Assessing Pantanal fauna through environmental DNA metabarcoding after the 2020 megafire

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Keywords: Biodiversity; species monitoring; conservation; eDNA.

Abstract – The environmental DNA (eDNA) metabarcoding is a methodology that, from environmental samples such as soil, water, air, and others, enables the simultaneous identification of multiple species, thus allowing for large-scale mapping of biological diversity in a specific study area. Due to its non-invasive sampling approach, where species are detected from the traces they leave in the environment, eliminating the need to isolate and capture organisms, eDNA metabarcoding emerges as a valuable tool in conservation strategies. This study aims to explore the use of eDNA methodology for biodiversity monitoring and environmental impact assessment caused by the 2020 megafire in the Pantanal of Brazil, focusing on vertebrates. Environmental samples were collected at two protected areas and their surrounding areas, Taiamã Ecological Station (TES) and Pantanal Matogrossense National Park (PMNP), Mato Grosso, Brazil. We identified in TES, 27 mammals, 56 fishes, 12 birds, 4 amphibians, and 4 reptiles, while in PMNP, 43 mammals, 45 fishes, 126 birds, 19 amphibians, and 11 reptiles. Soil sampling proved to be more efficient compared to water sampling: 26 species were exclusively identified in soil samples, while 9 were attributed to water samples. Here, we demonstrated that the primer 12SV5 only a superior efficacy

in identifying mammal and herpetofauna species compared to the other markers used (16Smam and MiBird). Moreover, we confirmed the complementary role of eDNA alongside camera trapping, and its advantage to estimate species richness with a single field expedition. We stress the need to optimize sample collection methods for the target group and to reduce the influence of contamination and water flow. This study highlights the importance of eDNA methodology as a crucial tool for biodiversity monitoring and environmental impact assessment, enabling rapid access to biodiversity and long-term monitoring.

Avaliando a fauna do Pantanal através do DNA ambiental metabarcoding após o megaincêndio de 2020

Palavras-chave: Biodiversidade; monitoramento; conservação; DNA ambiental.

Resumo – O DNA ambiental (eDNA) *metabarcoding* é uma metodologia que permite identificar, simultaneamente, múltiplas espécies a partir de amostras ambientais como solo, água, ar, entre outras, possibilitando mapeamentos da biodiversidade em larga escala. Devido à sua abordagem de amostragem não invasiva, em que as espécies são detectadas a partir dos vestígios deixados no ambiente, eliminando a necessidade de isolar e capturar organismos, o eDNA *metabarcoding* é considerado uma ferramenta importante e eficiente para estratégias de manejo e conservação. Este estudo teve como objetivo explorar o uso da análise de eDNA para monitorar a biodiversidade e avaliar o impacto ambiental causado pelo megaincêndio, em 2020, no Pantanal brasileiro sobre os vertebrados nativos. Amostras ambientais de água e solo foram coletadas na Estação Ecológica Taiamã (TES) e no Parque Nacional do Pantanal Matogrossense (PMNP), e em seus entornos, no estado do Mato Grosso. Por meio da análise de água, identificaram-se 27 espécies de mamíferos, 56 peixes, 12 aves, quatro anfíbios e quatro répteis na TES; enquanto no PMNP, por meio da análise de amostras de solo, foram identificadas 43 espécies de mamíferos, 45 peixes, 126 aves, 19 anfíbios e 11 répteis. A amostragem de solo identificou um maior número de espécies comparada à amostragem de água: 26 espécies foram identificadas exclusivamente em amostras de solo, enquanto apenas nove espécies foram exclusivas em amostras de água. Além disso, ficou evidente que o *primer* 12SV5 apresentou uma eficácia superior na identificação de espécies da mastofauna e da herpetofauna em comparação com os outros marcadores utilizados (16Smam e MiBird). Os resultados deste estudo confirmam que o eDNA *metabarcoding* complementa os levantamentos realizados por armadilhamento fotográfico, bem como suas vantagens para estimar a riqueza de espécies com uma única expedição de campo. Para aumentar a eficiência do método é necessário otimizar os métodos de coleta de amostras para o grupo-alvo de cada estudo e minimizar a influência da contaminação e do fluxo de água. Este estudo demonstra que a metodologia de eDNA *metabarcoding* é uma ferramenta fundamental para o monitoramento da biodiversidade e avaliação de impacto ambiental, permitindo acessar rapidamente a biodiversidade e monitorá-la em longo prazo.

Acceso a la fauna del Pantanal a través de metabarcoding de ADN ambiental después del megaincendio de 2020

Palabras clave: Biodiversidade; monitoreo; conservación; ADN ambiental.

Resumen – El metabarcoding de ADN ambiental (eDNA) es una metodología que, a partir de muestras ambientales como suelo, agua, aire y otras, permite la identificación simultánea de múltiples especies, permitiendo así mapear a gran escala la diversidad biológica en un área de estudio específica. Debido a su enfoque de muestreo no invasivo, donde las especies se detectan a partir de los rastros que dejan en el medio ambiente, eliminando la necesidad de aislar y capturar organismos, esta técnica emerge como una herramienta valiosa

en las estrategias de conservación. El presente estudio tiene como objetivo explorar el uso de la metodología de metabarcoding eDNA para el monitoreo de la biodiversidad y la evaluación del impacto ambiental en los vertebrados causado por el mega incendio de 2020 en el Pantanal brasileño. Se colectaron muestras ambientales en dos áreas protegidas y sus áreas circundantes, la Estación Ecológica Taiamã (TES) y el Parque Nacional Pantanal Matogrossense (PMNP), Mato Grosso, Brasil. A través de muestras de agua fueron identificadas en TES 27 especies de mamíferos, 56 de peces, 12 de aves, 4 de anfibios y 4 de reptiles, mientras que en PMNP, a través de muestras de suelo, fuero 43 especies de mamíferos, 45 de peces, 126 de aves, 19 de anfibios y 11 de reptiles. El muestreo de suelo demostró ser más eficiente en comparación con el muestreo de agua: 26 especies fueron identificadas exclusivamente en muestras de esta naturaleza, mientras que 9 se atribuyeron a muestras de agua. Fue observado que el marcador 12SV5 presentó una eficacia superior en la identificación de especies de mamíferos y herpetofauna en comparación con los otros marcadores utilizados (16Smam y MiBird). Además, se confirma el papel complementario del análisis de eDNA junto con la cámara trampa y su ventaja para estimar la riqueza de especies con una sola expedición de campo. También se destaca la necesidad de optimizar los métodos de recolección de muestras para el grupo objetivo y reducir la influencia de la contaminación y del flujo del curso de agua. Este estudio resalta la importancia de la metodología basada en el metabarcoding de eDNA como una herramienta crucial para la vigilancia de la biodiversidad y la evaluación del impacto ambiental, permitiendo acceder rápidamente a la biodiversidad y monitorearla a largo plazo.

Introduction

In 2020, the Brazilian Pantanal faced the largest wildfires recorded to date, with approximately 40,000 km^2 of its area burned, especially at the peak of the dry season, between August and November[1]. The 2020 fires particularly impacted two important protected areas and their surroundings in the Pantanal, Taiamã Ecological Station (35% of the area burned) and Pantanal Matogrossense National Park (97,5% burned) (ICMBio, unpublished data). Wildfire origins were identified from several causes, ranging from lightning strikes[2] to accidental and intentional use of fire by landowners[3]. After a two-year severe drought[4][5], areas not burned for over 20 years become vulnerable and susceptible to wildfires[6]. High temperatures combined with low relative humidity and strong winds, made wildfire behavior extreme [4][5], reaching the organic soil, surface, and tree canopy, making firefighting efforts extremely difficult.

In response to this catastrophic event, researchers sought to understand the causes of the wildfires, the factors that exacerbated them, and their consequences on the sociobiodiversity (see [7] [2][8][9][10]). Pantanal is the Brazilian biome with the highest average fire *foci* per square kilometer, but knowledge of wildfire impacts on Brazilian biodiversity is scarce, especially on its fauna[11]. One of the first estimates of wildfire impacts on the fauna unraveled that approximately 1,710 vertebrates/ km^2 were indirectly affected (at least 65 million individuals) plus four billion invertebrates[3], and another study estimated about 17 million vertebrate deaths[12]. Therefore, increasing in-depth knowledge of the biodiversity responses after these catastrophic events is paramount. To do this, novel analytical tools can facilitate fieldwork, increase spatial scale, and be less time-consuming.

Environmental DNA (eDNA) metabarcoding is a methodology that gained prominence in recent years, enabling the simultaneous identification of multiple species and allowing for the screening of biological diversity on large scales within a specific area using environmental samples such as soil, water, and air[13]. The species are detected by the DNA from the biological traces left behind, including shed cells, tissue fragments, body excretions, or gametes[14]. Due to its non-invasive sampling approach, which eliminates the need for isolating and capturing organisms, eDNA metabarcoding emerges as a valuable tool in conservation strategies. This methodology is also complementary to traditional sampling methods because it overcomes some limitations, facilitating the detection of rare and elusive species, such as mammals,

and demanding less fieldwork and sampling effort[15] [16][17]. Moreover, it holds the potential for early detection of invasive species, thus generating data to subsidize decision-making on ecosystem protection and management. This methodology has been successfully employed for biodiversity monitoring in several countries, including renowned environmental agencies such as the US Fish and Wildlife Service (USFWS) and the US Department of Agriculture (USDA) in the United States. In Brazil, Sales et al.[18] used eDNA metabarcoding to detect aquatic and terrestrial mammals in flowing water in Amazon and Atlantic Forest biomes and recommended using this methodology together with other minimally invasive methods to obtain a more detailed description of the mammalian biodiversity.

In this study, aiming to increase knowledge of wildfire impacts on vertebrate fauna, and to highlight the potential of eDNA metabarcoding for biodiversity monitoring and environmental impact assessment in Brazil, we employed this methodology to detect terrestrial vertebrates (especially mammals and herpetofauna) in two protected areas in the Pantanal, which were subject to the 2020 Pantanal megafires. In addition, we compared the results from eDNA with camera trapping data on mammals conducted concomitantly in the study areas, as a robustness test.

Materials and Methods

Study areas

The study was conducted in two strictly protected areas and their surroundings, Taiamã Ecological Station (TES) and Pantanal Matogrossense National Park (PMNP), both located in north Pantanal, in the municipalities of Cáceres and Poconé, respectively, in Mato Grosso State, Brazil (Figure 1). TES is an island delimited by the Paraguay River and its branch (locally named Bracinho River), comprising an area of 115 km^2 (Figure 1A). The PMNP, with an area of 1350 km^2, is located on the border between the states of Mato Grosso and Mato Grosso do Sul, near the confluence of the São Lourenço and Paraguay rivers. In the west of PMNP, beyond the Paraguay River, lies the "Serra do Amolar", where the Acurizal Private Natural Heritage Reserve is located, spanning 263 km^2, (Figure 1B).

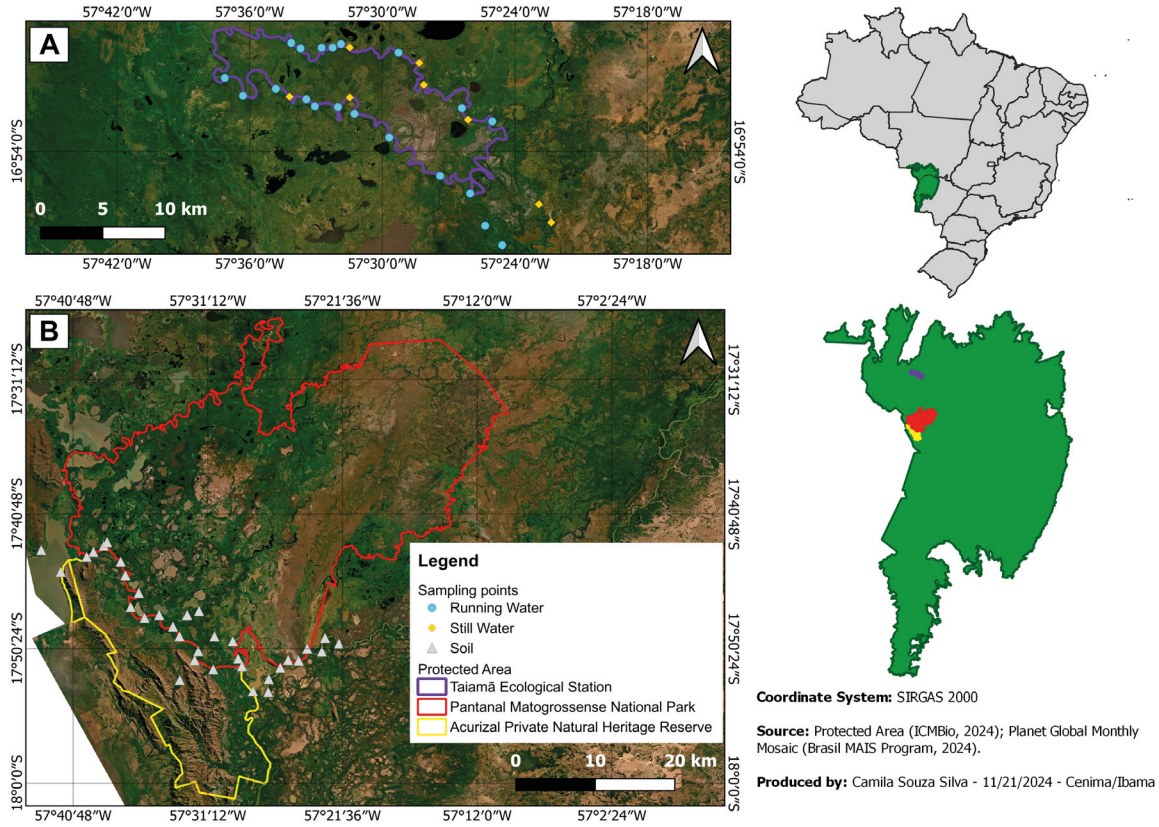

Figure 1 – Location of (A) Taiamã Ecological Station (TES) and (B) Pantanal Matogrossense National Park in Pantanal (PMNP), Mato Grosso, Brazil, depicting the sites where environmental DNA (eDNA) samples were collected.

eDNA sampling

In TES, in November 2021, one year after the fires, water samples were collected in lentic and lotic water bodies in triplicate for each sampling point. We classified samples as still water (lentic) when collected in ponds or puddles, and as flowing water (lotic), when collected in rivers or streams, totaling 28 collection points (Figure 1A). The collected water was poured into a syringe, and the plunger was placed and pushed manually at a flow rate of 1 mL for 10 s for filtration[19]. A minimum volume of 45 mL [20] was passed through a polyethersulfone membrane filter (0.22-micrometer pore size, 30 mm diameter, Kasvi) using a sterilized disposable syringe of 20 mL. All sampling equipment was handled with clean nitrile gloves, which were changed among sampling sites, and the equipment was cleaned with a 10% bleach solution after each collection. The filters were transported refrigerated at ~0°C and the membranes were removed from the filters and stored in a Longmire buffer[21] on ice until DNA extraction.

In PMPN, during August 2022, two years after the fires, soil samples were collected in duplicates from organic horizons at 34 sampling points distributed throughout the area (Figure 1B). At each point, at least 10 soil subsamples were collected within a maximum radius of 10 m from the center to increase the representativeness of the sampling. The samples were collected to a depth of up to 2 cm and homogenized to obtain a composite sample[22]. All sampling equipment was washed with a 10% bleach solution before use and between samplings to prevent contamination. Soil samples were separated into two aliquots of 25 ml and stored in sterile plastic containers containing a 25g sachet of silica gel and immediately refrigerated until DNA extraction.

DNA extraction, PCR amplification, and sequencing

Before DNA extraction from water samples, the filter membranes were eluted in the PW1 buffer from DNeasy PowerWater Kit (Qiagen). Then, the membranes containing the filtered DNA were vortexed for 30 seconds and 400 μl of the PW1 buffer was removed for extraction. DNA extraction was performed in a room dedicated to processing low-quantity DNA samples using the DNeasy PowerWater Kit (Qiagen), following the manufacturer's procedures. DNA extraction from soil samples was performed utilizing phosphate buffer (Na_2HPO_4; 0.12 M; pH ≅ 8) according to[23].

For both water and soil samples, we amplified two mini-barcode regions from the ribosomal mitochondrial genes (12S and 16S rRNA) using primers sets previously described for targeting vertebrates (12SV5F and 12SV5R, [24]) and mammals (16Smam1 and 16Smam2; [25]), respectively. Specifically for soil samples, the MiBird primers described by[26] were also used for detecting mammals. The PCR products were cleaned using magnetic beads (Agencourt AMPure XP® – Beckman Coulter), quantified using a Qubit fluorometer (Thermo Fisher, Waltham, Massachusetts, USA), normalized to a concentration of 20 ng/μl, and indexed using a Nextera Index kit® (Illumina, San Diego, California, USA), using a dual index strategy. The paired-end sequencing was performed on the Illumina iSeq® platform, using an iSeq v2 300 Cycle Reagent kit (2x150 bp).

Bioinformatics

The bioinformatics pipeline was organized in R 4.3.1 (R Core Team 2023). In brief, reads were initially submitted to the removal of undetermined bases and quality filtering (Q-scores ≥ 30). Only reads containing the expected index primer sequence corresponding to each sample were kept for subsequent downstream analysis. Error correction, read-pair merging, and chimera identification and removal were performed using the default settings of DADA2 functions[27]. No length truncation was performed since the primer removal step automatically removed uninformative regions, and resulting ASVs (Amplicon Sequence Variants) out of the expected amplicon length range for each marker (135–139 bp for 12SrRNA, 130–134 bp for 16SrRNA, and ~171bp for MiBird) were discarded. Subsequently, identified ASVs were clustered into OTUs (Operational Taxonomic Units) using SWARM v3.1.0 [28], applying the fastidious option and d=1. Taxonomic assignments were conducted using local alignment of the NCBI nucleotide collection using an automated BLAST+ 2.10.1 function with minimum similarity and minimum coverage (-perc_identity 90 and -qcov_hsp_perc 90). The OTUs were also compared with sequences available in GenBank for species identification using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The final dataset included only OTUs with > 90% similarity against the GenBank database and containing ≥ 4 reads (0.5% relative abundance of reads). All taxonomic assignments were manually curated. When a sequence had a match for two

or more species with equal similarity, we selected those with expected occurrence in the studied area. When a high percentage of matches was obtained (≥ 98%), but the species is not expected to occur in the Pantanal biome, we assigned the genus. This situation generally happens when the mini-barcode sequence from the species is unavailable in GenBank and matched with other species of the same genus (e.g., small mammals and amphibians). Results were obtained at the species (≥ 98%), genus (95-97.99%), and family levels (90-94.99%). Only mammalian and herpetofauna records were curated considering the occurrence in the study areas.

Camera trapping

From August to November 2021, 55 sampling stations were deployed in TES and its surroundings[10], and 34 stations were deployed in PMNP and surroundings from August to November 2022 [Concone et al. unpublished data]. Sampling stations were located at a mean distance of 1 km from each other, following the TEAM protocol[29]. A single unbaited camera trap (Bushnell, models 119949C and 119932C; Browning models Patriot and SPEC OPS ELITE HP4) was installed per station at ~40 cm above the ground, programmed to take three photos at 0.6 seconds intervals between bursts, and operating 24h/day. Cameras were active for about 95 days, totaling a sampling effort of 4,639 and 3,128 trap-days in TES and PMPN, respectively. We used the web platform Wildlife Insights [30] (https://www.wildlifeinsights.org/) to store, organize, and identify all species records.

Data analysis

For eDNA analysis, we summarized the number of reads of the taxonomic groups identified in TES and PMNP, excluding *Homo sapiens*, in pie charts. For mammal, reptile, and amphibian records, we detailed which species were recorded per primer used (12SV5, 16Smam, and MiBird), and if from soil or water samples. We also included species listed in the Brazilian red list highlighting the threat categories[31]. For mammal species, we used the vegan package[32] to calculate accumulation curves and to estimate the overall richness using 1st-order Jackknife and Bootstrap estimators for TES and PMNP, using the accumulated number of samples per area. As a robustness test, we compared the number of mammal species recorded by eDNA and camera traps in each area.

Mammals were categorized as small (< 1 kg), medium and large-sized (> 1 kg), and volant (bats) [33][34]. Mammalian nomenclature followed the list of the Brazilian Society of Mammalogists[35], and reptiles and amphibians followed lists[36][37]. We consider exotic species those occurring outside their natural range[38].

Results and Discussion

Overall results

From the 28 water samples triplicates collected in TES, 2,383,292 reads were obtained for the 16S rRNA gene and 1,694,445 reads for the 12S rRNA gene, corresponding to 464 OTUs (220 for 16S, 244 for 12S). Additionally, from the 34 soil sample duplicates in PMNP, 503,180 reads were obtained for the MiBird mini-barcode, 1,085,412 reads for the 16Smam, and 3,020,637 reads for the 12SV5, corresponding to 5,268 OTUs (1,148 for MiBird, 206 for 16Smam, and 3,914 for 12SV5). Mammals presented the higher number of reads in TES (75,4%), while in PMNP, birds presented the highest (45,7%) (Figure 2). The result in PMNP is explained by the MiBird primer, which can identify several avian species[26]; the results for birds will be presented in a future study given the large effort required for data curation. Among the primer sets used in this study, 12SV5, designed to identify vertebrates in general, exhibited the highest number of identifications with greater taxonomic resolution (Tables 1 and 2) considering our target groups (amphibians, reptiles, and mammals). As the outcome depends on the availability of reference sequences in the database, the taxonomic resolution can be improved with the deposition of more reference sequences[39] [40], especially those from species occurring in the hyperdiverse neotropical realm, as also stated in previous studies [41][42].

Combining the results from the primer sets, in TES, we identified 27 mammals, 56 fishes, 12 birds, four amphibians, and four reptiles, while in PMNP, 43 mammals, 45 fishes, 126 birds, 19 amphibians, and 11 reptiles were identified. It is noteworthy that 64% and 39% of the total reads obtained from TES and PMPN samples, respectively, were attributed to *Homo sapiens*. The presence of human DNA in eDNA metabarcoding studies is commonly attributed to contamination during handling (e.g. [41][42][43]), even when using procedures to avoid it. Domestic

species were also identified: *Bos taurus, Sus scrofa, Canis lupus familiaris, Felis catus, Ovis aries, Equus caballus, Rattus rattus,* and *Gallus gallus domesticus*. Despite precautions, the presence of domestic species might also be related to contamination from handling the collection apparatus in the field and even in laboratory procedures[44]. Therefore, we recommend using blocking primers for human and domestic species to prevent contamination[41][45], which can increase the amount and diversity of DNA detected in eDNA samples, and improve the detection of rare species[45].

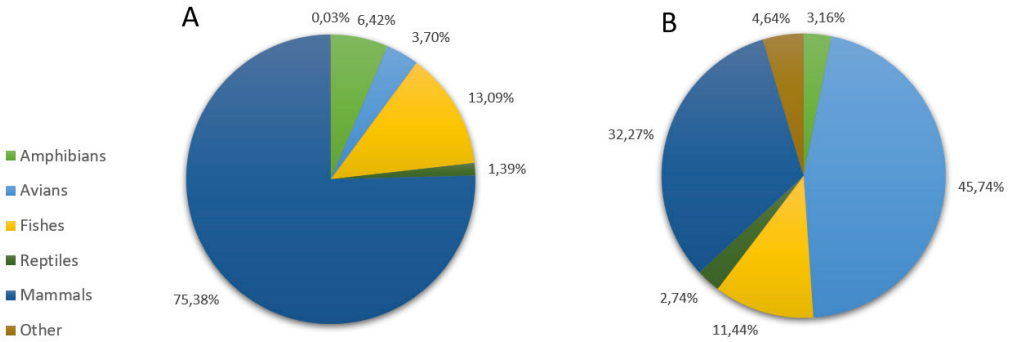

Figure 2 – Proportion of reads of environmental DNA (eDNA) metabarcoding analysis per taxonomic group (disregarding *Homo sapiens*) in (A) Taiamã Ecological Station (TES) and (B) Pantanal Matogrossense National Park (PMNP), Mato Grosso, Brazil.

Concerning herpetofauna, it is noteworthy that water samples were more successful in detecting species occurring in the region, although all species recorded are neither rare nor threatened (Table 2). However, it is still necessary to define a more suitable eDNA methodology for sampling reptiles and amphibians, including specific mini-barcodes for this group, which will enhance species detection. More records were obtained in soil samples at order or family levels, indicating the need to improve the genetic sequence banks to increase taxonomic resolution. The 12SV5 primer was also more effective in surveying herpetofauna (Table 2), highlighted by the record of *Caiman latirostris* in TES, which is not expected to occur in the area. This information is an alert to TES managers, indicating the possible introduction of this species. However, further studies are needed to confirm its presence using species-specific approaches, such as qPCRs, which improve detection efficiency[47].

We identified 49 native mammal species combining TES and PMNP records, divided into small (n=15), medium and large-sized (n=28), and volant (n=6), belonging to 10 orders and 23 families (Table 1). Eleven species are threatened in Brazil[31], most of which were impacted in some way by the 2020 wildfires[3]10][12][46]. Twenty-six species were exclusively identified in soil samples, while nine were attributed only to water samples. Compared to camera trap data obtained in the same period in both areas, 22% of all species recorded were shared in TES[10], and 35% in PNPM [Concone et al. unpublished data] (Table 1).

The eDNA metabarcoding enabled us to identify species with just one field expedition, different from camera trapping, which requires a minimum of two expeditions (installation and removal). Camera trapping is a widely employed method for sampling and monitoring mammals. Still, it requires an extended temporal coverage, is expensive considering equipment and fieldwork, demands significant upkeep, and typically overlooks small animals when aiming to sample the entire assemblage[32]. In addition, post-processing remains laborious as manual tagging of images is still required[22], although new platforms incorporating artificial intelligence are improving the identification process (e.g., https://www.wildlifeinsights.org/). The eDNA is complementary to camera trapping aiming at richness estimation, and the latter offers valuable insights into species-specific and assemblage responses, such as occupancy and relative abundance[22].

The number of species recorded by the 12SV5 primers was 22 in water and 34 in soil samples, 16Smam recorded 5 in water and 18 in soil, and MiBird recorded 17 in soil. Twenty-three species were exclusively recorded by the 12SV5 primer, 2 by the 16Smam, and 3 by the MiBird. Mammal richness per sampling point ranged from 0 to 7 (N = 28; 2.5 ± 2) in TES from 1 to 15 in PMNP (N = 32; 6.1 ± 3.4). Species accumulation curves per sampling point, considering records at the family, genus, and species levels, do not reach the asymptote in TES (Figure 3A), but showed signs of stability in PMNP (Figure 3B), suggesting sampling sufficiency. It is important to note that this outcome could be enhanced, considering that some identifications were made only at the family level, in response to the lack of reference sequences in databases for the species found in the Pantanal.

The presence of DNA in water bodies is important evidence of the current, or at least very recent, presence of species using aquatic habitats[35]. DNA released into the environment is susceptible to degradation and loss due to UV light exposure and microbial activity, typically remaining from two to four weeks[48][49][50]. For water sample collection, we tried prioritizing lentic water bodies because DNA can be transported downstream in flowing water, possibly introducing DNA from species that do not occur in the study areas[51]. Considering the flow of the Paraguay River (300 m^3/s) in 12 hours, DNA molecules could have traveled about 45 km. Therefore, some species identified only in lotic water bodies may not be present in the TES, such as *Akodon* sp., *Cerdocyon thous, Chrysocyon brachyurus,* and *Tayassu pecari*. Other species were also recorded by camera traps, such as *Hydrochoerus hydrochaeris, Myrmecophoga tridactyla,* and *Puma concolor* (Table 1), validating eDNA records in flowing water. In this sense, given the challenges encountered in using water samples for mammal surveys in TES, that is, the difficulty and potential contamination in opening the filtering apparatus and the scarcity of lentic water bodies, soil collection was employed in PMPN.

Table 1 – Mammals recorded using environmental DNA (eDNA) metabarcoding from water and soil samples in Taiamã Ecological Station (TES) and Pantanal Matogrossense National Park (PMPN), Mato Grosso, Brazil, depicting the species recorded by each primer (12SV5, 16Smam, and MiBird) in water samples collected in TES, from still (S) and flowing water (F), and soil samples collected in PMNP, and threat categories at national level[31]. Species captured by camera traps are highlighted in bold.

Class	Order	Family	Genus	Species (Extinction Risk Status)	Common Name	Primers			Sample	
						12SV5	16Smam	MiBird	Water	Soil
Mammal	Carnivora									
		Canidae								
			Cerdocyon	*Cerdocyon thous* (LC)	cachorro-do-mato				F	
			Chrysocyon	*Chrysocyon brachyurus* (VU)	lobo-guará				F	
			Lycalopex	*Lycalopex vetulus* (VU)	raposa-do-campo				F/S	
		Mephitidae								
			Conepatus	*Conepatus chinga* (LC)	jaritataca					
		Mustelidae							S	
			Eira	***Eira barbara*** (LC)	irara					
		Procyonidae								
			Nasua	***Nasua nasua*** (LC)	quati				F/S	
			Procyon	*Procyon cancrivorus* (LC)	mão-pelada				S	
		Felidae								
			Herpailurus	***Herpailurus yagouaroundi*** (VU)	gato-mourisco				S	
			Leopardus	*Leopardus sp.*						
			Leopardus	***Leopardus pardalis*** (LC)	jaguatirica					
			Panthera	***Panthera onca*** (VU)	onça-pintada				F/S	
			Puma	***Puma concolor*** (NT)	onça-parda				F	
	Cetartiodactyla									
		Cervidae								
			Subulo	***Subulo gouazoubira*** (LC)	veado-catingueiro					
		Tayassuidae								
			Dicotyles	***Dicotyles tajacu*** (LC)	cateto				F/S	
			Tayassu	*Tayassu pecari* (VU)	queixada				F	
	Chiroptera									
		Emballonuridae								
			Rhynchonycteris	*Rhynchonycteris naso* (LC)	morcego-de-tromba					
		Phyllostomidae								
			Artibeus	*Artibeus lituratus* (LC)	morcego-da-cara-branca					
			Carollia	*Carollia perspicillata* (LC)	morcego					
			Desmodus	*Desmodus rotundus* (LC)	morcego-vampiro-comum					
			Glossophaga	*Glossophaga soricina* (LC)	morcego-beija-flor					
		Vespertilionidae								
			Myotis	*Myotis riparius* (LC)	morcego					
	Cingulata									
		Dasypodidae								
			Dasypus	*Dasypus sp.*	tatu-galinha					
				Dasypus novemcinctus (LC)	tatu-galinha				F/S	
		Chlamyphoridae								
			Priodontes	*Priodontes maximus* (VU)	tatu-canastra					
			Cabassous	*Cabassous unicinctus* (LC)	tatu-de-rabo-mole					
			Cabassous	*Cabassous sp.*	tatu-de-rabo-mole				F/S	
	Didelphimorphia									
		Didelphidae								
			Caluromys	*Caluromys sp.*	cuíca					
			Caluromys	*Caluromys philander* (LC)	cuíca					
			Didelphis	*Didelphis sp.*	gambá					
			Gracilinanus	*Gracilinanus sp.*	cuíca				S	
			Marmosa	*Marmosa sp.*	cuíca				F/S	
			Marmosops	*Marmosops sp.*	cuíca				S	
			Philander	***Philander canus*** (LC)	cuíca-de-quatro-olhos					
			Metachirus	*Metachirus sp.*	cuíca					
	Lagomorpha									
		Leporidae								
			Sylvilagus	*Sylvilagus sp.*	tapiti					
	Perissodactyla									
		Tapiridae								
			Tapirus	***Tapirus terrestris*** (VU)	anta					
	Primates									
		Atelidae								
			Alouatta	***Alouatta caraya*** (NT)	bugio-preto				F/S	
		Cebidae								
			Callithrix	*Callithrix penicillata* (LC)	mico-estrela					
			Sapajus	***Sapajus cay*** (VU)	macaco-prego					
	Pilosa									
		Myrmecophagidae								
			Myrmecophaga	***Myrmecophaga tridactyla*** (VU)	tamanduá-bandeira				F	
	Rodentia									
		Caviidae								
			Hydrochoerus	***Hydrochoerus hydrochaeris*** (LC)	capivara				F	
		Erethizontidae								
				Coendou longicaudatus (LC)	ouriço				S	
		Dasyproctidae								
			Dasyprocta	***Dasyprocta azarae*** (LC)	cutia					
		Echimyidae								
			Thrichomys	*Thrichomys fosteri (LC)*	punaré					
		Cricetidae							F	
			Akodon	*Akodon sp.*	rato-do-chão				F	
			Holochilus	*Holochilus sp.*	rato-d'água				S	
			Oecomys	*Oecomys sp.*	rato-da-árvore				F/S	
			Oligoryzomys	*Oligoryzomys sp.*	rato-do-mato				F/S	
			Rhipidomys	*Rhipidomys macrurus* (LC)	rato-da-árvore					

Table 2 – Reptile and amphibian species recorded using environmental DNA (eDNA) metabarcoding analysis from water and soil samples in Taiamã Ecological Station (TES) and Pantanal Matogrossense National Park (PMPN), Mato Grosso, Brazil, depicting the species recorded by each primer (12SV5, 16Smam, and MiBird) in water samples collected in TES, from still (S) and flowing water (F), and soil samples collected in PMNP, and threat categories at national level[31].

Class	Order	Family	Genus	Species (Extinction Risk Status)	Common Name	Primers			Sample	
						12SV5	16Smam	MiBird	Water	Soil
Reptilia	Squamata									
		Boidae	*Eunectes*	*Eunectes notaeus* (LC)	sucuri-amarela				F	
		Mabuyidae								
		Iguanidae								
	Crocodylia									
		Alligatoridae								
			Caiman	*Caiman latirostris* (LC)	jacaré-do-papo-amarelo				F/S	
			Caiman	*Caiman yacare* (LC)	jacaré-do-Pantanal				F	
			Paleosuchus	*Paleosuchus palpebrosus* (LC)	jacaré-paguá				F	
	Testudines									
		Chelidae								
Amphibia	Anura	Microhylidae								
			Elachistocleis	*Elachistocleis bicolor* (LC)	Sapo-guarda-de-duas-cores				F/S	
		Leptodactylidae								
			Leptodactylus	*Leptodactylus podicipinus* (LC)	rã-do-ventre-salpicado				F/S	
		Hylidae								
			Pseudis	*Pseudis paradoxa* (LC)	rã-d'água				F/S	
			Trachycephalus	*Trachycephalus typhonius* (LC)	sapo-cunauaru				F/S	
		Hylodidae								
		Bufonidae								

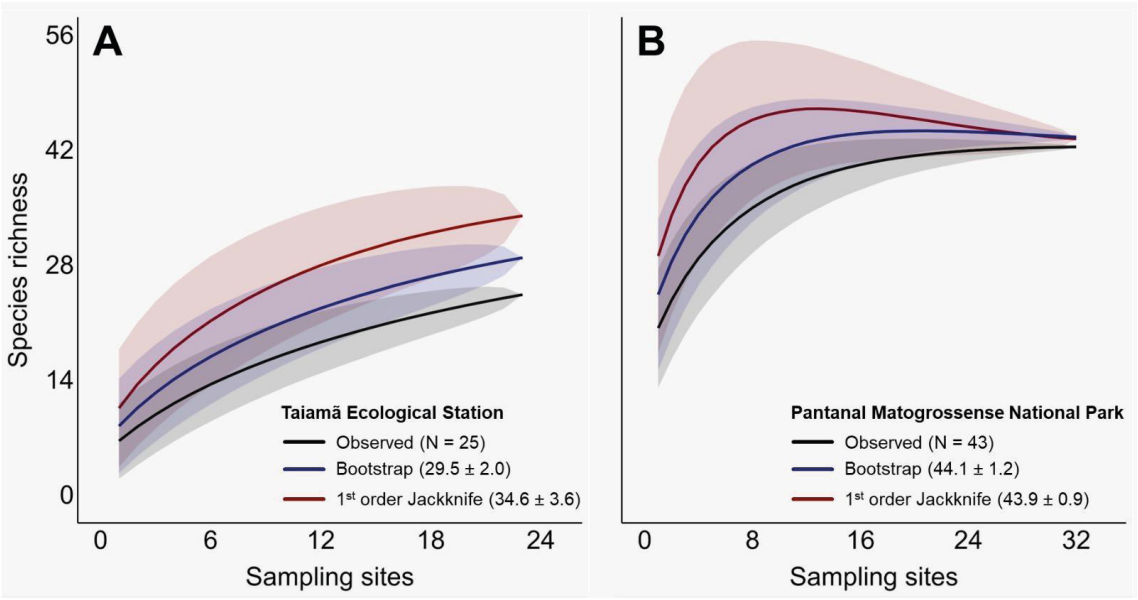

Figure 3 – Species accumulation curves in Taiamã Ecological Station (TES) and Pantanal Matogrossense National Park (PMPN), Mato Grosso, Brazil, by the number of sampling sites. Shaded areas correspond to the standard deviation estimated from 1000 random permutations.

Conclusion

In this study, despite employing other primer sets, the 12SV5 demonstrated superior efficacy in identifying mammal and herpetofauna species. However, since human DNA is abundant in the samples or due to contamination and easily amplified by this primer, it can compromise DNA amplification from rare species, usually found in lower amounts. Moreover, we reinforce the complementary role of eDNA used together with camera trapping for richness estimation. While cameras afford prolonged animal observation and insights into community dynamics, eDNA offers comprehensive surveying of species richness, especially considering short-field expeditions. The findings concerning herpetofauna

were modest compared to those mammals, highlighting the need for improvements to study this group, such as primers specifically designed for reptile and amphibian identification, such as COI and 16S rRNA[52][53]. Additionally, optimizing water collection is essential to mitigate the influence of river currents on records and minimize contamination from human DNA. This study underscores the significance of eDNA methodology as a crucial tool for biodiversity monitoring, capable of illuminating the impacts of human-induced or natural catastrophes, particularly on endangered species.

Ethical standards

The study was conducted under SISBIO #79107-1 and SisGen #ABF0E81 permits.

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