ÄBÖ \mathbb{E} O
 —i ſ Oi | „ z ö

i Åå t $\alpha =$ sG` \pm ÈAK, \pm ÅÅ[116]- „ , 11.7.2, å. ©t
 å M¹ \mathbb{E} 7, OØi „ q ſ üZ ſ * i ſ Åg = 1/2 + ähúS { / { \mathbb{E} \$
 { /, L{ i ſ i - t $\alpha =$ sG` \pm ÈAK \pm , \mathbb{E} sG< \mathbb{E} 95% ö- ná:
 ö \: OØi „ ýp

$$g(\text{elevation}) = \text{logit}^{-1}(\cdot_{og} + \cdot_1 \text{elevation}) \quad (19)$$

$$g(\text{reserve}) = \text{logit}^{-1}(\cdot_{og} + \cdot_2 \text{reserve}) \quad (\text{for the LSU model only}) \quad (20)$$

$$p_g(\text{leeches}) = 1 - (1 - \text{logit}^{-1}(\cdot_{og}))^{\text{leeches}=100} \quad (21)$$

ÚÍ 1 Ó HO(ý p_g(elevation)- OÝ ð: 1 " Ý » Ý : ö (ý
 p_g(reserve)- wÓØ! Ý : ö ù" Ž ì pn - Ù> ØØ! , sG< à
 : " K<(ú! KM/ Æ " : tř AE i l ô, ` tEAKfùØØ! , í
 " ì • (Ŧ * i f ù o, 1, 2E o, Oj <Öä ð=...Åpeí Ù> j - å
 . -t * i f , ñsG<
 : tÄO\$* úàpnùÅbqi f Oi | Ø , ô | , i Ôf t \$* pn
 Koi f Oi | , * | Ŧ , * / Ŧ * f 7, - ÅBO, i f Oi | , E* /
 á; : ÅBO, i f Oi | , * / Ŧ * á; : , O-i f Oi | s 9nzi,j
 - , i f ñsG< ùŽt * | Ŧ , i j - t pñKô, ® øs' v
 (tÅŒKÖtøsúp ö, sú ì Ø• (È~• | z' ! < Poisson GLMs
 Åå tř * i f Oi | Kt < Ç7HfKô, sú ì Ŧ * f 7, , ÅBi f
 Oi | Ŧ * f 7, , Wp, ùp<ÜLPR • , Ŧ * á; : , ÅBŒO-i
 f Oi | Ŧ * á; : , 7, p, ùp<ÜLPR • , v(tÅŒKÖœf ûp, >
 W'
 ð = Ä

i • (úŽO—` f ¶ z_{ij} --O, Jaccardø < | ñG< e Ç"
 á; : Kô " i ð=Ä , Ø , å 1 Ó, v Ö: < ÅDorazio[28] ñKéry
 and Royle[116] • (^ | Ŧ , NMDS ' • þt AE JaccardÝ » è 5
 s distance = (1 similarity) • (vegan- , Ordsurfy p t Öt - f ØØ!
 • (Ward AE Rý p hclust(, method = "ward.D2") 9n JaccardÝ » ùá; :
 ÜLZ{ úŽZ{ , Óœ á; : « Ä + ù" ŽNwÔ - wÓñØwÔ
 , : B i • (Cramer's V e Ŧ \$* úàpn, Z{ 9M| i Ç(ÅbqO
 þ 9nÙ * Z{ ù * á; : ÜL@r f i AE Ý ð: ..., ð=Ä , z ØØ
 : t. © å " i ð=(Ù> Z{ : BKô/, UØ , i • (` f ¶
 z_{ij}, ñ7, e j -t * i f (NwÔ - wÓñØwÔZ{ : B- , ` f s
 X(á; : , Ó< , ñG< ñ95% ö- ná: ô
 : tÄOÙ\$* úàpn@t s, á; : Kô ð=Ä Ø ! , q« | , i
 • (R-, ade4::coinertia ùt * pn- Ŧ * á; : , , Ki f é5ÜL t Oi '
 • , i • (RVÚp[89]t Oi ' (ade4::RV.rtest- (999f ' KÖv>W
 , i Ø• (999f ' , MantelKÖAŒt JaccardÝ » ñG< (\$* úàpnK
 Ø, øs'

6. pnï · ·

, å \ @(, Illumina HiSeq/MiSeqK • pnGò ñNCBI, SRA Sequence
 Read Archive pn" - ù" , BioProject accession number/ PRJNA624712
 [https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA624712] , pn
 å OTU-ñ• pn h < (eEP™pn6- D> ö_ř å f GitHubQÙ i , ` n
 ! < , - - -(https://github.com/bakerccm/leeches-public/releases/tag/v1.1;
 doi:10.5281/zenodo.5914708) i @ (, MIDORIpn i å f http://www.reference-
 midori.info } ž , , ç' Sú à Ä Ä pn e ê Sallehl ° 2017, °
 f (GigaScience 6(8): gix053) wSÁv f - , h1Œh2@ , NCBI ÷ (f à þ ¥
 : https://academic.oup.com/gigascience/article/6/8/gix053/3958782) PanTHERIAp
 nï å f https://doi.org/10.6084/m9.figshare.c.3301274.v1. — i @øs å \
 , i f h(eEP™pn2Œpn3- D>

7. , i · ·

i , i á o f • Ŧ Ý E pn — OOTUs , i
 å , i https://github.com/jiyinqiu/ailaoshan_leeches_method_code [107]- .
 — i f { t s , i Å https://github.com/dougywu/screenforbio-
 mbc-ailaoshan/releases/tag/1.3 [24] M 1 ` n ! < , • å

÷ Å <https://github.com/bakerccm/leeches-public/releases/tag/v1.1>
(doi:10.5281/zenodo.5914708) [31]

References

- [1] Ida Bærholm Schnell, Philip Francis Thomsen, Nicholas Wilkinson, Morten Rasmussen, Lars R.D. Jensen, Eske Willerslev, Mads F. Bertelsen, and M. Thomas P. Gilbert. Screening mammal biodiversity using DNA from leeches. *Current Biology*, 22(8):R262 – R263, 2012.
- [2] P G Taylor. Reproducibility of ancient DNA sequences from extinct Pleistocene fauna. *Molecular Biology and Evolution*, 13(1):283–285, 1996.
- [3] Tiayba Riaz, Wasim Shehzad, Alain Viari, François Pompanon, Pierre Taberlet, and Eric Coissac. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, 39(21):e145–e145, 2011.
- [4] Matthieu Leray, Joy Y Yang, Christopher P Meyer, Suzanne C Mills, Natalia Agudelo, Vincent Ranwez, Joel T Boehm, and Ryuji J Machida. A new versatile primer set targeting a short fragment of the mitochondrial coi region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, 10:34, 2013.
- [5] Ida Bærholm Schnell, Kristine Bohmann, and M. Thomas P. Gilbert. Tag jumps illuminated – reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, 15(6):1289–1303, 2015.
- [6] M. L. Zepeda-Mendoza, K. Bohmann, A. Carmona Baez, and M. T. Gilbert. DAMe: a toolkit for the initial processing of datasets with PCR replicates of double-tagged amplicons for DNA metabarcoding analyses. *BMC Research Notes*, 9:255, 2016.
- [7] Tom van der Valk, Francesco Vezzi, Mattias Ormestad, Love Dalén, and Katerina Guschanski. Index hopping on the Illumina HiSeqX platform and its consequences for ancient DNA studies. *Molecular Ecology Resources*, 20(5):1171–1181, 2020.
- [8] Mikkel Schubert, Stinus Lindgreen, and Ludovic Orlando. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes*, 9:88, 2016.
- [9] J N Joshi and N A Fass. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33), 2011.
- [10] Heng Li. BFC: correcting illumina sequencing errors. *Bioinformatics*, 31(17):2885–2887, 2015.
- [11] Andre P Masella, Andrea K Bartram, Jakub M Truszkowski, Daniel G Brown, and Josh D Neufeld. PANDAseq: paired-end assembler for Illumina sequences. *BMC Bioinformatics*, 13(1):1–7, 2012.
- [12] Torbjørn Rognes, Tomáš Flouri, Ben Nichols, Christopher Quince, and Frédéric Mahé. VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4:e2584, 2016.
- [13] F. Mahe, T. Rognes, C. Quince, C. de Vargas, and M. Dunthorn. Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ*, 3:e1420, 2015.
- [14] T. G. Frøslev, R. Kjøller, H. H. Bruun, R. Ejrnæs, A. K. Brunbjerg, C. Pietroni, and A. J. Hansen. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, 8:1188, 2017.

- [15] Jan Axtner, Alex Crampton-Platt, Lisa A Hörig, Azlan Mohamed, Charles C Y Xu, Douglas W Yu, and Andreas Wilting. An efficient and robust laboratory workflow and tetrapod database for larger scale environmental DNA studies. *GigaScience*, 8(4):giz029, 2019.
- [16] P. Somervuo, S. Koskela, J. Pennanen, R. H. Nilsson, and O. Ovaskainen. Unbiased probabilistic taxonomic classification for DNA barcoding. *Bioinformatics*, 32(19):2920–2927, 2016.
- [17] P. Somervuo, D. W. Yu, C. C. Y. Xu, Y. Q. Ji, J. Hultman, H. Wirta, and O. Ovaskainen. Quantifying uncertainty of taxonomic placement in DNA barcoding and metabarcoding. *Methods in Ecology and Evolution*, 8(4):398–407, 2017.
- [18] Ryuji J. Machida, Matthieu Leray, Shian-Lei Ho, and Nancy Knowlton. Metazoan mitochondrial gene sequence reference datasets for taxonomic assignment of environmental samples. *Scientific Data*, 4:170027, 2017.
- [19] Faezah Mohd Salleh, Jazmín Ramos-Madrigal, Fernando Peñaloza, Shanlin Liu, S Sinding Mikkel-Holger, P Patel Riddhi, Renata Martins, Dorina Lenz, Jörns Fickel, Christian Roos, Mohd Shahir Shamsir, Mohammad Shahfiz Azman, K Lim Burton, J Rossiter Stephen, Andreas Wilting, and M Thomas P Gilbert. An expanded mammal mitogenome dataset from Southeast Asia. *GigaScience*, 6(8):1–8, 2017.
- [20] Scott Chamberlain, Eduard Szoecs, Zachary Foster, Zebulun Arendsee, Carl Boettiger, Karthik Ram, Ignasi Bartomeus, John Baumgartner, James O’Donnell, Jari Oksanen, Bastian Greshake Tzovaras, Philippe Marchand, Vinh Tran, Maëlle Salmon, Gaopeng Li, and Matthias Grenié. *taxize: taxonomic information from around the web*, 2019. R package version 0.9.7.
- [21] Alexey M Kozlov, Jiajie Zhang, Pelin Yilmaz, Frank Oliver Glöckner, and Alexandros Stamatakis. Phylogeny-aware identification and correction of taxonomically mislabeled sequences. *Nucleic Acids Research*, 44(11):5022–5033, 2016.
- [22] Torrey W Rodgers, Charles C Y Xu, Jacalyn Giacalone, Karen M Kapheim, Kristin Saltonstall, Marta Vargas, Douglas W Yu, Panu Somervuo, W Owen McMillan, and Patrick A Jansen. Carrion fly-derived DNA metabarcoding is an effective tool for mammal surveys: Evidence from a known tropical mammal community. *Molecular Ecology Resources*, 17(6):e133–e145, 2017.
- [23] Szymon M Kiełbasa, Raymond Wan, Kengo Sato, Paul Horton, and Martin C Frith. Adaptive seeds tame genomic sequence comparison. *Genome Research*, 21(3):487–493, 2011.
- [24] Doug Yu. Ailaoshan version with unweighted and weighted PROTAX and M_tDORI 1.2, 2020.
- [25] Scott Chamberlain. rredlist: ‘IUCN’ red list client, 2018. R package version 0.6.0.
- [26] Kate E. Jones, Jon Bielby, Marcel Cardillo, Susanne A. Fritz, Justin O’Dell, C. David L. Orme, Kamran Safi, Wes Sechrest, Elizabeth H. Boakes, Chris Carbone, Christina Connolly, Michael J. Cutts, Janine K. Foster, Richard Grenyer, Michael Habib, Christopher A. Plaster, Samantha A. Price, Elizabeth A. Rigby, Janna Rist, Amber Teacher, Olaf R. P. Bininda-Emonds, John L. Gittleman, Georgina M. Mace, and Andy Purvis. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology*, 90(9):2648–2648, 2009.

- [27] R. M. Dorazio, J. A. Royle, B. Soderstrom, and A. Glimskar. Estimating species richness and accumulation by modeling species occurrence and detectability. *Ecology*, 87(4):842–854, 2006.
- [28] Robert M. Dorazio, Nicholas J. Gotelli, and Aaron M. Ellison. *Modern methods of estimating biodiversity from presence-absence surveys*, pages 277–302. InTech, Rijeka, Croatia, 2011.
- [29] Darryl I MacKenzie, James D Nichols, Gideon B Lachman, Sam Droege, J Andrew Royle, and Catherine A Langtimm. Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, 83(8):2248–2255, 2002.
- [30] Investigation Group of Ailaoshan Nature Reserve. *Comprehensive survey of Ailaoshan Nature Reserve*. Yunnan Ethnic Press, Kunming, Yunnan, 1988.
- [31] Christopher CM Baker, Yinqui Ji, Viorel D Popescu, Jiaxin Wang, Chunying Wu, Zhengyang Wang, Yuanheng Li, Lin Wang, Chaolang Hua, Zhongxing Yang, Chunyan Yang, Charles CY Xu, Alex Diana, Qingzhong Wen, Naomi E Pierce, and Douglas W Yu. Measuring protected-area vertebrate biodiversity using leech iDNA. GitHub repository. [<https://github.com/bakerccm/leeches-public/releases/tag/v1.1>] [doi:10.5281/zenodo.5914708], 2021.
- [32] B. J. Callahan, P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7):581–583, 2016.
- [33] William A. Link and John R. Sauer. Extremes in ecology: avoiding the misleading effects of sampling variation in summary analyses. *Ecology*, 77(5):1633–1640, 1996.
- [34] Donald B. Rubin. Bayesianly justifiable and relevant frequency calculations for the applied statistician. *The Annals of Statistics*, 12(4):1151–1172, 1984.
- [35] Andrew Gelman. Prior distributions for variance parameters in hierarchical models. *Bayesian Analysis*, 1(3):515–533, 2006.
- [36] Martyn Plummer. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling, 2017. Version 4.3.0.
- [37] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2019.
- [38] Martyn Plummer. rjags: Bayesian graphical models using MCMC, 2018. R package version 4.8.
- [39] Ken Kellner. *jagsUI: A wrapper around ‘rjags’ to streamline ‘JAGS’ analyses*, 2019. R package version 1.5.1.
- [40] Andrew Gelman and Donald B. Rubin. Inference from iterative simulation using multiple sequences. *Statistical Science*, 7(4):457–472, 1992.
- [41] Stephen P. Brooks and Andrew Gelman. General methods for monitoring convergence of iterative simulations. *Journal of Computational and Graphical Statistics*, 7(4):434–455, 1998.
- [42] Gábor Csárdi and Tamás Nepusz. The igraph software package for complex network research. *InterJournal Complex Systems*, page 1695, 2006.
- [43] Convention on Biological Diversity. Aichi Biodiversity Targets, 2010.
- [44] Lauren Coad, James EM Watson, Jonas Geldmann, Neil D Burgess, Fiona Leverington, Marc Hockings, Kathryn Knights, and Moreno Di Marco. Widespread

- shortfalls in protected area resourcing undermine efforts to conserve biodiversity. *Frontiers in Ecology and the Environment*, 17(5):259–264, 2019.
- [45] James E. M. Watson, Emily S. Darling, Oscar Venter, Martine Maron, Joe Walston, Hugh P. Possingham, Nigel Dudley, Marc Hockings, Megan Barnes, and Thomas M. Brooks. Bolder science needed now for protected areas. *Conservation Biology*, 30(2):243–248, 2016.
 - [46] Sean L Maxwell, Victor Cazalis, Nigel Dudley, Michael Hoffmann, Ana S L Rodrigues, Sue Stoltz, Piero Visconti, Stephen Woodley, Naomi Kingston, Edward Lewis, Martine Maron, Bernardo B N Strassburg, Amelia Wenger, Harry D Jonas, Oscar Venter, and James E M Watson. Area-based conservation in the twenty-first century. *Nature*, 586(7828):217–227, 2020.
 - [47] W. H. Xu, Y. Xiao, J. J. Zhang, W. Yang, L. Zhang, V. Hull, Z. Wang, H. Zheng, J. G. Liu, S. Polasky, L. Jiang, Y. Xiao, X. W. Shi, E. M. Rao, F. Lu, X. K. Wang, G. C. Daily, and Z. Y. Ouyang. Strengthening protected areas for biodiversity and ecosystem services in china. *PNAS*, 114(7):1601–1606, 2017.
 - [48] B. A. Bryan, L. Gao, Y. Q. Ye, X. F. Sun, J. D. Connor, N. D. Crossman, M. Stafford-Smith, J. G. Wu, C. Y. He, D. Y. Yu, Z. F. Liu, A. Li, Q. X. Huang, H. Ren, X. Z. Deng, H. Zheng, J. M. Niu, G. D. Han, and X. Y. Hou. China’s response to a national land-system sustainability emergency. *Nature*, 559(7713):193–204, 2018.
 - [49] Ruidong Wu, Hugh P Possingham, Guangzhi Yu, Tong Jin, Junjun Wang, Feiling Yang, Shiliang Liu, Jianzhong Ma, Xi Liu, and Haiwei Zhao. Strengthening China’s national biodiversity strategy to attain an ecological civilization. *Conservation Letters*, 68(2):e12660, 2019.
 - [50] Guopeng Ren, Stephen S. Young, Lin Wang, Wei Wang, Yongcheng Long, Ruidong Wu, Junsheng Li, Jianguo Zhu, and Douglas W. Yu. Effectiveness of China’s National Forest Protection Program and nature reserves. *Conservation Biology*, 29(5):1368–1377, 2015.
 - [51] Ruidong Wu, S Zhang, Douglas W Yu, P Zhao, X Li, Longzhu Wang, Qian Yu, Jian Ma, Ai Chen, and Yongcheng Long. Effectiveness of China’s nature reserves in representing ecological diversity. *Frontiers in Ecology and Evolution*, 9:383–389, 2011.
 - [52] Jonas Geldmann, Andrea Manica, Neil D Burgess, Lauren Coad, and Andrew Balmford. A global-level assessment of the effectiveness of protected areas at resisting anthropogenic pressures. *PNAS*, 116(46):23209–23215, 2019.
 - [53] William F Laurance, D Carolina Useche, Julio Rendeiro, Margareta Kalka, Corey J A Bradshaw, Sean P Sloan, Susan G Laurance, Mason Campbell, Kate Abernethy, Patricia Alvarez, Víctor Arroyo-Rodríguez, Peter Ashton, Julieta Benitez-Malvido, Allard Blom, Kadiri S Bobo, Charles H Cannon, Min Cao, Richard Carroll, Colin Chapman, Rosamond Coates, Marina Cords, Finn Danielsen, Bart De Dijn, Eric Dinerstein, Maureen A Donnelly, David Edwards, Felicity Edwards, Nina Farwig, Peter Fashing, Pierre-Michel Forget, Mercedes Foster, George Gale, David Harris, Rhett Harrison, John Hart, Sarah Karpany, W John Kress, Jagdish Krishnaswamy, Willis Logsdon, Jon Lovett, William Magnusson, Fiona Maisels, Andrew R Marshall, Deedra McClearn, Divya Mudappa, Martin R Nielsen, Richard Pearson, Nigel Pitman, Jan van der Ploeg, Andrew Plumptre, John Poulsen, Mauricio Quesada, Hugo Rainey, Douglas Robinson, Christiane Roetgers, Francesco Rovero, Frederick Scatena, Christian Schulze, Douglas Sheil,

Thomas Struhsaker, John Terborgh, Duncan Thomas, Robert Timm, J Nicolas Urbina-Cardona, Karthikeyan Vasudevan, S Joseph Wright, Juan Carlos Arias-G, Luzmila Arroyo, Mark Ashton, Philippe Auzel, Dennis Babaasa, Fred Babweteera, Patrick Baker, Olaf Banki, Margot Bass, Inogwabini Bila-Isia, Stephen Blake, Warren Brockelman, Nicholas Brokaw, Carsten A Brühl, Sarayudh Bunyavejchewin, Jung-Tai Chao, Jerome Chave, Ravi Chellam, Connie J Clark, José Clavijo, Robert Congdon, Richard Corlett, H S Dattaraja, Chittaranjan Dave, Glyn Davies, Beatriz de Mello Beisiegel, Rosa de Nazaré Paes da Silva, Anthony Di Fiore, Arvin Diesmos, Rodolfo Dirzo, Diane Doran-Sheehy, Mitchell Eaton, Louise Emmons, Alejandro Estrada, Corneille Ewango, Linda Fedigan, François Feer, Barbara Fruth, Jacalyn Giacalone Willis, Uromi Goodale, Steven Goodman, Juan C Guix, Paul Guthiga, William Haber, Keith Hamer, Ilka Herbinger, Jane Hill, Zhongliang Huang, I-Fang Sun, Kalan Ickes, Akira Itoh, Natália Ivanauskas, Betsy Jackes, John Janovec, Daniel Janzen, Mo Jiangming, Chen Jin, Trevor Jones, Hermes Justiniano, Elisabeth Kalko, Aventino Kasangaki, Timothy Killeen, Hen-biao King, Erik Klop, Cheryl Knott, Inza Koné, Enoka Kudavidanage, José Lahoz da Silva Ribeiro, John Lattke, Richard Laval, Robert Lawton, Miguel Leal, Mark Leighton, Miguel Lentino, Cristiane Leonel, Jeremy Lindsell, Lee Ling-Ling, K Eduard Linsenmair, Elizabeth Losos, Ariel Lugo, Jeremiah Lwanga, Andrew L Mack, Marlucia Martins, W Scott McGraw, Roan McNab, Luciano Montag, Jo Myers Thompson, Jacob Nabe-Nielsen, Michiko Nakagawa, Sanjay Nepal, Marilyn Norconk, Vojtech Novotný, Sean O'Donnell, Muse Opiang, Paul Ouboter, Kenneth Parker, N Parthasarathy, Kátia Pisciotta, Dewi Prawiradilaga, Catherine Pringle, Subraj Rajathurai, Ulrich Reichard, Gay Reinartz, Katherine Renton, Glen Reynolds, Vernon Reynolds, Erin Riley, Mark-Oliver Rödel, Jessica Rothman, Philip Round, Shoko Sakai, Tania Sanaiotti, Tommaso Savini, Gertrud Schaab, John Seidensticker, Alhaji Siaka, Miles R Silman, Thomas B Smith, Samuel Soares de Almeida, Navjot Sodhi, Craig Stanford, Kristine Stewart, Emma Stokes, Kathryn E Stoner, Raman Sukumar, Martin Surbeck, Matthias Tobler, Teja Tscharntke, Andrea Turkalo, Govindaswamy Umapathy, Merlijn van Weerd, Jorge Vega Rivera, Meena Venkataraman, Linda Venn, Carlos Verea, Carolina Volkmer de Castilho, Matthias Waltert, Benjamin Wang, David Watts, William Weber, Paige West, David Whitacre, Ken Whitney, David Wilkie, Stephen Williams, Debra D Wright, Patricia Wright, Lu Xiankai, Pralad Yonzon, and Franky Zamzani. Averting biodiversity collapse in tropical forest protected areas. *Nature*, 489(7415):290–294, 2012.

- [54] Li Yiming and David S. Wilcove. Threats to vertebrate species in China and the United States. *BioScience*, 55(2):147–153, 2005.
- [55] Paul J Ferraro, Toshihiro Uchida, and Jon M Conrad. Price premiums for eco-friendly commodities: are ‘green’ markets the best way to protect endangered ecosystems? *Environmental and Resource Economics*, 32(3):419–438, 2005.
- [56] A Zabel and B Roe. Optimal design of pro-conservation incentives. *Ecological Economics*, 69:126–134, 2009.
- [57] T Dietz, Elinor Ostrom, and Paul C Stern. The struggle to govern the commons. *Science*, 302(5652):1907–1912, 2003.
- [58] Lydia Beaudrot, Jorge A Ahumada, Timothy O’Brien, Patricia Alvarez-Loayza, Kelly Boekee, Ahimsa Campos-Arceiz, David Eichberg, Santiago Espinosa, Eric Fegraus, Christine Fletcher, Krisna Gajapersad, Chris Hallam, Johanna Hurtado, Patrick A Jansen, Amit Kumar, Eileen Larney, Marcela Guimaraes Moreira Lima, Colin Mahony, Emanuel H Martin, Alex McWilliam, Badru Mugerwa, Mireille

- Ndoundou-Hockemba, Jean Claude Razafimahaimodison, Hugo Romero-Saltos, Francesco Rovero, Julia Salvador, Fernanda Santos, Douglas Sheil, Wilson R Spironello, Michael R Willig, Nurul L Winarni, Alex Zvoleff, and Sandy J Andelman. Standardized assessment of biodiversity trends in tropical forest protected areas: the end is not in sight. *PLoS Biology*, 14(1):e1002357, 2016.
- [59] Paul D Meek, Guy A Ballard, Jess Sparkes, Mark Robinson, Brad Nesbitt, and Peter J S Fleming. Camera trap theft and vandalism: occurrence, cost, prevention and implications for wildlife research and management. *Remote Sensing in Ecology and Conservation*, 5:160–168, 2019.
 - [60] Paul Glover-Kapfer, Carolina A Soto-Navarro, and Oliver R Wearn. Camera-trapping version 3.0: current constraints and future priorities for development. *Remote Sensing in Ecology and Conservation*, 5(3):209–223, 2018.
 - [61] Andrew Tilker, Jesse F Abrams, An Nguyen, Lisa Hörig, Jan Axtner, Julie Louvrier, Benjamin M Rawson, Hoa Anh Quang Nguyen, Francois Guegan, Thanh Van Nguyen, Minh Le, Rahel Sollmann, and Andreas Wilting. Identifying conservation priorities in a defaunated tropical biodiversity hotspot. *Diversity and Distributions*, 10(1):100331–100315, 2020.
 - [62] K. Bohmann, A. Evans, M. T. P. Gilbert, G. R. Carvalho, S. Creer, M. Knapp, D. W. Yu, and M. de Bruyn. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*, 29(6):358–367, 2014.
 - [63] Kristine Bohmann, Ida B Schnell, and M Thomas P Gilbert. When bugs reveal biodiversity. *Molecular Ecology*, 22(4):909–911, 2013.
 - [64] Sébastien Calvignac-Spencer, Fabian H Leendertz, M Thomas P Gilbert, and Grit Schubert. An invertebrate stomach’s view on vertebrate ecology. *BioEssays*, 35(11):1004–1013, 2013.
 - [65] Ida Bærholm Schnell, Rahel Sollmann, Sébastien Calvignac-Spencer, Mark E Siddall, Douglas W Yu, Andreas Wilting, and M Thomas P Gilbert. iDNA from terrestrial haematophagous leeches as a wildlife surveying and monitoring tool – prospects, pitfalls and avenues to be developed. *Frontiers in Zoology*, 12(1):302, 2015.
 - [66] Rosie Drinkwater, Ida Bærholm Schnell, Kristine Bohmann, Henry Bernard, Géraldine Veron, Elizabeth L Clare, M Thomas P Gilbert, and Stephen J Rossiter. Using metabarcoding to compare the suitability of two blood-feeding leech species for sampling mammalian diversity in North Borneo. *Molecular Ecology Resources*, 19(1):105–117, 2019.
 - [67] Jan F Gogarten, Ariane Düx, Benjamin Mubemba, Kamilla Pléh, Constanze Hoffmann, Alexander Mielke, Jonathan Müller Tiburtius, Andreas Sachse, Roman M Wittig, Sébastien Calvignac-Spencer, and Fabian H Leendertz. Tropical rainforest flies carrying pathogens form stable associations with social nonhuman primates. *Molecular Ecology*, 28(18):4242–4258, 2019.
 - [68] Arthur Kocher, Benoit de Thoisy, François Catzeflis, Sophie Valière, Anne-Laure Bañuls, and Jérôme Murienne. iDNA screening: disease vectors as vertebrate samplers. *Molecular Ecology*, 26(22):6478–6486, 2017.
 - [69] Ida Bærholm Schnell, Kristine Bohmann, Sebastian E Schultze, Stine R Richter, Daithí C Murray, Mikkel-Holger S Sinding, David Bass, John E Cadle, Mason J Campbell, Rainer Dolch, David P Edwards, Thomas N E Gray, Teis Hansen, Anh Nguyen Quang Hoa, Christina Lehmkühl Noer, Sigrid Heise-Pavlov, Adam F

- Sander Pedersen, Juliet Carl Ramamonjisoa, Mark E Siddall, Andrew Tilker, Carl Traeholt, Nicholas Wilkinson, Paul Woodcock, Douglas W Yu, Mads Frost Bertelsen, Michael Bunce, and M Thomas P Gilbert. Debugging diversity - a pan-continental exploration of the potential of terrestrial blood-feeding leeches as a vertebrate monitoring tool. *Molecular Ecology Resources*, 18(6):1282–1298, 2018.
- [70] M Tessler, S R Weiskopf, L Berniker Systematics and, and 2018. Bloodlines: mammals, leeches, and conservation in southern Asia. *Systematics and Biodiversity*, 16(5):488–496, 2018.
 - [71] Niccolo Alfano, Anisha Dayaram, Jan Axtner, Kyriakos Tsangaras, Marie-Louise Kampmann, Azlan Mohamed, Seth T. Wong, M. Thomas P. Gilbert, Andreas Wilting, and Alex D. Greenwood. Non-invasive surveys of mammalian viruses using environmental DNA. *bioRxiv*, page 2020.03.26.009993, 2020.
 - [72] Jesse F Abrams, Lisa Hörig, Robert Brozovic, Jan Axtner, Alex Crampton-Platt, Azlan Mohamed, Seth T Wong, Rahel Sollmann, Douglas W Yu, and Andreas Wilting. Shifting up a gear with iDNA: from mammal detection events to standardized surveys. *Journal of Applied Ecology*, 18(3):511–512, 2019.
 - [73] S. R. Weiskopf, K. P. McCarthy, M. Tessler, H. A. Rahman, J. L. McCarthy, R. Hersch, M. M. Faisal, and M. E. Siddall. Using terrestrial haematophagous leeches to enhance tropical biodiversity monitoring programmes in Bangladesh. *Journal of Applied Ecology*, 55(4):2071–2081, 2018.
 - [74] Conrad P D T Gillett, Andrew J Johnson, Iain Barr, and Jiri Hulcr. Metagenomic sequencing of dung beetle intestinal contents directly detects and identifies mammalian fauna. *bioRxiv*, page 074849, 2016.
 - [75] D L Wu and C C Luo. Effect of human activity on community structure of small mammals in Ailao Mountain. *Zoological Research*, 14(1):35–41, 1993.
 - [76] Z. J. Wang, C. Carpenter, and S. S. Young. Bird distribution and conservation in the Ailao Mountains, Yunnan, China. *Biological Conservation*, 92(1):45–57, 2000.
 - [77] Hongmei Li, Xin Zhang, Dingqi Rao, and Hongyu Zhang. Research on the reptiles diversity in the east of Xinping Ailaoshan Nature Reserve. *Hubei Agricultural Sciences*, 51(16):3557–3559, 2012.
 - [78] W S Luo, S Y Zhao, Z Q Luo, and Q Wang. Population and distribution of *Nomascus concolor* in Jingdong jurisdiction of Ailaoshan National Nature Reserve. *Sichuan Journal of Zoology*, 26(3):600–603, 2007.
 - [79] Hongmei Li, Huixian Zhu, Lin Wang, and Jiazhong Liu. Biological characteristics and protection of *Tylototriton shanjing* at Mount Ailao in Xinping. *Journal of Chongqing College of Education*, 23(6):16–18, 2010.
 - [80] Hongmei Li. The distribution and perniciousness of *Rhabdophis subminiatus* at Ailaoshan in Xinping County. *Hubei Agricultural Sciences*, 50(4):800–801, 2011.
 - [81] Guosong Li, Xianming Yang, Hongyu Zhang, and Wei Li. Population and distribution of western black crested gibbon (*Nomascus concolor*) at Ailao Mountain, Xinping, yunnan. *Zoological Research*, 32(6):675–683, 2011.
 - [82] Dejun Kong, Fei Wu, Pengfei Shan, Jianyun Gao, Dao Yan, Weixiong Luo, and Xiaojun Yang. Status and distribution changes of the endangered green peafowl (*Pavo muticus*) in china over the past three decades (1990s–2017). *Avian Research*, 9(1):427, 2018.

- [83] X.L. He, K Luo, Z Y Lu, and L X Lin. Preliminary camera-trapping survey on wild mammals and birds in Ailaoshan National Nature Reserve, Yunnan Province, China. *Acta Theriologica Sinica*, 38(3):318–322, 2018.
- [84] Andrew J Tyre, Brigitte Tenhumberg, Scott A Field, Darren Niejalke, Kirsten Parris, and Hugh P Possingham. Improving precision and reducing bias in biological surveys: estimating false-negative error rates. *Ecological Applications*, 13(6):1790–1801, 2003.
- [85] Laurent Lelouch, Sandrine Pavoine, Frédéric Jiguet, Hervé Glotin, and Jérôme Sueur. Monitoring temporal change of bird communities with dissimilarity acoustic indices. *Methods in Ecology and Evolution*, 5(6):495–505, 2014.
- [86] Y. Q. Ji, L. Ashton, S. M. Pedley, D. P. Edwards, Y. Tang, A. Nakamura, R. Kitching, P. M. Dolman, P. Woodcock, F. A. Edwards, T. H. Larsen, W. W. Hsu, S. Benedick, K. C. Hamer, D. S. Wilcove, C. Bruce, X. Y. Wang, T. Levi, M. Lott, B. C. Emerson, and D. W. Yu. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters*, 16(10):1245–1257, 2013.
- [87] K Y Zhang, Y P Zhang, Y H Liu, and Y R Li. Vertical distribution characteristics of rainfall in the Ailao mountain. *Scientia Geographica Sinica*, 14(2):144–150, 1994.
- [88] Z Q Zhang. Status quo of the biodiversity of Ailaoshan Nature Reserve and countermeasures for protection and management. *Forest Inventory and Planning*, 32(3):68–70, 2007.
- [89] Yves Escoufier. Le traitement des variables vectorielles. *Biometrics*, 29(4):751–760, 1973.
- [90] Ronald W. Davies, L. R. Linton, and F. J. Wrona. Passive dispersal of four species of freshwater leeches (Hirudinoidea) by ducks. *Freshwater Invertebrate Biology*, 1(4):40–44, 1982.
- [91] Christopher T. Rota, Robert J. Fletcher Jr, Robert M. Dorazio, and Matthew G. Betts. Occupancy estimation and the closure assumption. *Journal of Applied Ecology*, 46(6):1173–1181, 2009.
- [92] M. Fahmy, K.M. Williams, M. Tessler, S.R. Weiskopf, E. Hekkala, and M.E. Siddall. Multilocus metabarcoding of terrestrial leech bloodmeal iDNA increases species richness uncovered in surveys of vertebrate host biodiversity. *Journal of Parasitology*, 106(6):843–853, 2020.
- [93] Shanlin Liu, Xin Wang, Lin Xie, Meihua Tan, Zhenyu Li, Xu Su, Hao Zhang, Bernhard Misof, Karl M Kjer, Min Tang, Oliver Niehuis, Hui Jiang, and Xin Zhou. Mitochondrial capture enriches mito-DNA 100 fold, enabling PCR-free mitogenomics biodiversity analysis. *Molecular Ecology Resources*, 16(2):470–479, 2016.
- [94] Cheri M Ackerman, Cameron Myhrvold, Sri Gowtham Thakku, Catherine A Freije, Hayden C Metsky, David K Yang, Simon H Ye, Chloe K Boehm, Tinna-Sólveig F Kosoko-Thoroddsen, Jared Kehe, Tien G Nguyen, Amber Carter, Anthony Kulesa, John R Barnes, Vivien G Dugan, Deborah T Hung, Paul C Blainey, and Pardis C Sabeti. Massively multiplexed nucleic acid detection with Cas13. *Nature*, 582(7811):277–282, 2020.

- [95] Paul D. N. Hebert, Peter M. Hollingsworth, and Mehrdad Hajibabaei. From writing to reading the encyclopedia of life. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1702):20150321, 2016.
- [96] Jan F Gogarten, Constanze Hoffmann, Mimi Arandjelovic, Andreas Sachse, Kevin Merkel, Paula Dieguez, Anthony Agbor, Samuel Angedakin, Gregory Brazzola, Sorrel Jones, Kevin E Langergraber, Kevin Lee, Sergio Marrocoli, Mizuki Murai, Volker Sommer, Hjalmar Kühl, Fabian H Leendertz, and Sébastien Calvignac-Spencer. Fly-derived DNA and camera traps are complementary tools for assessing mammalian biodiversity. *Environmental DNA*, 2(1):63–76, 2019.
- [97] J. A. Royle and W. A. Link. Generalized site occupancy models allowing for false positive and false negative errors. *Ecology*, 87(4):835–841, 2006.
- [98] D. A. Miller, J. D. Nichols, B. T. McClintock, E. H. C. Grant, L. L. Bailey, and L. A. Weir. Improving occupancy estimation when two types of observational error occur: non-detection and species misidentification. *Ecology*, 92(7):1422–1428, 2011.
- [99] Jim E. Griffin, Eleni Matechou, Andrew S. Buxton, Dimitrios Bormpoudakis, and Richard A. Griffiths. Modelling environmental DNA data; Bayesian variable selection accounting for false positive and false negative errors. *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, 69(2):377–392, 2020.
- [100] Piero Visconti, Stuart H. M. Butchart, Thomas M. Brooks, Penny F. Langhammer, Daniel Marnewick, Sheila Vergara, Alberto Yanosky, and James E. M. Watson. Protected area targets post-2020. *Science*, 364:239–241, 2019.
- [101] Vanessa M. Adams, Piero Visconti, Victoria Graham, and Hugh P. Possingham. Indicators keep progress honest: A call to track both the quantity and quality of protected areas. *One Earth*, 4(7):901–906, 2021.
- [102] J. Andrew Royle and James D. Nichols. Estimating abundance from repeated presence-absence data or point counts. *Ecology*, 84(3):777–790, 2003.
- [103] J. Andrew Royle. N-mixture models for estimating population size from spatially replicated counts. *Biometrics*, 60(1):108–115, 2004.
- [104] Connor M. Wood, Viorel D. Popescu, Holger Klinck, John J. Keane, R.J. Gutiérrez, Sarah C. Sawyer, and M. Zachariah Peery. Detecting small changes in populations at landscape scales: a bioacoustic site-occupancy framework. *Eco-logical Indicators*, 98:492–507, 2019.
- [105] Marie-Louise Kampmann, Ida Bærholm Schnell, Randi Holm Jensen, Jan Axtner, Adam F. Sander, Anders J. Hansen, Mads F. Bertelsen, Alex D. Greenwood, M. Thomas P. Gilbert, and Andreas Wilting. Leeches as a source of mammalian viral DNA and RNA - a study in medicinal leeches. *European Journal of Wildlife Research*, 63(2):36, 2017.
- [106] Rory Gibb, David W. Redding, Kai Qing Chin, Christl A. Donnelly, Tim M. Blackburn, Tim Newbold, and Kate E. Jones. Zoonotic host diversity increases in human-dominated ecosystems. *Nature*, 584(7821):398–402, 2020.
- [107] Yinqiu Ji. Ecec_alaishan_leeches_bioinfo_pipeline, 2020.
- [108] Antoine Guisan, Stuart B. Weiss, and Andrew D. Weiss. GLM versus CCA spatial modeling of plant species distribution. *Plant Ecology*, 143(1):107–122, 1999.

- [109] Gentile Francesco Ficetola, Eric Coissac, Stéphanie Zundel, Tiayyba Riaz, Wasim Shehzad, Julien Bessière, Pierre Taberlet, and Francois Pompanon. An *in silico* approach for the evaluation of DNA barcodes. *BMC Genomics*, 11(1):434, 2010.
- [110] Emiliano Mori, Luca Nerva, and Sandro Lovari. Reclassification of the serows and gorals: the end of a neverending story? *Mammal Review*, 49(3):256–262, 2019.
- [111] T.D. Phan, S. Nijhawan, S Li, and L. Xiao. *Capricornis sumatraensis*. The IUCN Red List of Threatened Species 2020, page e.T162916735A162916910, 2020.
- [112] James D. Nichols, Larissa L. Bailey, Allan F. O’Connell Jr., Neil W. Talancy, Evan H. Campbell Grant, Andrew T. Gilbert, Elizabeth M. Annand, Thomas P. Husband, and James E. Hines. Multi-scale occupancy estimation and modelling using multiple detection methods. *Journal of Applied Ecology*, 45(5):1321–1329, 2008.
- [113] Benedikt R. Schmidt, Marc Kéry, Sylvain Ursenbacher, Oliver J. Hyman, and James P. Collins. Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. *Methods in Ecology and Evolution*, 4(7):646–653, 2013.
- [114] Margaret E. Hunter, Sara J. Oyler-McCance, Robert M. Dorazio, Jennifer A. Fike, Brian J. Smith, Charles T. Hunter, Robert N. Reed, and Kristen M. Hart. Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. *PLoS ONE*, 10(4):e0121655, 2015.
- [115] Robert M. Dorazio and Richard A. Erickson. eDNAoccupancy: An R package for multiscale occupancy modelling of environmental DNA data. *Molecular Ecology Resources*, 18(2):368–380, 2018.
- [116] Marc Kéry and J. Andrew Royle. *Applied Hierarchical Modeling in Ecology*, volume 1. Elsevier, London, UK, 2016.