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Using reliable predator identification to investigate feeding habits of Neotropical carnivores (Mammalia, Carnivora) in a deforestation frontier of the Brazilian Amazon

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Abstract: Accurate identification of predator species is a critical requirement to investigate their diet using faecal samples. We used non-invasive sampling and two methods of predator identification to investigate the diets of sympatric carnivores in a highly deforested region of the Brazilian Amazon. Of 108 scats, 81 could be identified at the species level using DNA sequencing and/or trichology. The former performed better than the latter (81.5% vs. 54.3% of the identified samples), and results were quite congruent (89.7% concordance in the 29 samples that could be assessed with both approaches). Nine species were identified, out of which four (crab-eating fox, ocelot, puma and jaguar) presented a sufficient number of samples to allow dietary analyses. The crab-eating fox was the most generalist ($BA=0.92$); ocelots focused on small- to

medium-sized prey; pumas fed mostly on medium-sized items; and jaguars mostly targeted large-sized prey. A considerable overlap was observed between ocelots and pumas in all estimations ($O=0.47-0.83$). The presence of jaguars in the same region could be driving pumas to select medium- and small-sized prey. The results of this study highlight the importance of reliable predator identification and the need for in-depth ecological studies in areas where carnivore species are sympatric.

Keywords: carnivorans; diet; DNA barcoding; trichology.

Introduction

Carnivores are considered to be very important ecosystem components (Miller and Rabinowitz 2002), with their absence resulting in biodiversity loss and other ecological changes (Terborgh et al. 2001). Competition for food resources associated with dietary flexibility has been inferred to play a relevant role in various carnivore guilds. Therefore, dietary studies of sympatric carnivores are crucial to understand these processes, as they allow comparative assessments of their feeding strategies and competitive interactions.

Dietary studies of wild carnivores often rely on the identification of prey items in faecal samples. In an area harbouring several sympatric carnivores, it is therefore critical to reliably identify the species of origin of each sample (Farrell et al. 2000). Traditional methods to identify predator species from faecal samples include monitoring latrine sites, using tracks found near the scat or features such as odour and morphology (size, shape) (Pedó et al. 2006, De la Torre and De la Riva 2009). However, these techniques have often been found to be inconsistent and unreliable, as there are frequent overlaps in these features among sympatric carnivore species (Farrell et al. 2000). Consequently, other techniques have been developed. One of these methods, trichology, employs microscopy of predator guard hairs (swallowed during self-grooming), and has been used in numerous dietary studies (e.g. Gatti et al. 2006, Quadros and Monteiro-Filho 2006a,b, Silva-Pereira 2009, Rocha-Mendes et al. 2010). Another

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technique is to use DNA sequences (i.e. a DNA barcoding approach), which has been shown to be a powerful tool for identifying carnivore species (Farrell et al. 2000, Chaves et al. 2012). As a consequence, several studies have already used DNA-based approaches to identify carnivores in dietary studies (e.g. Farrell et al. 2000, Napolitano et al. 2008, Martínez-Gutiérrez et al. 2015, Morin et al. 2016). As both trichology and DNA barcoding are now commonly used in these studies, it is interesting to compare their performance with the same set of samples to assess their relative efficiency and reliability.

The Amazon forest hosts ~25% of global biodiversity, including jaguars *Panthera onca* (Linnaeus, 1758), pumas *Puma concolor* (Linnaeus, 1771) and ocelots *Leopardus pardalis* (Linnaeus, 1758) (Malhi et al. 2008, Macdonald and Loveridge 2010). However, this biome has been suffering impacts such as climate change and deforestation (Malhi et al. 2008, Barlow et al. 2016). Currently, the epicentre of Amazon deforestation is known as the “deforestation arc”, located in Eastern and Southern Amazonia. The region of Alta Floresta, included in this arc, is one of the most deforested areas of the Brazilian Amazon, and thus has become highly fragmented, resulting in both habitat loss and isolation of the remaining natural patches. Michalski et al. (2008) reported high deforestation rates in the area since the early 1980s, with the original forest cover declining from 91% in 1984 to 42% in 2004 and 35% in 2016 (Michalski and Peres 2016) on the southern bank

of the Teles Pires River, driven by economic activities such as cattle ranching and farming. In this region, Michalski and Peres (2007) recorded 15 carnivore species, from the top predator, jaguar, to the opportunistic crab-eating fox *Cerdocyon thous* (Linnaeus, 1766).

Few studies have so far analysed the diet of carnivores in the Amazon forest (e.g. Rosas et al. 1999, Ramalho 2006, Cabral et al. 2010, these studies used latrine sites, tracks found near the scat, odour and/or size to identify predators) and no published study on carnivore diet has focused on the southern Brazilian Amazon. Therefore, in the present study, we aimed to compare two methods of predator species identification based on scat samples (hair microscopy vs. DNA barcoding) so as to make broader recommendations on their applicability in this field. In addition, incorporating these results in predator identification, we aimed to describe and compare the diet of sympatric carnivores in one of this area.

Materials and methods

Study area

We carried out this study in Alta Floresta municipality (09°53'S, 56°28'W), located in northern Mato Grosso state, in the southern Brazilian Amazon. The study area (Figure 1)

Figure 1: Map of the study area in Alta Floresta, Brazil, and the classified Landsat 5 TM image showing the location of the 108 carnivore scat samples (orange circles) included in the analyses.

encompassed at least 7295 km² (Michalski and Peres 2017). The climate was classified by Köppen (1948) as Amazon (tropical monsoon climate), also known as a “tropical wet climate”. The average annual precipitation is 2350 mm. The mean annual temperature is 24.5°C, and there is high relative air humidity (80–85%) (Radam-Brasil 1983).

Faecal sampling and identification of predator species from scats

Carnivoran scats were collected opportunistically from October 2007 to December 2008, and identified at the species level using two different approaches: a molecular (DNA barcoding) assay and a trichological approach (microscopy-based analysis of guard hairs). The former is based on the polymerase chain reaction (PCR) amplification and sequencing of short segments of the mitochondrial DNA (mtDNA), followed by comparisons to a reference database (Michalski et al. 2011, Chaves et al. 2012). For this molecular approach, a portion of ~5 cm in length of each scat was collected, immersed in 96% ethanol and stored at –20°C prior to DNA extraction. The remaining portion of each scat was stored in a zip-lock bag for use in the hair microscopy-based carnivore identification and in the dietary analysis.

The identification using hair microscopy was performed independently from the DNA-based approach. We searched for predator hairs contained in the scats, and then isolated them from the remaining material; this procedure was possible as prey hairs are much more numerous and form tufts, while predator hairs are usually scarce and dispersed individually. Subsequently, carnivore hairs were prepared on glass slides following the technique developed by Quadros and Monteiro-Filho (2006b). Hair microstructures (hair medulla and cuticular scales) were compared to a collection of slides prepared specifically for this study, using hairs collected from reference specimens of multiple carnivoran species housed in the mammalogy collection of Museu de Ciências Naturais – Fundação Zoológica do Rio Grande do Sul (MCN-FZB/RS).

To test the efficacy and reliability of these methods, we: (i) evaluated the number of samples identified by each of them relative to the total number; and (ii) evaluated if both methods identified the same species in cases where both could be applied and compared their results with a “blind test”. In addition, we tested the identification method that is based on the perimeter of the scats, using a tape measure on all scat samples that were not deformed. Using the identifications originated by molecular and/or hair microscopy methods, we compared the perimeter of

scats between different species, and tested its significance using Student’s t-test with a 99% confidence level.

Dietary data collection

Faecal samples were washed with flowing water using two sizes of fine-mesh filters (1.0 and 0.5 mm). The organic material was then separated, with remaining muscles of vertebrates and invertebrates being immersed in ethanol 70%, whilst feathers, plants, teeth, claws, bones, etc. were sun-dried in thin paper bags for 3 days to avoid fungal growth (adapted from Carss and Parkinson 1996). For every scat sample, we created a record with information on all the food items it contained, which were initially identified to a broad taxonomic level (i.e. mammal, bird, non-avian reptile, amphibian, etc.).

We identified food items by examining the undigested material, which varied among prey groups. Small mammals were identified through the comparative study of claws, hairs and molars with specimens housed in the Laboratório de Mamíferos – Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo (LMUSP). Medium-sized and large mammals were identified using a hair reference collection built from specimens housed in the following museums: Museu de Ciências e Tecnologia – Pontifícia Universidade Católica do Rio Grande do Sul (MCT-PUCRS), Museu de Ciências Naturais – Fundação Zoológica do Rio Grande do Sul (MCN-FZB) and Museu Nacional – Universidade Federal do Rio de Janeiro (MN-UFRJ). Bird feet, beaks and feathers were compared macroscopically to a reference collection from MCT-PUCRS, in addition to slides with feathers used in microscopical identification compared to an identification key (Brom 1986). A reference collection from the Laboratório de Ictiologia of Universidade Federal do Rio Grande do Sul (UFRGS) was used for fish identification. Reptiles and invertebrates were identified by zoologists from UFRGS working on these groups.

Dietary analysis

Quantitative dietary analyses were performed only for species with sufficient sample size ($n \geq 8$). The frequency of occurrence (FO) was quantified by the presence of the food item in the total faecal samples. In addition, we measured its proportion of occurrence (PO) by dividing the total number of occurrences of that particular prey by the total number of items (Wang 2002).

The *FO* tends to overestimate the relative consumption of small items (Villa-Meza et al. 2002) and underestimates the relative consumption of large prey (Weaver 1993). To overcome this problem, some studies with pumas (Ackerman et al. 1984) and bobcats (*Lynx rufus*, Baker et al. 1993, 2001) developed a correction algorithm based on feeding trials in captivity using known prey of different body sizes. Studies with Asian and South American fauna have used these equations in mammalian carnivores with body sizes similar to the original species (Villa-Meza et al. 2002, Andheria et al. 2007, Martins et al. 2008). In the present study, the correction algorithm for puma was used for the puma and jaguar samples ($Y = 1.98 + 0.035X$), while the bobcat correction was used for ocelots ($Y = 16.63 + 4.09X$), where X is the live weight of the prey (Ackerman et al. 1984, Villa-Meza et al. 2002, Azevedo 2008, Martins et al. 2008). We only applied the index to the mammalian prey of these three felids, as this was the group for which we were able to perform the most precise identification. It was also the most frequent category of vertebrate prey in the diet of all three cats. Live weight estimates of prey items were based on published references (Ackerman et al. 1984, Emmons 1987, Bonvicino et al. 2008). With respect to smaller prey (<1 kg), we did not calculate the correction factor because predators usually ingest the entire animal (Ackerman et al. 1984, Campos 2009).

The relative biomass (*RB*) of each item i in the diet of species k was calculated using the correction factor (Y) and the *FO* (Ackerman et al. 1984, Villa-Meza et al. 2002, Andheria et al. 2007): $RB_{ki} = [(FO_{ki} \cdot Y_{ki}) / \sum_{i=1}^n FO_{ki} \cdot Y_{ki}] \cdot 100$. Prey items were divided into three body size categories: small (<1 kg), medium (1–15 kg), and large (>15 kg) (Iriarte et al. 1990). To evaluate the importance of each prey item in the diet of jaguars, pumas and ocelots, the index of relative importance (*IRI*) from Pinkas et al. (1971) was measured as: $IRI = (PO + RB)FO$.

We used the normalized Levins' measure of standardized niche breadth (Hulbert 1976, see also Krebs 1999) to estimate the food niche breadth of each of these three carnivorous species, as follows:

$$\hat{B}_A = (\hat{B} - 1) / (n - 1)$$

where B is Levins' measurement ($\hat{B} = 1 / \sum_{i=1}^n \hat{p}_i^2$), $\hat{p}_i$ is the fraction of items in the diet that belong to food category i ; and n is the number of possible resource states. The values of niche breadth can range from 0 (small diversity of prey consumed at high frequencies, i.e. the predator is more of a specialist than a generalist) to 1 (resources are used in similar frequencies, i.e. the predator is more of a generalist) (Krebs 1999).

The dietary niche overlap (O) between these three felids was calculated using two indices in order to compare their results: Pianka's (1973) measure:

$$O_{12} = O_{21} = \sum_{i=1}^n (p_{ki} \cdot p_{li}) / \sqrt{\sum_{i=1}^n p_{ki} \cdot \sum_{i=1}^n p_{li}},$$

and Czekanowski's index (1913), also known as Sørensen's index (1948):

$$O_{12} = O_{21} = 1.0 - 0.5 \cdot \left[\sum_{i=1}^n (p_{ki} - p_{li}) \right],$$

where p_{ki} and p_{li} are the proportions of resource i in the diet of the species k and l , respectively.

These indices range from 0 (no overlap) to 1 (complete overlap) (Sørensen 1948, Krebs 1999). The niche overlap was measured considering the *PO* of all vertebrate items, and using the *RB* of mammalian prey for both indices. The bootstrap of niche overlap between species was calculated using the function `niche.overlap.boot`, with 999 iterations and a confidence interval between 0.025 and 0.975. We carried out these analyses using the package “spaa” (Zhang 2016) of the software R 3.2.3. (R Development Core Team 2015). We also tested these same data (*PO* and *RB*) for significance of niche overlap by comparing the observed values with values obtained by randomizing the original matrices (5000 iterations), using the default procedure (RA3) implemented in the package “EcoSimR” 0.1.0 (Gotelli et al. 2015) in R. Additionally, we used the Bray-Curtis similarity coefficient (Bray and Curtis 1957) to examine the similarity in the *PO* of vertebrate items among the three species. The Bray-Curtis similarity coefficient for species k is defined as:

$$S = \left[\sum (x_{ki} - x_{kj}) / (\sum (x_{ki} + x_{kj})) \right]$$

where x_{ki} is the count of the i th prey species for predator species k . We used the package “vegan” (Oksanen et al. 2017) in R to calculate Bray-Curtis similarity and to perform a subsequent cluster analysis.

Arthropod fragments and plant material were not included in the analyses of the three felid species (Villa-Meza et al. 2002). However, these items were included in the *Cerdocyon thous* analysis, as they may play a more important role in its diet (Jácomo et al. 2004, Gatti et al. 2006, Rocha et al. 2008). Thus, the niche overlap between felids and *C. thous* was not calculated in this study.

Results

Methods of predator identification

A total of 108 faecal samples was collected, and of these, 81 could be identified at the species level. Of these samples, 66 (81.5%) were identified using the molecular method (as reported by Michalski et al. 2011), while 44 (54.3%) were identified with trichology. Twenty-nine samples could be identified with both methods (Table 1), allowing a comparison of their performance. We observed a concordance of 89.7% in species identification, and thus a 10.3% ($n = 3$)

discrepancy in the identifications (Table S1). We chose to preferentially follow the molecular identification, as this approach always included reagent-only controls, decreasing the chance of contamination, while trichology involves a subjective assessment of hair microstructural patterns.

We used the 66 scats that were successfully identified with the molecular approach, along with the 15 samples that were only identified with trichology to investigate the diets of predator species. In this combined sample set, we identified nine different predator species (Table 1). With regard to the trichology approach, of all the samples that contained guard hairs ($n = 65$), we identified 44 to the species level, five at the family level (all Felidae, Table S1)

Table 1: Number of faecal samples from Alta Floresta, Brazil, whose source predator species could be successfully identified with the molecular approach, trichology or both methods.

Predators	Molecular ^a	Hair microscopy	Congruence in both methods	Total
Felidae				
<i>Panthera onca</i>	13	7	4	16
<i>Puma concolor</i>	7	2	1	8
<i>Puma yagouaroundi</i>	2	1 (2) ^b	0	3
<i>Leopardus pardalis</i>	16	10 (11) ^b	8	18
Canidae				
<i>Cerdocyon thous</i>	24	18 (19) ^b	13	29
Mustelidae				
<i>Lontra longicaudis</i>	1	1	0	2
<i>Pteronura brasiliensis</i>	2	0	0	2
<i>Eira barbara</i>	0	2	0	2
<i>Speothos venaticus</i>	1	0	0	1
Total	66	41 (44) ^b	26 ^c	81

^aData from Michalski et al. (2011). ^bNumbers in parentheses include samples that were identified by hair microscopy and were not congruent with the molecular identification; as we chose to preferentially follow the molecular identification, these numbers are only an indication of how many samples were identified with hair microscopy, including cases of putatively incorrect identification. ^cThe total number of samples identified by both methods was 29; however, three samples were differently identified by both methods (see Table S1).

Table 2: Size variation in carnivoran scats ($n = 81$) from Alta Floresta, Brazil.

Predators	N	DS	Min–max (mm)	CI (95%)	Mean $\pm$ SD	t-test	p-Value
Felidae							
<i>Panthera onca</i>	8	8	60–140	64.9–116.8	90.9 $\pm$ 28.1	8.6	<0.01
<i>Puma concolor</i>	1	6	106	–	–	–	–
<i>Puma yagouaroundi</i>	0	3	–	–	–	–	–
<i>Leopardus pardalis</i>	10	8	35–85	47.2–73.9	60.6 $\pm$ 18.7	10	<0.01
Canidae							
<i>Cerdocyon thous</i>	13	17	40–110	59.0–83.9	71.5 $\pm$ 20.6	13	<0.01
<i>Speothos venaticus</i>	0	1	–	–	–	–	–
Mustelidae							
<i>Eira barbara</i>	1	1	70	–	–	–	–
<i>Lontra longicaudis</i>	1	1	63	–	–	–	–
<i>Pteronura brasiliensis</i>	0	2	–	–	–	–	–

The table indicates the circumference of scat samples identified with the molecular and/or trichology approaches. N, number of scats that could be measured; DS, number of deformed scats; CI, confidence interval; SD, standard deviation.

and 16 samples remained unidentified due to the high level of hair degradation and/or unclear microstructural patterns.

The circumference of scats was assessed for samples already identified by the other methods (molecular and/or hair microscopy). However, 58.03% of scats (including all the puma samples) were deformed, precluding adequate measurement. The assessment of the remaining samples (Table 2) revealed overlap in the circumference among almost all species. In addition, for the species with sufficient samples to apply t-tests, we observed that all comparisons were non-significant (jaguar $\times$ ocelot: $t=2.49$, $df=9.67$, $p=0.03$; jaguar $\times$ crab-eating fox: $t=1.61$, $df=9.57$, $p=0.14$; and ocelot $\times$ crab-eating fox: $t=1.32$, $df=20.3$, $p=0.20$).

Dietary analysis

Prey items were surveyed for all the identified predator species (Tables 3 and 4; Table S2; Table S3), but quantitative dietary analyses were performed only for *Panthera onca*, *Puma concolor*, *Leopardus pardalis* and *Cerdocyon thous*, which had larger sample sizes. For the other predator species (*Puma yagouaroundi*, *Lontra longicaudis*, *Pteronura brasiliensis*, *Eira barbara*), we only provide a qualitative description of their diet (Table S3).

Cerdocyon thous – Thirty different items were recognized in the crab-eating fox scats. Twenty-three were animal matter (76.67%), and seven were plant matter (23.33%). Among the animal items, we identified mammals (2.23%), birds (1.12%), reptiles (8.38%) and invertebrates (88.27%). In the most frequent vertebrate category (reptiles), we found three species of lizards, one identified at the family level (Teiidae), another at the suborder level (Lacertilia) and another at the order level (Squamata); we also identified two species of snakes, one of which was identified at the family level (Colubridae) and the other at the suborder level (Serpentes). The insect order Coleoptera was the most frequent among all animal matter. Seeds were the most consumed item by the crab-eating fox ($FO=96.55$ and $PO=60.92$), which was the carnivorous species that presented the largest variety of items (Table 3). Additionally, we found a human waste item (plastic) in one of the samples.

Panthera onca – Ten vertebrate prey categories were detected in the jaguar diet (Table S3). Mammals appeared in all scat samples and presented the highest frequency in the jaguar diet ($FO=100$ and $PO=84.21$), with 68.75% being large species and 31.25% being medium-sized ones. The most frequent items were *Tayassu pecari* and *Pecari*

Table 3: Frequency of occurrence (FO) and proportion of occurrence (PO) of prey consumed by the crab-eating fox (*Cerdocyon thous*) in Alta Floresta.

	FO N	(Scats=29) %	PO N	(Items=459) %
Mammals				
<i>Agouti paca</i>	1	3.448	1	0.218
Unidentified rodent	3	10.344	3	0.655
Birds				
<i>Turdus</i> sp.	1	3.448	1	0.218
Anatidae	1	3.448	1	0.218
Reptiles				
Colubridae	4	13.793	4	0.873
Teiidae	2	6.896	2	0.437
Lacertilia	7	24.138	7	1.528
Serpentes	1	3.448	1	0.218
Unidentified Squamata	1	3.448	1	0.218
Invertebrates				
Gastropoda	1	3.448	1	0.218
Arthropods				
Trichodactylidae	1	3.448	1	0.218
Crustacea	1	3.448	1	0.218
Diptera	2	6.896	2	0.437
Scarabaeidae	15	51.724	90	19.651
Carabidae	1	3.448	1	0.218
Coleoptera	8	27.586	40	8.734
Formicidae	3	10.345	3	0.655
Apidae	1	3.448	1	0.218
Lepidoptera	1	3.448	1	0.218
Hemiptera	1	3.448	1	0.218
Orthoptera	8	27.586	8	1.747
Auchenorrhyncha	1	3.448	2	0.437
Unidentified Arthropoda	5	17.241	6	1.31
Plants				
Poaceae (seed)	7	24.138	18	3.93
Arecaceae	1	3.448	2	0.437
Unidentified palm	4	13.793	5	1.092
Fruit	4	13.793	4	0.873
Seed #1	4	13.793	55	12.01
Seed #2	7	24.138	177	38.646
Seed #3	1	3.448	18	3.93
Human waste				
Plastic	1	3.448	1	0.218

tajacu, the former with the highest RB, which was also corroborated by the IRI, which showed it as the most important prey item for this species. It is noteworthy that we found one livestock prey (*Bos taurus indicus*) in one jaguar sample (Table 4).

Puma concolor – Seven vertebrate categories were found in the puma scats (Table 4; Table S3). Rodents were the most frequent item ($FO=50$ and $PO=57.16$). The item with the highest RB and IRI was *Dasypus* sp. (Table 4).

Leopardus pardalis – For the ocelot scats, we identified 19 different vertebrate categories. *Proechimys* sp. was the

Table 4: Frequency of occurrence (*FO*), proportion of occurrence (*PO*), correction factor (*Y*), relative biomass consumed (*RB*) and index of relative importance (*IRI*) for mammalian prey items in faecal samples of pumas (*Puma concolor*), jaguars (*Panthera onca*) and ocelots (*Leopardus pardalis*) in Alta Floresta, Brazil.

Prey item ^a	Jaguar					Puma					Ocelot							
	FO (%)	PO (%)	Weight (kg)	Y (kg/scat)	RB (%)	IRI	FO (%)	PO (%)	Weight (kg)	Y (kg/scat)	RB (%)	IRI	FO (%)	PO (%)	Weight (kg)	Y (kg/scat)	RB (%)	IRI
Mammals																		
Large prey size (>15 kg)																		
<i>Bos taurus indicus</i> (livestock) ^b	6.25	5.20	169.00	7.90	6.32	72.02												
<i>Tayassu pecari</i>	25.00	21.05	33.50	3.15	29.75	1270.00												
<i>Pecari tajacu</i>	25.00	21.05	19.50	2.66	25.13	1154.40												
<i>Mazama</i> sp.	12.50	10.52	22.60	2.77	13.07	294.94												
Medium prey size (1–15 kg)																		
<i>Tayassu pecari</i> (young)							12.5	14.29	3.00	0.29	27.33	7.90	5.26	3.03	3.00	0.29	0.06	16.29
<i>Tamandua tetradactyla</i>													5.26	3.03	5.50	0.4	0.09	16.42
<i>Dasylops</i> sp.	6.25	5.20	3.50	2.10	4.96	63.50	12.5	14.29	3.50	2.10	29.26	61.53	21.05	12.12	3.50	0.31	0.28	261.03
<i>Curculio paca</i>	18.75	15.78	7.50	2.24	15.87	593.47												
<i>Dasyprocta azarae</i>	6.25	5.20	2.70	2.07	4.89	63.09							5.26	3.03	2.70	0.28	0.06	16.28
Small prey size (<1 kg)																		
<i>Proechimys</i> sp.							12.5	14.29	0.25	0.25	23.93	6.05	31.58	18.18	0.25	0.25	0.34	584.91
<i>Rhagamys</i> sp.													5.26	3.03	0.02	0.02	0.00	15.97
<i>Euryoryzomys</i> cf. <i>emmonsae</i>													5.26	3.03	0.09	0.09	0.02	16.05
<i>Oryzomys</i> cf. <i>amazonicus</i> .							12.5	14.29	0.08	0.08	7.19	0.55	5.26	3.03	0.08	0.08	0.02	16.04
<i>Necomys lasiurus</i>							12.5	14.29	0.06	0.06	5.67	0.34	15.79	9.09	0.06	0.06	0.04	144.18
<i>Metachirus nudicaudatus</i>													5.26	3.03	0.07	0.24	0.05	16.23
Unidentified small rodent							12.5	14.29	0.07	0.07	6.62	0.46	10.53	6.06	0.24	0.07	0.03	64.13

^aOther items (invertebrates) were not included in this table, which is why the *PO* does not add up to 100%. ^bLivestock reference (male, 205 days): ABCZ/SUT/SMG-2000.L.

Table 5: Levins' measure of niche breadth ($\hat{B}$) and standardized niche breadth ($\hat{B}_A$) for four predators from Alta Floresta: crab-eating fox (*Cerdocyon thous*), jaguars (*Panthera onca*), puma (*Puma concolor*) and ocelot (*Leopardus pardalis*).

	$\hat{B}$	$\hat{B}_A$
Crab-eating fox	21.173	0.917
Jaguar	10.362	0.339
Puma	7	0.857
Ocelot	12.663	0.364

most frequent item, followed by *Dasypus* sp. *Proechimys* sp. exhibited the highest *RB*, and the *IRI* showed it as the most important prey item in the ocelot diet (Table 4).

The niche breadth (*B* and *BA*) of jaguar, puma and ocelot was lower than that of the crab-eating fox (Table 5). Among the three felids, the ocelot and puma presented the largest niche overlap (Table 6), using both indices (Pianka and Czekanowski). Using *PO* and *RB*, the results were not significantly different from random expectations (Figures 2 and 3). Additionally, the Bray-Curtis similarity coefficients between the *PO* of vertebrate prey in the jaguar, puma and ocelot diet indicated a medium to high similarity between the puma and ocelot diets (Figure 4).

Discussion

Predator species identification

The data collected in this study allowed an assessment of two methods of carnivoran faecal identification, and

provided insights into the diet of carnivorans in one of the most deforested areas of the Brazilian Amazon. This area presents harsh conditions for fieldwork, posing challenges to finding scats on trails. Furthermore, scat removal by dung beetles is an added problem, as they can remove up to 71% of faecal samples in 24 h (Norris and Michalski 2010). Given these issues, it is challenging to obtain large numbers of scats in the area, making it critical to perform careful predator identification of the obtained samples to maximize the reliability of the dietary assessments, in light of limited sample sizes.

The DNA-based method has been successful in dietary studies (e.g. Farrell et al. 2000, Napolitano et al. 2008, Martínez-Gutiérrez et al. 2015, Morin et al. 2016) and so has the trichology approach (e.g. Silva-Pereira 2009, Rocha-Mendes et al. 2010). In this comparative study, the molecular method was more successful than the trichology approach, given the larger number of samples identified by the former vs. the latter. The DNA-based method seemed to be affected by the scat age (e.g. if it was too old or dry); overall, we considered 93 samples as feasible for molecular identification, while the others were excluded due to severe deterioration. Samples obtained in the tropics are often exposed to high humidity, warm temperatures, frequent rain and intense sunlight, all of which can rapidly degrade DNA (Michalski et al. 2011). The hair microscopy approach depended on finding predator guard hairs in the faecal sample ($n=65$), and also on the level of degradation of these hairs. Both methods faced some obstacles, but mostly presented consistent identification, and complemented each other when considering the total set of identified samples. As for the identification method based on the circumference of scats, the results showed that it presents very low reliability, agreeing with previous

Table 6: Pianka's and Czekanowski's indices [observed (*O*) and bootstrap (Bot, mean $\pm$ SD)] of niche overlap between jaguar (*Panthera onca*), puma (*Puma concolor*) and ocelot (*L. pardalis*) from Alta Floresta, Brazil, calculated with two categories: proportion of occurrence of vertebrate prey (*PO*) and relative biomass of mammalian prey (*RB*).

Type of data	Index	Pair of species	<i>O</i>	Bot	Bot (CI1)	Bot (CI2)
<i>PO</i> (vertebrates)	Pianka	Jaguar-puma	0.052	0.055 $\pm$ 0.054	0.00	0.18
		Jaguar-ocelot	0.089	0.100 $\pm$ 0.077	0.00	0.28
		Puma-ocelot	0.689	0.677 $\pm$ 0.119	0.43	0.85
	Czekanowski	Jaguar-puma	0.052	0.054 $\pm$ 0.058	0.00	0.20
		Jaguar-ocelot	0.113	0.118 $\pm$ 0.064	0.00	0.26
		Puma-ocelot	0.476	0.463 $\pm$ 0.114	0.21	0.65
<i>RB</i> (mammals)	Pianka	Jaguar-puma	0.067	0.073 $\pm$ 0.082	0.00	0.27
		Jaguar-ocelot	0.081	0.094 $\pm$ 0.082	0.00	0.31
		Puma-ocelot	0.833	0.810 $\pm$ 0.141	0.4	0.97
	Czekanowski	Jaguar-puma	0.050	0.06 $\pm$ 0.072	0.00	0.26
		Jaguar-ocelot	0.099	0.104 $\pm$ 0.084	0.00	0.31
		Puma-ocelot	0.674	0.647 $\pm$ 0.141	0.29	0.85

Figure 2: (A) Observed utilization matrix of ocelots (*Leopardus pardalis*), pumas (*Puma concolor*) and jaguars (*Panthera onca*) for the original proportion of occurrence (PO) of vertebrate prey data from Alta Floresta, using Czekanowski's and Pianka's indices. (B) Simulated data matrix generated using 5000 repetitions.

The area of each circle is proportional to the utilization of a resource category by a species. If no circle is shown, the utilization was 0. The result for Pianka's index was not significantly different from the expected by chance (observed index = 0.28, mean of simulated index = 0.31, $p = 0.38$), as was also observed for the Czekanowski's index (observed index = 0.21, mean of simulated index = 0.25, $p = 0.24$).

Figure 3: (A) Observed utilization matrix of ocelots (*Leopardus pardalis*), pumas (*Puma concolor*) and jaguars (*Panthera onca*) for the relative biomass (RB) of mammal prey data matrix from Alta Floresta, Brazil, assessed with two different indices: Czekanowski and Pianka. (B) Simulated data matrix generated using 5000 repetitions.

The area of each circle depicted is proportional to the utilization of a resource category by a species; if no circle is shown, the utilization was 0. The result for both indices was not significantly different from random expectations (Pianka: observed = 0.33, mean of simulated index = 0.29, $p = 0.68$; Czekanowski: observed = 0.27, mean of simulated index = 0.27, $p = 0.58$).

Figure 4: Cluster analysis of Bray-Curtis similarity indices of vertebrate prey species identified in faecal samples of jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) in Alta Floresta, Brazilian Amazon.

studies (Foran et al. 1997, Farrell et al. 2000, Prugh and Ritland 2005). Deformation in faecal samples, overlap in measurement among species and no significant difference between species, all argue against the use of this method.

Carnivoran diet in a highly deforested region of the Brazilian Amazon

An initial assessment including all the sampled predator species demonstrated that the majority of them consumed more mammals relative to other groups, except for the crab-eating fox and the semi-aquatic predators [neotropical otter (*Lontra longicaudis*) and giant otter (*Pteronura brasiliensis*) – the former had a more diverse diet and the latter had a diet based on fish]. Among the species with sufficient samples to perform quantitative analyses, the crab-eating fox was the one with the largest number of prey categories, indicating that its predation strategy seems generalist and opportunistic. Insects were the most consumed animals per scat, and Coleoptera was the most commonly observed order, as reported in a previous study (Rocha et al. 2008). Among the vertebrate prey, we found different results relative to previous studies, with Squamata being more frequent than mammals. The most frequent items based on their *PO* were seeds/fruits, which agrees with other studies conducted in tropical areas (Jácomo et al. 2004, Gatti et al. 2006). We also found plastic in the faeces of this species. Given the advance of urban and rural landscapes over the forest in this region, the contact of humans and wildlife has increased. Additionally, as the crab-eating fox seems to have considerable flexibility in its habitat use (Bianchi et al. 2016), including human-dominated landscapes (Pedó et al. 2006,

Rocha et al. 2008), this would also increase its exposure to anthropogenic waste, which may explain the presence of this material in its faeces.

The most important prey item for jaguars was the white-lipped peccary (*Tayassu pecari*), followed by the collared peccary (*Pecari tajacu*), both of which were reported among the three most important items in almost every dietary study of this species (Scognamillo et al. 2003, Azevedo and Murray 2007, Azevedo 2008). Both species of peccaries are a group-living species, and may pose a high injury risk for predators, as documented in some studies. For example, there is at least one record of a sub-adult female jaguar killed by collared peccaries (Scognamillo et al. 2003). Even with these risks, the results provided by this study suggest that jaguars may select for an effective energy gain by preying upon peccaries. The consumption of livestock by this predator was observed in the study, an occurrence that has been well documented by other studies in Brazil (Azevedo 2008, Silveira et al. 2008, Cavalcanti and Gese 2010, Marchini and Macdonald 2012) and in the study region in particular (Michalski et al. 2006). This is occurring due to the expansion of cattle ranching, which has severely increased deforestation in the Brazilian Amazon, replacing natural areas with extensive pastures; as a consequence, livestock has become an alternative prey for large felids.

The puma exhibited a broad range of prey size and feeding strategies in Alta Floresta. The results of the present study found puma to prey primarily on medium-sized mammals, as was observed by Emmons (1987) for the western Amazon. Ocelots preyed primarily on small- to medium-sized mammals, similarly to patterns reported in previous studies (Emmons 1987, Wang 2002, Bianchi et al. 2014). In this species' diet, three species of small mammals were different from those consumed by the puma. An interesting observation was that these prey species are typical of forest areas, while those shared with puma are typical of edge areas between forest and open landscapes. In one particular case, we observed that an ocelot had preyed upon a juvenile white-lipped peccary, probably as an opportunistic event, as there is no record of ocelots preying upon large-bodied peccaries (Villa-Meza et al. 2002, Moreno et al. 2006, Abreu et al. 2008, Bianchi et al. 2010, 2014, Santos et al. 2014). This observation was similar to Villa-Meza et al.'s (2002) results in Mexico, which included evidence of subadult white-tailed deer (*Odocoileus virginianus*) in ocelot scats, suggesting that ocelots may capture non-adult individuals of larger prey.

Using different methods of estimation, our results indicated that the trophic niches of jaguars and pumas were the least overlapping of the three pairs tested, suggesting

that they are not competing strongly for the same food resources in this region. We also observed a large overlap in diet between pumas and ocelots. Although the sample size for pumas was low, we can hypothesize that jaguars prioritize large-sized prey, driving pumas to seek smaller ones, consequently increasing the competition between pumas and ocelots. Other studies observed similar results in areas where jaguars and pumas are sympatric: large and medium-sized prey species comprised the bulk of the jaguar diet, while pumas concentrated on medium-sized items. Thus, jaguars may influence prey selection by pumas, inducing character displacement in the latter towards smaller body size (Iriarte et al. 1990, Scognamillo et al. 2003, Azevedo 2008). Smaller body size as well as smaller prey size could increase the competition for food resources between pumas and ocelots. Accordingly, previous studies of pumas and ocelots observed that, when jaguars do not coexist with them, both species present lower dietary overlap (Iriarte et al. 1990, Moreno et al. 2006, Azevedo 2008, Martins et al. 2008). It is important to emphasise that with our number of samples we may not have results entirely consistent, especially concerning the perimeter of the scats and in niche overlap values, and we recognize that future studies with additional samples could achieve different results.

Conclusion

Despite the low number of samples evaluated in our study, it could be used as a guide for dietary and methodological comparative studies due to its innovative analytical methods. The use of a reliable method of species identification is crucial for studies based on faecal samples. In this study, we demonstrate that the two currently used methods are largely congruent, and can complement each other to maximize sample size in areas where obtaining a large number of samples is challenging. Regarding the dietary comparisons, we concluded that the three largest Neotropical felids can interfere with each other's diet in an asymmetric pattern, with jaguars driving the process and leading to shifts in the competitive dynamics of the other two. Additionally, our results also indicated that anthropogenic activities (wood harvesting and cattle ranching, favouring the expansion of rural and urban areas) are already affecting the feeding behaviour of carnivores in the area. As human activities are taking place over much of the Amazon, pristine habitats for prey and predators are decreasing, while conflicts between carnivores and humans tend to increase. The data generated in this study can contribute, as an initial assessment, to improve the

understanding of carnivoran ecology in the southern Amazon, which should be useful in the context of conservation planning on behalf of this highly impacted region.

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