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Strong trophic specialization on guanaco within the native prey community -DNA metabarcoding of puma diet in Torres del Paine, Patagonia

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Understanding dietary composition and prey-use patterns of apex predators is fundamental for interpreting predator-prey dynamics and ecosystem functioning. In Patagonia, the diet of the puma (*Puma concolor*) has been described primarily using conventional scat analyses, while molecular approaches offer additional taxonomic resolution for detecting low-frequency prey taxa. Here, we used vertebrate DNA metabarcoding to characterize the native vertebrate component of puma diet in the Cerro Guido-Torres del Paine landscape of southern Chile. We analyzed 19 molecularly confirmed puma scat samples using mitochondrial 16S metabarcoding and quantified dietary composition using frequency of occurrence (FOO), relative read abundance (RRA), and dietary breadth. Guanaco (*Lama guanicoe*) was the dominant native vertebrate prey taxon, occurring in 68.4% of scat samples and accounting for more than 95% of native-prey reads. Dietary breadth based on native vertebrate prey was low (Levins' $B = 1.05$; standardized $B_A = 0.017$), indicating a highly concentrated native-prey dietary profile. Temporally matched camera-trap data showed that guanaco accounted for approximately 5% of independent detections during the scat-sampling months, whereas it occurred in more than two-thirds of the sampled scats; however, camera-trap detections were interpreted as an index of relative occurrence rather than true prey availability. DNA metabarcoding also detected several low-frequency native vertebrate taxa, including *Conepatus chinga*, *Rhea pennata*, and *Geranoaetus melanoleucus*. Overall, the results show that the native vertebrate prey detected in the sampled puma scats was strongly concentrated on guanaco during the study period, while additional native

taxa occurred at comparatively low frequencies. By combining vertebrate DNA metabarcoding with temporally matched camera-trap occurrence data, this study provides a molecular baseline for future investigations of spatial and temporal variation in puma diet and predator–prey relationships in Patagonia.

KEYWORDS

camera trapping, dietary breadth, DNA metabarcoding, guanaco, Patagonia, *Puma concolor*

1 Introduction

Predators can influence ecosystem structure through their effects on prey populations, species interactions, and trophic processes (Estes et al., 2011; Ripple et al., 2014; Breviglieri et al., 2017). Understanding the degree to which apex predators specialize on particular prey species is therefore fundamental for predicting predator responses to environmental change and for interpreting predator–prey dynamics across ecological systems. Although many large carnivores exhibit broad dietary capabilities at continental scales, local populations frequently concentrate on a limited subset of available prey, resulting in varying degrees of trophic specialization (Elbroch and Wittmer, 2013; Karandikar et al., 2022).

The puma (*Puma concolor*) is among the most widely distributed terrestrial carnivores in the Americas and occupies a broad diversity of habitats and prey communities (Iriarte et al., 1990; Prude and Cain, 2021; Karandikar et al., 2022). Throughout southern South America, pumas consume a wide range of vertebrates, including large herbivores, medium-sized mammals, birds, and occasionally domestic animals (Yáñez et al., 1986; Rau et al., 1991; Rau and Jiménez, 2002; Guerisoli et al., 2021). Nevertheless, numerous studies from Patagonia have identified guanaco (*Lama guanicoe*) as the dominant prey species, suggesting a predator–prey relationship strongly centered on a single large herbivore (Wilson, 1984; Iriarte et al., 1991; Franklin et al., 1999; Bank et al., 2002; Elbroch and Wittmer, 2013; De la Torre et al., 2009).

Most knowledge regarding puma diet in Patagonia derives from scat morphology or kill-site investigations (Iriarte et al., 1991; Franklin et al., 1999; Gelin et al., 2017). These methods have been instrumental in describing broad dietary patterns but may underestimate prey diversity because highly digested remains, rare prey items, and cryptic trophic interactions are often difficult to identify accurately (Pompanon et al., 2012; Deagle et al., 2019). Consequently, uncertainty remains regarding whether pumas in Patagonian ecosystems function as strict trophic specialists or whether they exploit a broader vertebrate prey spectrum that is incompletely characterized by conventional approaches.

DNA metabarcoding has emerged as a powerful tool for dietary reconstruction, enabling high-resolution taxonomic identification of prey species from fecal samples (Pompanon et al., 2012; Deagle et al., 2019; Ando et al., 2020; Wang et al., 2022). Compared with traditional morphological approaches, metabarcoding can improve the detection of low-frequency, highly degraded, soft-bodied, or morphologically indistinguishable prey taxa and reveal trophic interactions that might otherwise remain unresolved (Berry et al., 2017; Galan et al., 2018; Ando et al., 2020; Zabala et al., 2025). By

increasing prey detection and taxonomic resolution, metabarcoding also complements conventional morphological analyses rather than replacing them, providing a more comprehensive characterization of vertebrate predator diets (Cuff et al., 2023). Consequently molecular approaches offer an opportunity to reassess trophic specialization and dietary breadth in apex predators with unprecedented taxonomic resolution.

In this study, we used the mitochondrial vertebrate 16S molecular marker to characterize puma diet within a Patagonian steparic grassland ecosystem of southern Chile. Specifically, we aimed to (1) identify native vertebrate prey species consumed by pumas, (2) evaluate whether DNA metabarcoding detects low-frequency prey taxa not commonly reported by traditional dietary analyses, and (3) quantify dietary breadth within the native vertebrate prey component. Given previous evidence from Patagonia, we predicted that guanaco would dominate the native prey spectrum and the native vertebrate dietary profile would be strongly concentrated on a principal prey species.

2 Methodology

2.1 Study area

This study was conducted at Cerro Guido Farm, an area adjacent to Torres del Paine National Park. The study area is located within the Torres del Paine Biosphere Reserve (UNESCO) in southern Chilean Patagonia and comprises five distinct vegetation formations: Patagonian steppe, pre-Andean shrubland, Magellan Forest, Andean desert, and Sphagnum moss bog (Vela-Ruiz Figueroa and Repetto-Giavelli, 2017). During the summer months, from October to March, the average maximum temperature in the park reaches 15.3°C, while the average minimum temperature is around 5.9°C. In contrast, during the winter season, from May to September, the average maximum temperature drops to 6.9°C, and the average minimum temperature lowers to 0.9°C. However, temperatures can occasionally reach as low as -10°C during the winter months (Centro del Clima y la Resiliencia (CR) 2 Explorador, 2023). The park exhibits a significant annual variation in precipitation, particularly in areas closer to the southern ice fields, which are in the western side of the park. In these regions, annual precipitation levels can reach up to 1000 mm/year. On the other hand, in the eastern area of the park, precipitation amounts average around 400 mm/year. The summer season experiences the highest levels of precipitation throughout the park, while winter is

characterized by predominantly snowfall (Vela-Ruiz Figueroa and Repetto-Giavelli, 2017).

The ranch encompasses a heterogeneous landscape composed of Patagonian steppe, shrublands, deciduous *Nothofagus* forests, and Andean environments rich with rocky boulders and hilltops. The region experiences a cold-temperate climate characterized by strong winds, low temperatures, and marked seasonal variation in precipitation.

Cerro Guido Farm supports cattle and sheep production, contributing additional domestic animal resources within the broader landscape. The location and spatial extent of the study area, together with the distribution of fecal sampling sites, camera-trap stations, and monitored eagle nests, are shown in [Supplementary Figure S1](#). In addition, the area is located within one of Patagonia's most important tourism destinations, generating substantial human activity both within and around the study area (Cifuentes-Ibarra et al., 2023). The region supports a diverse vertebrate community, including native herbivores such as guanacos (*L. guanicoe*), introduced prey species such as the European hare (*Lepus europaeus*), and a variety of other vertebrates that may contribute to the diet of pumas (*P. concolor*) (Franklin et al., 1999; Ohrens et al., 2021; Elbroch et al., 2024). As the apex predator of this ecosystem, the puma plays a key role in trophic regulation and ecosystem functioning (Franklin et al., 1999; LaBarge et al., 2022).

2.2 Sample collection and handling

Puma diet was assessed using fecal samples (scats) collected non-invasively within the Cerro Guido–Torres del Paine landscape. Sampling was conducted during June, July, August, and November of 2024 and 2025, corresponding to the late winter and spring periods in southern Patagonia. The spatial distribution of fecal sample collection sites within the study area is shown in [Supplementary Figure S1](#). Candidate puma scats were initially identified in the field following standardized morphological and contextual criteria described by Guarda et al. (2010), including characteristics of the feces and associated field signs where available. Samples were collected, labeled, and stored frozen until molecular processing.

Puma presence within the study area was also monitored throughout the study period using camera traps and direct field observations, confirming the occurrence of multiple individuals within the sampled landscape. In some cases, scat samples were collected following direct observations of recognizable pumas, allowing those samples to be associated with known individuals. However, individual identity could not be assigned to all collected scats. The metabarcoding marker was therefore used to confirm species identity rather than individual identity, and the dietary dataset was interpreted at the study-area level rather than as representing 19 independent puma individuals or individual-level dietary patterns.

A total of 20 candidate puma scats were initially submitted for molecular analysis. Predator identity was subsequently verified using metabarcoding sequence data. No separate host-specific molecular assay was conducted; rather, species identity was evaluated from host-derived sequences recovered in the metabarcoding

dataset. One sample (FC14) did not contain detectable *Puma concolor* DNA and instead yielded only eagle DNA, indicating that the sample was inconsistent with puma origin and had likely been misclassified during field identification. This sample was therefore excluded from subsequent dietary analyses. *Puma concolor* DNA was detected in each of the remaining 19 samples, which were retained as molecularly confirmed puma scats for dietary analyses.

2.3 DNA extraction and quality assessment

All laboratory procedures, including DNA extraction, library preparation and metabarcoding sequencing were conducted at the AUSTRAL-omics laboratory (Universidad Austral de Chile, Valdivia Chile). DNA extraction was performed using the DNeasy PowerSoil Pro Kit (Qiagen). Approximately 250 mg of fecal material were transferred to a 2 mL PowerBead Pro tube and 800 μ L of CD1 reagent were added. Samples were subjected to mechanical disruption using bead beating (two cycles of 30 seconds at 3,400 strokes), followed by enzymatic digestion with proteinase K (20 mg/mL) and lysozyme (50 mg/mL). The mixture was incubated for 4 hours at 37°C and subsequently for 12 hours at 55°C.

Following lysis, DNA binding, washing, and elution were performed using MB Spin Columns (Qiagen). For elution, 50 μ L of buffer C6 were added directly to the center of the column, incubated for 1 hour at room temperature, and then centrifuged at 8,000 $\times g$ for 1 minute. A negative extraction control was included during the extraction procedure. DNA concentration was quantified using a Qubit 4.0 fluorometer with the dsDNA High Sensitivity Assay Kit.

2.4 Amplification of the molecular marker V16S-U

Dietary characterization targeted vertebrate prey using the mitochondrial V16S-U marker (Wang et al., 2023). DNA concentrations oscillate between 0.11 to 74 ng/ μ L and all samples were normalized prior to amplification. Amplicons were generated following the Illumina 16S Metagenomic Sequencing Library Preparation protocol using a first PCR with marker-specific primers linked to Illumina Nextera XT adapters and barcode sequences to enable multiplexing.

Amplification was performed using Platinum SuperFi II Master Mix (Thermo Fisher Scientific). Thermal cycling conditions consisted of an initial denaturation at 98°C for 2 minutes, followed by 35 cycles of denaturation at 98°C for 10 seconds, annealing at 60°C for 10 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

The primers used were:

V16S-U For: ACGAGAAGACCCYRYGRARCTT

V16S-U Rev: TCTHRRANAGGATTGCGCTGTTA

Negative controls for both extraction and PCR were included. Amplification success was evaluated using agarose gel electrophoresis.

Among the 20 candidate scat samples initially processed, the expected amplification band was observed in 10 samples during the

first PCR round. Samples without detectable amplification were subjected to an additional clean-up step and re-amplified to evaluate potential PCR inhibition; however, this did not improve amplification. Additional subsamples from the same original scats, maintained under frozen storage, were then subjected to a second DNA extraction and PCR attempt. Five additional samples subsequently yielded detectable bands, resulting in 15 of the 20 candidate samples showing visible amplification, while five samples remained without a detectable band. One of the PCR-positive samples, FC14, was subsequently excluded following taxonomic assignment because it did not contain detectable *Puma concolor* DNA and instead yielded eagle DNA, as described in Section 2.2. Thus, among the 19 molecularly confirmed puma scats retained for dietary analyses, 14 showed detectable amplification by agarose gel electrophoresis.

2.5 Library preparation

PCR products were purified using magnetic beads to remove non-specific amplification products and primer dimers and were subsequently quantified using the Qubit dsDNA High Sensitivity Assay Kit. For library construction, equal nanograms of amplicons were pooled together along with defined volumes of extraction and PCR negative controls. For samples with low amplicon concentration (<0.1 ng/ μ L), 2 μ L of PCR product were added to the pool.

Sequencing libraries were generated through a second PCR using KAPA HiFi HotStart ReadyMix (Roche) to incorporate Illumina P5 and P7 adapters together with Nextera XT indices. The amplification program consisted of an initial denaturation at 95°C for 3 minutes, followed by 8 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. Final libraries were purified using magnetic beads, eluted in 22 μ L of 10 mM Tris, and evaluated for integrity using agarose gel electrophoresis. Fragment size was verified using a Fragment Analyzer (Agilent Technologies).

2.6 Quantification of libraries and sequencing

Final libraries were quantified by fluorometry and converted to molar concentration (nM) for sequencing preparation. The libraries were diluted to a final concentration of 6 nM, pooled, and denatured using 0.2 M NaOH for 5 minutes. The final library pool was adjusted to a concentration of 820 pM and combined with a PhiX control library to increase sequence diversity.

Sequencing was performed on an Illumina NextSeq2000 platform using a 600-cycle kit with paired-end reads and FastQ output configuration. A total of 2,989,997 raw reads were obtained across all samples and controls, with less than 0.1% of reads corresponding to negative controls.

2.7 Bioinformatic processing and taxonomic assignment

Raw paired-end sequences were demultiplexed using Cutadapt v2.10 (Martin, 2011), assigning reads to samples based on sample-specific indices. Adapter and primer sequences were removed using

strict matching parameters (error rate = 0.0, no indels). Quality filtering was conducted using Trimmomatic v0.39 (Bolger et al., 2014) and PRINSEQ v0.20.4 (Schmieder and Edwards, 2011). Reads containing ambiguous bases, low-quality bases (Phred score $< Q30$), or low average quality were removed. Only reads with an average Phred quality score $\geq Q30$ were retained for downstream analyses.

Denosing and inference of amplicon sequence variants (ASVs) were performed using the DADA2 pipeline (Callahan et al., 2016). Sequencing error rates were estimated separately for forward and reverse reads. Reads were dereplicated, and ASVs were inferred independently for each sample. Forward and reverse reads were merged to reconstruct full-length amplicons, and chimeric sequences were identified and removed using a consensus approach.

To minimize the inclusion of sequencing artefacts and low-frequency errors, ASVs represented by fewer than 50 reads across all samples were excluded from further analyses. Low-abundance filtering is commonly used in metabarcoding analyses to reduce potential sequencing artefacts, although the choice of threshold can influence taxon detection (Drake et al., 2022). Taxonomic assignment was obtained using the Ribosomal Database Project (RDP) Naive Bayesian Classifier implemented in DADA2 with a minimum bootstrap confidence threshold of 50% (Wang et al., 2007). For vertebrate identification, a custom reference database was generated using CRABS v1.0.3 (Jeunen et al., 2023) from NCBI GenBank (accessed February 2026) based on the amplified marker and subsequently curated to ensure adequate representation of Chilean vertebrate species relevant to the study area. Additional mitochondrial V16S-U sequences corresponding to target species absent from the initial reference database, together with Sanger sequences generated in this study, were incorporated to produce the final reference database used for taxonomic assignment. Raw sequencing data generated in this study are available through the NCBI Sequence Read Archive under BioProject accession PRJNA1475511.

2.8 Camera trapping

Camera traps were deployed as part of a long-term puma monitoring program designed to document puma presence and facilitate the identification of different individuals across the study area. Individual pumas were distinguished from camera-trap imagery using a combination of morphological characteristics, including nose pigmentation patterns, marks behind the ears, sex when reproductive organs were visible, and distinctive scars. These observations confirmed the presence of multiple pumas within the monitored landscape. However, camera-based individual identification was not used to assign individual identity to scat samples unless scat collection was directly associated with an observed animal.

A total of 28 camera traps (CTs) were deployed at Cerro Guido between May 2024 and December 2025 as part of a puma monitoring program, accumulating 2,688 trap-nights of sampling effort. The spatial distribution of camera-trap stations in relation to fecal sampling locations is shown in Supplementary Figure S1. Camera-trap records were subsequently used to assess the relative occurrence of native prey and associated fauna across the fecal sampling area. To estimate prey species richness and relative frequency of

detection, records were filtered using a 24-hour independence criterion (Da Silva et al., 2026; Royle et al., 2009), whereby consecutive detections of the same species at the same camera within a 24-hour period were considered part of the same event.

To ensure temporal consistency between dietary and camera-trap occurrence data, only camera-trap records obtained during the same months in which fecal samples were collected (June, July, August, and November of 2024 and 2025) were retained for comparison with the dietary data. The number of independent detection events for each species was therefore used as an index of relative occurrence during the dietary sampling period rather than as an estimate of absolute abundance or true prey availability. Because camera-trap detection probabilities may differ among species as a consequence of variation in movement, habitat use, behavior, body size, and interaction with camera locations, these data were interpreted descriptively when comparing the relative occurrence of prey taxa with their detection in puma scats.

2.9 Data analysis

Sequence read counts were processed and analyzed in R (R Core Team, 2024). Prior to analysis, reads corresponding to *P. concolor* and *Homo sapiens* were removed to exclude predator DNA and potential contamination. Sequences that could not be assigned to species level were retained as “Unidentified” but were excluded from ecological interpretation. Both camera-trap occurrence comparisons and dietary analyses were restricted to native vertebrate species. Domestic animals and non-native exotic species were excluded prior to analysis to focus on ecological relationships between pumas and native prey. All calculations of relative camera-trap occurrence, dietary occurrence, relative read abundance, and dietary breadth were therefore based exclusively on native vertebrate taxa.

Amplicon sequence variant (ASV) counts were aggregated to species level by summing read counts across all ASVs assigned to the same taxon. Subsequent analyses were conducted using this species-level dataset.

Diet composition was assessed using frequency of occurrence (FOO) and relative read abundance (RRA). FOO was calculated as the proportion of samples in which each prey taxon was detected, with presence defined as detection of ≥ 1 read per sample. To evaluate the robustness of dietary patterns, a secondary assessment was conducted using a more conservative detection threshold of ≥ 10 reads.

RRA was calculated as the proportion of total prey reads represented by each taxon across all samples. RRA values were used to describe the relative contribution of prey taxa to the overall diet but were interpreted cautiously due to known biases in metabarcoding data, including differential amplification efficiency and variation in mitochondrial DNA copy number among taxa.

Dietary breadth was quantified using Levins' niche breadth index (B) (Levins, 1968), calculated as:

$$B = \frac{1}{\sum p_i^2}$$

where (p_i) represents the proportional contribution of each prey taxon based on RRA. A standardized Levins' index (B_A) was

also calculated to facilitate comparison across systems:

$$B_A = \frac{(B - 1)}{(n - 1)}$$

where (n) is the number of prey taxa. Lower values of (B_A) indicate a narrower dietary niche and greater trophic concentration.

To compare dietary detections with the occurrence of native vertebrate taxa recorded by camera traps, independent 24-hour camera-trap detection events collected during the same months as fecal sampling were summarized by species. The number of independent detections for each taxon was converted to a percentage of total camera-trap detections and used as an index of relative camera-trap occurrence. Dietary occurrence was represented by frequency of occurrence (FOO) derived from DNA metabarcoding of the scat samples. These measures were compared descriptively to examine differences between the relative frequency with which native vertebrate taxa were detected by camera traps and their occurrence in puma scats. Because camera-trap detection probabilities may differ among species, camera-derived frequencies were not interpreted as estimates of absolute abundance or true prey availability, and no formal inference of prey selection or avoidance was made.

To assess variation in diet at the individual sample level, read counts were converted to proportional composition within each sample and visualized using stacked bar plots. All data processing and visualization were performed in R using packages including *dplyr*, *tidyr*, *tidyverse* and *ggplot2*.

3 Results

A total of 2,989,997 paired-end reads were generated from the sequencing run. After quality filtering, paired-end merging, denoising, and chimera removal, 831,339 high-quality non-chimeric reads representing 2,221 ASVs were retained. Application of the abundance filter (minimum of 50 reads per ASV across all samples) retained 820,189 reads representing 1,810 ASVs for taxonomic assignment. Taxonomic classification identified vertebrate sequences corresponding to native prey, domestic animals, non-native exotic species, *Puma concolor*, *Homo sapiens*, and sequences that could not be assigned to species level. Only 0.34% of reads in the ≥ 50 -read taxonomic dataset could not be resolved to species level and were excluded from species-level ecological analyses. Reads assigned to *P. concolor* and *H. sapiens* were removed before dietary analyses, while domestic and non-native vertebrate taxa were excluded from the principal native-prey analyses presented here. Consequently, the dietary metrics reported in the main analysis were calculated using native vertebrate prey taxa.

Among the 19 molecularly confirmed puma scat samples, guanaco (*Lama guanicoe*) was detected in 13 samples (68.4%), representing the most frequently detected native vertebrate prey taxon. Other native vertebrate taxa were detected at lower frequencies, including *Geranoaetus melanoleucus* (15.8%), *Conepatus chinga* (10.5%), and *Rhea pennata* (5.3%) (Figure 1). Sequences assigned to domestic and non-native exotic species were also detected but were excluded from the native-prey analysis presented here. Therefore, all dietary metrics reported in the main analysis

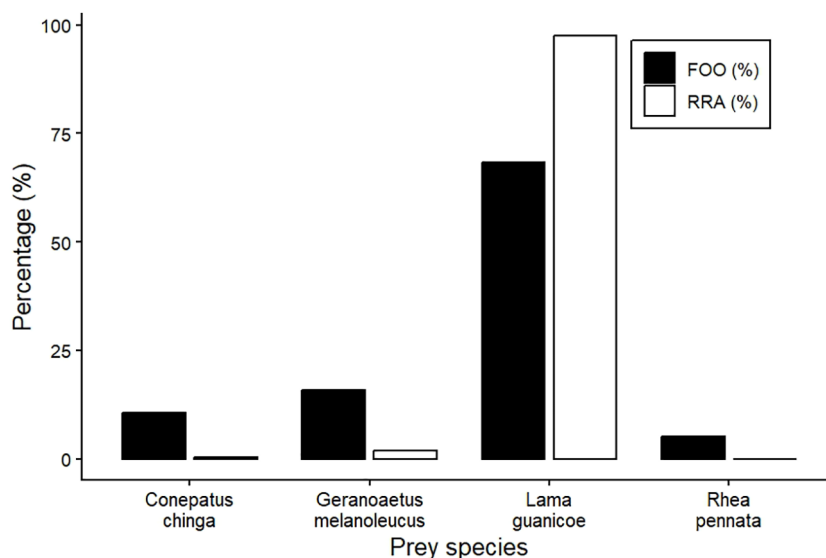


FIGURE 1

Frequency of occurrence (FOO%) and relative read abundance (RRA%) of native vertebrate prey taxa detected in puma (*Puma concolor*) fecal samples ($n = 19$) using DNA metabarcoding. FOO represents the percentage of samples in which each prey taxon was detected, whereas RRA represents the proportion of total prey reads assigned to each taxon. Together, these complementary metrics describe both the frequency and relative contribution of prey taxa to the diet.

refer specifically to native vertebrate prey taxa. Dietary patterns remained broadly similar when applying a more conservative detection threshold (≥ 10 reads). Guanaco remained the dominant prey species under this threshold, occurring in 11 out of 19 samples (57.9%), whereas *G. melanoleucus* and *C. chinga* retained the same occurrence values as under the ≥ 1 read threshold. *Rhea pennata*, which was detected under the ≥ 1 read threshold, was not retained under the ≥ 10 read threshold due to low read counts.

Relative read abundance analysis indicated a highly uneven distribution of prey reads among native vertebrate taxa. *Lama guanicoe* accounted for the majority of sequencing reads across samples, whereas all other taxa contributed only minor proportions to the total read composition (Figure 2). Niche breadth calculated from the RRA of native vertebrate prey was low, with Levins' niche breadth was $B = 1.05$, and the standardized Levins' index was $B_A = 0.017$, indicating a highly concentrated molecular dietary profile within the native prey dataset. Among the 16 scat samples containing identifiable native vertebrate prey, most were dominated by a single prey species, with guanaco comprising the majority of reads in many cases. A smaller number of samples contained multiple prey taxa, showing that more than one native prey taxon could be detected within individual scat samples. Other prey species were generally present at low proportions within samples and did not dominate individual scat-level dietary profiles (Figure 2). Together, these results show that the native vertebrate prey detected in the sampled scats was strongly concentrated on guanaco, with comparatively limited representation of other taxa.

3.2 Camera-trap occurrence and dietary detections

Camera-trap surveys conducted during the scat collection period recorded 1,627 independent 24-hour detection events. *Lycalopex* spp. accounted for the highest proportion of camera-

trap detections among the native vertebrate taxa included in the comparison (28.0%) was followed by *C. chinga* (12.2%) (Figure 3). In contrast, guanaco (*L. guanicoe*) accounted for 5.0% of independent camera-trap detections during the corresponding sampling months. Despite its lower relative detection frequency in the camera-trap dataset, guanaco was the most frequently detected native prey taxon in puma scats (68.4% FOO).

Relative camera-trap detection frequency and dietary occurrence showed contrasting patterns among native vertebrate taxa (Figure 3; Table 1). Guanaco (*L. guanicoe*) accounted for 4.98% of independent camera-trap detections but was detected in 68.4% of the molecularly confirmed puma scat samples. In contrast, *Lycalopex* spp. accounted for 28.03% of camera-trap detections but were not detected in the sampled scats. *C. chinga* represented 12.23% of camera-trap detections and occurred in 10.53% of scat samples. *G. melanoleucus* and *R. pennata* represented only 0.25% and 0.12% of camera-trap detections, respectively, but were detected in 15.79% and 5.26% of scat samples. These comparisons are descriptive because camera-trap detection frequency represents an index of relative occurrence rather than true prey availability, and detection probability may differ among species.

4 Discussion

This study provides molecular evidence of a strongly guanaco-dominated native vertebrate prey profile in the sampled puma scats from the Cerro Guido–Torres del Paine landscape. When analyses were restricted to native vertebrate prey, guanaco overwhelmingly dominated the dietary dataset, accounting for 68.4% of prey occurrences and more than 95% of total prey reads. The extremely low dietary breadth observed (Levins' standardized niche breadth,

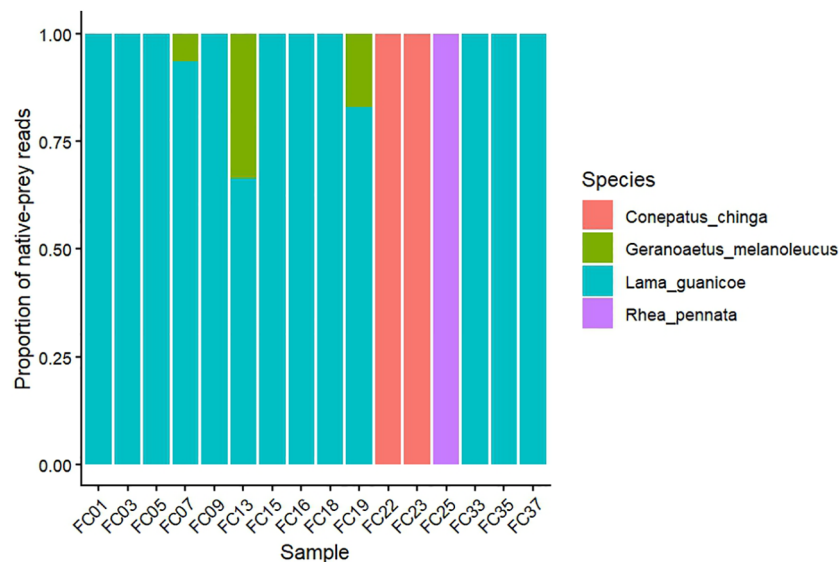


FIGURE 2

Sample-level dietary composition of puma (*Puma concolor*) based on relative read abundance of native vertebrate prey taxa across fecal samples. Sixteen of the 19 molecularly confirmed puma scats contained identifiable native vertebrate prey and are shown here; the remaining three samples contained no identifiable native vertebrate prey and were therefore not represented in the stacked-bar plot. Each bar represents one scat sample, and the height of each colored segment represents the proportion of native-prey sequencing reads assigned to that taxon within the sample. Read proportions were calculated after restricting the analysis to native vertebrate prey taxa and should not be interpreted as direct estimates of prey biomass consumed.

($B_A = 0.017$)) indicates a highly concentrated molecular dietary profile within the native vertebrate prey dataset, rather than broad use of the native prey taxa detected in the sampled scats. These findings are consistent with previous studies from Patagonia, which have repeatedly identified guanaco as the principal prey of pumas in terms of both occurrence and biomass contribution (Wilson, 1984; Iriarte et al., 1991; Franklin et al., 1999; Bank et al., 2002; Elbroch

and Wittmer, 2013; Zanón Martínez et al., 2016). The metabarcoding approach used here also enabled species-level detection of several low-frequency native vertebrate taxa alongside the dominant guanaco signal, providing additional resolution of the native-prey spectrum represented in the sampled scats.

The strong representation of guanaco in the sampled scats, despite its relatively low frequency in the camera-trap dataset,

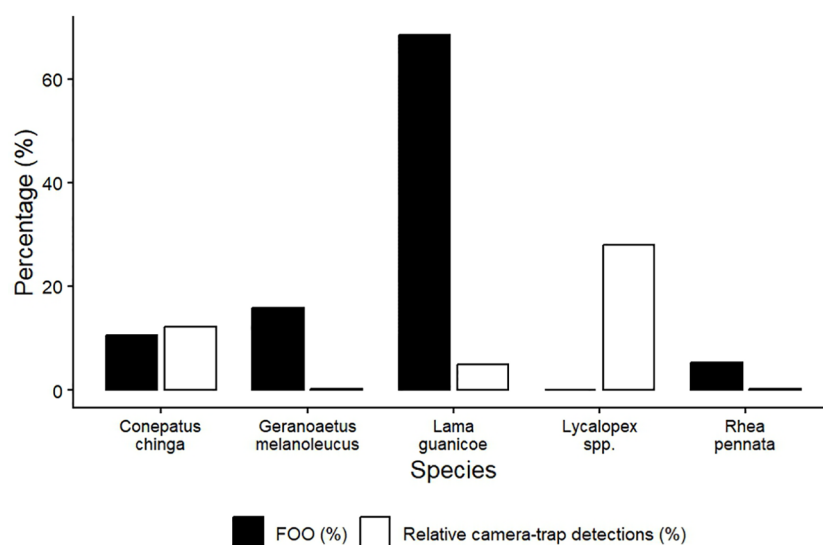


FIGURE 3

Comparison of dietary frequency of occurrence and relative camera-trap detection frequency of native vertebrate taxa in the Cerro Guido–Torres del Paine landscape. Dietary occurrence was quantified as frequency of occurrence (FOO%) from vertebrate DNA metabarcoding of 19 molecularly confirmed puma (*Puma concolor*) scat samples. Relative camera-trap occurrence was calculated from independent 24-hour detection events for each taxon. Camera-trap data were restricted to the same months in which scat samples were collected (June, July, August, and November of 2024 and 2025). Camera-trap detection frequencies are interpreted as an index of relative occurrence rather than as estimates of absolute abundance or true prey availability.

TABLE 1 Relative camera-trap detection frequency and dietary frequency of occurrence (FOO) of native vertebrate taxa during the scat-sampling period.

Species	Camera-trap detections (%)	FOO_percent
<i>Geranoaetus melanoleucus</i>	0.25	15.79
<i>Rhea pennata</i>	0.12	5.26
<i>Lama guanicoe</i>	4.98	68.42
<i>Conepatus chinga</i>	12.23	10.53
<i>Lycalopex spp</i>	28.03	0.00

suggests that encounter frequency alone may not explain its prominence in the native-prey diet. Guanaco accounted for less than 5% of independent camera-trap detections during the sampling period but occurred in more than two-thirds of the molecularly confirmed puma scats. This contrast should not be interpreted as formal evidence of prey selection, because camera-trap detections provide an index of relative occurrence rather than a direct measure of prey availability and may be influenced by species-specific differences in detectability, movement, habitat use, and behavior. Nevertheless, the energetic profitability of guanaco represents one plausible ecological explanation for its high dietary representation. Large-bodied prey can provide substantially greater energetic returns per successful hunt than smaller prey species, which may favor their use by larger carnivores when they are accessible (Karandikar et al., 2022). Previous studies in Patagonia have likewise documented the importance of guanaco in puma diets (Bank et al., 2002; Elbroch and Wittmer, 2013), supporting the ecological plausibility of this interpretation. However, prey profitability, encounter rates, kill success, and energetic intake were not directly measured in the present study, and therefore the mechanisms underlying guanaco dominance cannot be determined from these data alone. Camera-trap records were restricted to the months in which fecal samples were collected (June, July, August, and November); consequently, the observed contrast between camera-trap occurrence and dietary detections reflects conditions during the sampled periods rather than year-round predator-prey relationships.

Because the present study was designed to evaluate trophic relationships within the native vertebrate prey community, introduced species and domestic animals were excluded from both the dietary analyses and the camera-trap occurrence comparisons. Consequently, the ecological patterns described here should be interpreted specifically as interactions between pumas and native vertebrate prey rather than as a characterization of the complete prey spectrum exploited by pumas in Patagonia. Previous studies have demonstrated that introduced species, particularly the European hare (*Lepus europaeus*), can contribute substantially to puma diets under some ecological conditions (Iriarte et al., 1991; Franklin et al., 1999; Zanón Martínez et al., 2016; Guerisoli et al., 2021; Gheler-Costa et al., 2018). Accordingly, the results presented here describe the composition of the native vertebrate component of the sampled diet and should not be interpreted as a complete reconstruction of all prey consumed by pumas in the study landscape.

Within the native prey community, a notable contrast was observed for *Lycalopex* spp. Although foxes represented the most

frequently detected native vertebrate in the camera-trap survey, accounting for more than 28% of independent detections, they were not detected in any of the 19 molecularly confirmed puma scat samples. This absence should not be interpreted as evidence of active avoidance, particularly given the limited number of scat samples and the fact that camera-trap detections represent an index of relative occurrence rather than true prey availability. Previous studies from Patagonia have similarly reported foxes only as occasional prey of pumas. Iriarte et al. (1991) included *Lycalopex* spp. within a pooled carnivore category representing approximately 3.5% of prey occurrences, while Franklin et al. (1999) likewise described foxes as minor dietary components. Together, these observations are consistent with foxes being infrequently represented in puma diets in this region, although their absence from the present scat dataset does not allow us to determine whether this reflects prey preference, encounter rates, hunting behavior, or other ecological processes.

Although guanaco overwhelmingly dominated the native vertebrate prey profile, DNA metabarcoding also detected several additional native vertebrate taxa, including *Conepatus chinga*, *Rhea pennata*, and *Geranoaetus melanoleucus*. Individually, these species contributed little to overall native-prey read composition, indicating that the sampled diet was not exclusively composed of guanaco despite its strong dominance. Similar patterns have been reported elsewhere in South America, where pumas exhibit considerable dietary plasticity across their geographic range while local diets may be strongly dominated by one or a few prey species (Guerisoli et al., 2021; Karandikar et al., 2022). The low-frequency detections observed here indicate occasional inclusion of alternative native vertebrate taxa in the sampled scats; however, their energetic importance and the circumstances under which they were consumed cannot be determined from metabarcoding data alone.

Among the low-frequency prey detections, *G. melanoleucus* was particularly noteworthy. To our knowledge, *G. melanoleucus* has not previously been reported as a dietary item of pumas in Patagonia, making its detection in the present study particularly noteworthy. Birds are generally considered minor components of puma diets, and avian prey may be difficult to resolve to species level when highly digested remains are examined using morphological methods alone. The detection of *G. melanoleucus* therefore highlights the capacity of DNA metabarcoding to provide species-level taxonomic resolution for low-frequency dietary components. However, the presence of DNA from this taxon in puma scats does not by itself demonstrate active predation. Metabarcoding cannot distinguish among active predation, scavenging, secondary ingestion, or other feeding pathways, and the ecological interpretation of this low-frequency detection should therefore remain cautious.

The application of DNA metabarcoding provided detailed taxonomic information on the native vertebrate component of puma diet in this ecosystem. In addition to confirming the overwhelming importance of guanaco, the method detected several low-frequency native vertebrate taxa, including taxa that may be difficult to identify to species level from highly digested remains using morphological examination alone (Pompanon et al., 2012; Deagle et al., 2019; Zabala et al., 2025). These findings demonstrate the

utility of metabarcoding for resolving low-frequency dietary components, although the present study did not directly compare molecular and morphological identification methods. Consequently, the results should not be interpreted as demonstrating that metabarcoding necessarily provides a more complete dietary reconstruction than conventional methods in this system. Rather, the molecular dataset provides a baseline for future comparisons across seasons, years, and landscapes using consistent molecular approaches. Such temporal and spatial replications will be particularly valuable in Patagonian ecosystems, where tourism, wildlife management, livestock production, and environmental change have the potential to alter predator–prey interactions and the composition of prey consumed by puma over time (Cifuentes-Ibarra et al., 2023; Elbroch et al., 2024).

Several limitations should be considered when interpreting these findings. First, the relatively small sample size ($n = 19$) limits inference regarding rare prey taxa and potential temporal variation in diet. Although multiple pumas were documented within the study landscape, individual identity could not be assigned to all scat samples; therefore, repeated sampling of the same individual or feeding event cannot be excluded. The dataset should consequently be interpreted as 19 molecularly confirmed puma scat samples rather than as representing 19 independent puma individuals. Second, sampling was restricted to June, July, August, and November of 2024 and 2025, and therefore the dietary patterns observed here may not represent year-round puma diet. In addition, dietary breadth was calculated specifically for the native vertebrate component of the diet; because domestic and introduced prey were excluded from the principal analyses, the resulting niche-breadth estimates should not be interpreted as measures of the complete trophic niche of pumas in the study area. Third, camera-trap data were restricted to the months corresponding to fecal collection and were used as an index of relative species occurrence rather than as a measure of absolute abundance or true prey availability. Species-specific differences in movement, habitat use, behavior, and detectability may therefore influence comparisons between camera-trap detections and dietary occurrence. Finally, although DNA metabarcoding is highly effective for identifying prey DNA and detecting low-frequency taxa, relative read abundance may be influenced by differential amplification efficiency, DNA degradation, and variation in mitochondrial DNA copy number among taxa (Deagle et al., 2019; Wang et al., 2022). Metabarcoding also cannot reliably distinguish among active predation, scavenging, secondary ingestion, or other feeding pathways, nor can it directly estimate prey biomass consumed, kill rates, or prey age classes (Schenk et al., 2019). Consequently, DNA metabarcoding should be viewed as complementary to established approaches such as camera trapping, kill-site investigations, and behavioral observations.

Collectively, our findings show that the native vertebrate component of the sampled puma diet was strongly concentrated on guanaco during the study period. Although DNA metabarcoding detected several low-frequency native vertebrate taxa, these contributed minimally to overall dietary composition represented in the sampled scats and did not alter the pronounced dominance of guanaco. Rather than demonstrating population-

level trophic specialization, the results provide evidence of a narrow, guanaco-dominated native-prey dietary profile across the 19 molecularly confirmed puma scat samples. By integrating vertebrate DNA metabarcoding with temporally matched camera-trap occurrence data, this study provides a molecular baseline for evaluating puma–prey relationships in this Patagonian landscape and for future investigations of spatial and temporal variation in puma diet.

5 Conclusion and future directions

This study shows that the native vertebrate component of the sampled puma diet in the Cerro Guido–Torres del Paine landscape was strongly dominated by guanaco (*Lama guanicoe*) during the study period. Across all dietary metrics, guanaco (*L. guanicoe*) overwhelmingly dominated the native-prey profile, while other native vertebrate taxa contributed only minimally to overall dietary detections and read composition. Together, frequency of occurrence, relative read abundance, and dietary breadth indicate a highly concentrated, guanaco-dominated native-prey dietary profile across the 19 molecularly confirmed puma scat samples.

Beyond confirming the dominant role of guanaco, vertebrate DNA metabarcoding enabled species-level identification of several low-frequency native vertebrate taxa, including prey components that may be difficult to resolve from highly digested remains using morphological approaches alone. The combination of molecular dietary data with temporally matched camera-trap occurrence data provided complementary information on prey detected in puma scats and vertebrate occurrence within the study landscape. However, camera-trap detections should be interpreted as an index of relative occurrence rather than true prey availability.

Future studies incorporating larger sample sizes, year-round sampling, temporal replication across multiple years, individual identification or genetic assignment of scat samples where feasible, and complementary field approaches such as camera trapping, kill-site investigations, and movement ecology will improve understanding of spatial and temporal variation in puma foraging ecology. Integrating these approaches will also help clarify how environmental change, prey dynamics, and human activities influence predator–prey interactions across Patagonian ecosystems.

Overall, this study provides a molecular baseline for investigating puma diet in Patagonia and highlights the utility of DNA metabarcoding for characterizing both dominant and low-frequency native vertebrate dietary components.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI Sequence Read Archive, accession PRJNA1475511.

Ethics statement

The animal study was approved by Pontificia Universidad Católica de Chile. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

NK: Visualization, Formal analysis, Writing – original draft, Writing – review & editing. SH: Funding acquisition, Writing – review & editing, Data curation, Investigation. CA: Conceptualization, Validation, Writing – review & editing. AS: Data curation, Writing – review & editing, Methodology, Formal analysis. SQ-C: Methodology, Writing – review & editing, Data curation. MJ: Writing – review & editing, Investigation. SR: Writing – review & editing, Investigation. PV: Writing – review & editing, Data curation, Investigation. DÁ: Data curation, Writing – review & editing. CB: Funding acquisition, Supervision, Writing – review & editing, Methodology, Conceptualization.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2026.1931122/full#supplementary-material>

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