Biota Neotropica 24(4): e20241628, 2024
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Molecular and camera trap data confirm the occurrence of *Dasypus septemcinctus* (Linnaeus, 1758) in Mato Grosso do Sul State, Brazil

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DESBIEZ, A.L.J., MASSOCATO, G.F., MELO-DIAS, M., YOGUI, D. et al. **Molecular and camera trap data confirm the occurrence of *Dasypus septemcinctus* (Linnaeus, 1758) in Mato Grosso do Sul State, Brazil.** Biota Neotropica 24(4): e20241628. https://doi.org/10.1590/1676-0611-BN-2024-1628

Abstract: Understanding the distribution range of a species is crucial for conservation efforts. Yet, precise confirmation of their presence throughout predicted areas can be a challenge. Molecular tools, associated with traditional survey methods, can help in species identification, settling the misidentification of specimens. This study uses a combination of a molecular approach with morphological features to confirm records of the seven-banded armadillo (*Dasypus septemcinctus*) in Mato Grosso do Sul (MS), Brazil, at the southern edge of its known range. Despite extensive camera trapping and roadkill monitoring efforts, we obtained only two records of *D. septemcinctus*, both in the northeast region of MS. Molecular analysis confirmed the morphological identification, eliminating any doubt about the identification of the specimen. The confirmation of *D. septemcinctus* occurrence in the state of MS, not only expands our knowledge of this widely distributed but poorly studied species, but also highlights the importance of a local park (Parque Natural Municipal do Pombo) for Xenarthra conservation..
Keywords: *DNA barcode; camera trap; roadkill; seven-banded armadillo.*

Dados moleculares e de armadilhas fotográficas confirmam a ocorrência de *Dasypus septemcinctus* (Linnaeus, 1758) no Estado de Mato Grosso do Sul, Brasil

Resumo: Compreender a área de distribuição de uma espécie é crucial para os esforços de conservação. No entanto, a confirmação precisa da sua presença nas áreas previstas pode ser um desafio. Ferramentas moleculares, associadas aos métodos tradicionais de levantamento, podem auxiliar na identificação de espécies, resolvendo erros de identificação de espécimes. Este estudo utiliza uma combinação de abordagem molecular e características morfológicas para confirmar registros do tatu-mirim (*Dasypus septemcinctus*) em Mato Grosso do Sul (MS), Brasil, no extremo sul de sua distribuição conhecida. Apesar dos extensos esforços de captura fotográfica e monitoramento de atropelamentos, nós obtivemos apenas dois registros de *D. septemcinctus*, ambos na região nordeste do MS. A análise molecular confirmou a identificação morfológica, eliminando qualquer dúvida sobre a identificação do espécime. A confirmação da ocorrência de *D. septemcinctus* no estado de MS, não só amplia nosso conhecimento sobre esta espécie amplamente distribuída, mas pouco estudada, mas também destaca a importância de um parque local (Parque Natural Municipal do Pombo) para a conservação de Xenarthra.
Palavras-chave: *código de barras de DNA; armadilha fotográfica; atropelamentos; tatu-mirim.*

Introduction

Understanding the distribution range of species is of special interest for biodiversity conservation initiatives (Goldsmith 2012, Goodenough & Hart 2017, IUCN – SSC Species Conservation Planning Sub-Committee 2017). By monitoring species occurrence localities, it is possible to understand ecological aspects of species, such as habitat selection and environmental requirements, to help evaluate local and landscape variables influencing their persistence, to better model species distribution, as well as to infer potential threat factors and species threat status (Syfert et al. 2014, Fois et al. 2018). Although it seems a simple task, the confirmation of the predicted presence of species throughout their range with precise coordinates and identification can be a challenge. Traditional survey methods of vertebrates include live trapping, direct observations, sign surveys, and camera trapping. To complement this approach, molecular tools can be employed to identify species when biological samples are available (Hebert et al. 2003, Saranholi et al. 2023). This can help to settle misidentification of specimens (e.g., Feijó et al. 2019). This approach has been helpful for dead animals when the carcass loses some of its morphological characteristics (Barragán-Ruiz et al. 2023, Kipplel et al. 2015) or from other biological vestiges, the noninvasive samples, such as feces or hair found in the environment (Beja-Pereira et al. 2009).

Distribution delimitation can be a challenge even for widely distributed groups. For instance, Xenarthra is a group occurring from Argentina to the United States (Taulman et al. 1996, Aguiar & Fonseca 2008) and comprises 14 genera and 42 species of armadillos, tree sloths and anteaters (Miranda et al. 2018, Barthe et al. 2024). Among armadillos, a total of 11 living species of the *Dasypus* genus are currently recognized, and this genus has the highest number of species and widest distribution (Feijó et al. 2018, 2019, Barthe et al. 2024). Furthermore, several species from this genus have overlapping and sometimes poorly understood distributions (Feijó et al. 2018).

The seven-banded armadillo *Dasypus septemcinctus* is the smallest species in the *Dasypus* genus. Recently, morphological and molecular data analyses suggested that this species is represented by three subspecies: *Dasypus septemcinctus septemcinctus, D. s. hybridus,* and *D. s. cordobensis* (Feijó et al. 2018; Feijó et al. 2019). *Dasypus s. cordobensis* distribution is limited to Argentina, in Cordoba region (Soilbelzon et al. 2012). *Dasypus s. septemcinctus* occurs in Brazil, eastern Paraguay, Bolivia, and northern Argentina. In Brazil, it is widely distributed and inhabits savannas (Cerrado), dry forests (Caatinga), central and southern portion of the Atlantic Forest, and eastern Amazon, on the right banks of the Madeira and Lower Amazon rivers (Feijó et al. 2018). The southern and northern distribution limits of *D. s. septemcinctus* and *D. s. hybridus,* respectively, are not well understood and proximity of their distribution limits in Rio Grande do Sul state, and northern Argentina is possible (Feijó et al. 2018).

One of the factors that hinders the characterization of the exact distribution of *D. septemcinctus* may be the difficulty in identifying the species through camera trap photos or sightings. *Dasypus septemcinctus* is morphologically similar to other species of the genus. For example, *D. septemcinctus* is smaller than *D. novemcinctus* and many of the records based on field observations can easily mistake adults of *D. septemcinctus* as juveniles of *D. novemcinctus* (Silva & Henriques 2009). The distribution of these species overlaps extensively (Anacleto et al. 2014, Loughry et al. 2014). This may lead to underestimations of presence and abundance of *D. septemcinctus* throughout its distribution. *D. septemcinctus* has a length of head and body length of usually less than 270 mm, long ears averaging 31 mm, a tail shorter than the head-body length, and is characterized by a carapace with fewer (6 or 7) movable bands, while *D. novemcinctus* has a carapace with nine movable bands (Eisenberg & Redford 1999, Nowak 1999, Wetzel et al. 2008, Feijó et al. 2018, Feijó 2020).

Despite its wide predicted distribution, encompassing the State of Mato Grosso do Sul (MS), Brazil (Anacleto et al. 2014), there are no records confirmed by molecular analysis of *D. septemcinctus* for MS. Only two punctual camera trap registers were previously reported (Silveira et al. 2006; Homem et al. 2020). The first primary record of the species was made by Silveira et al. (2006) during a mammal survey carried out between February 2002 and March 2003 in the Rio Negro Pantanal State Park, between the municipalities of Corumbá (MS) and Aquidauana (MS). In the municipality of Três Lagoas, in eastern Mato Grosso do Sul State, Homem et al. (2020) reported four images pertaining to *D. septemcinctus* in an area composed of *Eucalyptus* plantations, Atlantic Forest and Cerrado mosaic. Subsequently, *D. septemcinctus* was also included in the most recent checklist of mammals from Mato Grosso do Sul (Tomas et al. 2011). The photographic record of the species was obtained from the Mammal Reference Image Collection of Embrapa Pantanal (Tomas et al. 2017). In these cases, precise taxonomic identification of *D. septemcinctus* is difficult due to the type of record. Two museum specimens previously recorded as *D. septemcinctus* in the municipality of Maracaju (MS) were identified later as *D. novemcinctus* (Feijó et al. 2018, Santos et al. 2019), reinforcing that a misidentification may occur between the two species even with collected specimens. Two other previous records of *D. septemcinctus* were obtained by interview and vestige, at Taquarussu municipality (MS), located in an Atlantic Forest area (high Parana River), and a record was obtained from a road-killed animal at Ribas do Rio Pardo municipality (MS), located in a savanna area (Santos et al. 2019). It is important to obtain robust records by reliably identifying specimens in the field in order to elucidate aspects of its geographic distribution, habitat use, and population status and density. Here, we use a combination of a molecular approach with morphological features to confirm records of *D. septemcinctus* for the State of Mato Grosso do Sul in midwestern Brazil and contribute to the characterization of the geographic distribution of this poorly known species.

Material and Methods

1. Camera trap survey

We conducted a camera trap survey in the Parque Natural Municipal do Pombo (PNMP), located in the municipality of Três Lagoas, Brazilian state of Mato Grosso do Sul (20º21'S, 52º38'W) (Figure 1). This area comprises of 8,032 hectares of native Cerrado vegetation. The camera trap grid was originally set up by the Giant Armadillo Conservation Program to monitor the giant armadillo population in the park. Seventy camera traps (HF2X Hyperfire; Reconyx, Holmen, USA) were installed in a 1 by 1 km grid layout across the park (Figure 1b) and set to rapid-fire mode, capturing 25-second videos without intervals, between April 2022 and March 2023, totaling over 20,000 camera trap days.

To investigate whether the camera trap sampling effort was sufficient to capture the xenarthran richness in the area, and the potential for recording new species through additional sampling, a rarefaction curve was constructed using the Estimate S 9.1.0 software (Colwell 2013) with Jackknife 1 richness estimator. We used the database of the 70 camera traps installed in the PNMP for one year and we considered a

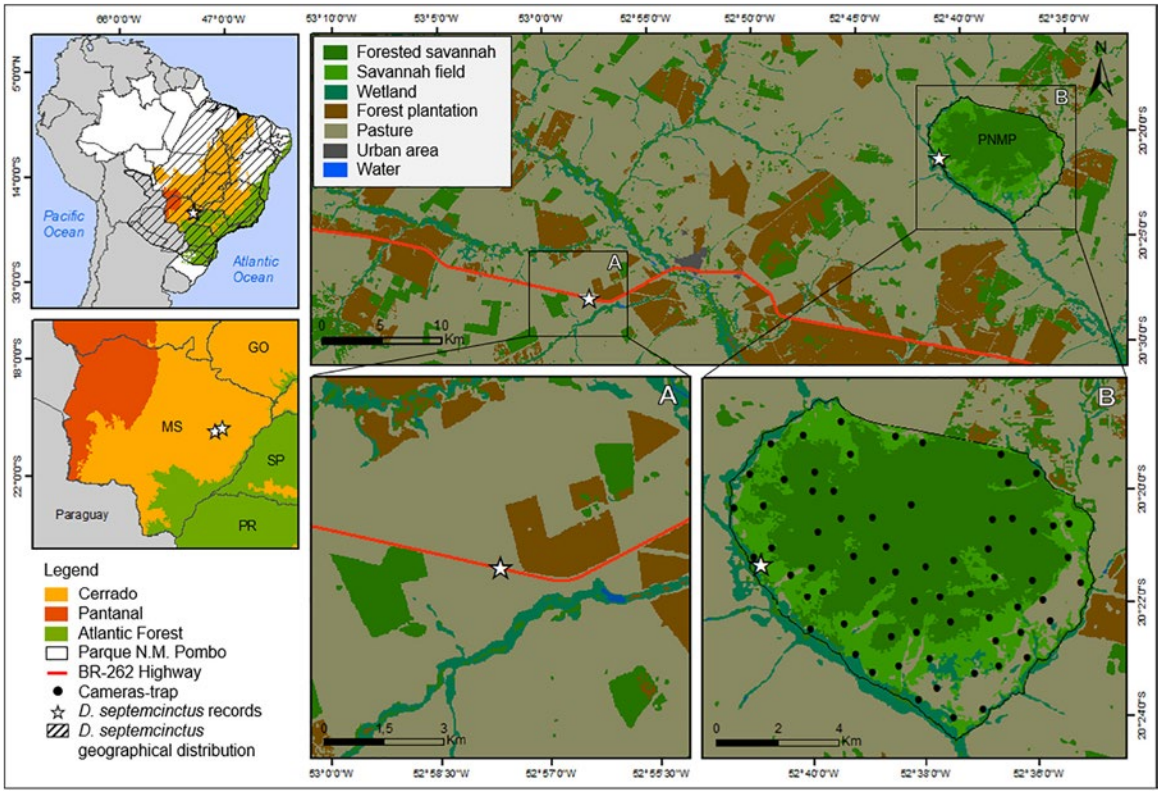

Figure 1. Location of the two new records of *Dasypus septemcinctus* in the Brazilian state of Mato Grosso do Sul, as well as the geographic distribution of the species, the range of biomes in the state, and the land cover and use at the study site. (A) Locations of *D. septemcinctus* carcasses found along the BR-262 Highway and (B) the camera trap record of the species in the Parque Natural Municipal do Pombo (PNMP).

month as a sampling effort unit (n = 12). To ensure the independence of the photographic records and avoid pseudo replication, we only used photos of the same species taken with an interval of at least 1h between the photos (Srbek-Araújo & Chiarello 2013) in the same camera.

We calculated the relative frequency (RF) by camera traps for each species of Xenarthra. The species taxonomic classification followed the Official List of Brazilian Mammals from the Brazilian Society of Mammalogy (Abreu et al. 2022). The threat status of species at the national level followed the Red List of Threatened Species of Brazil (ICMBio 2018), and at the global level followed the International Union for Conservation of Nature's Red List of Threatened Species (IUCN 2023).

2. Roadkill monitoring

Since 2013 over 100,000 km of roads have been systematically surveyed by car (40–60 km/h) to register road-killed animals (see: Ascensão et al. 2017, Ascensão et al. 2021). Each *Dasypus* observation was classified to species level (when possible) and its location recorded with hand-held GPS, and a photograph taken before carcasses were removed from the road to avoid recounting of road accidents. For some individuals tissue samples (muscle, ear, hair, and skin) were collected for further genetic analysis. Surveys included the federal roads BR-262 and BR-267, and the state roads MS-040 and MS-338. All roads have two-lanes and are paved. The land use around these roads is dominated by grassland/pasture and agriculture, followed by remnant native vegetation and riparian areas. Urban areas had relatively low representation in the study area and were not surveyed in road transects.

3. Species-level molecular identification of specimens

A tissue sample (muscle) from a road-killed specimen suspected to be *D. septemcinctus* was collected and preserved in ethanol 70%. The DNA was extracted from the sample using a salt extraction protocol (Aljanabi & Martinez 1997). Then, for the DNA-based species identification, PCRs (polymerase chain reactions) were performed to amplify mitochondrial fragments of cytochrome c oxidase subunit 1 (COI) and cytochrome b (CytB) genes using the COIX-L2 and LCO1490 (Lara-Ruiz et al. 2008) and CytB-L and CytB-R (Schetino et al. 2017) primer pairs, respectively. PCRs were performed in a Veriti thermal cycler (Applied Biosystems) following the protocols described for each gene (COI, Lara-Ruiz et al. 2008, CytB, Schetino et al. 2017), using 50°C of annealing temperature. Both forward and reverse sequencing were performed on an automatic sequencer ABI3730XL (Applied Biosystems), and the electropherograms were checked and analyzed in the Geneious 7.1.7 software (Kearse et al. 2012). Yet using this latter software, the forward and reverse sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) and a consensus sequence for each gene was obtained. The obtained sequences were deposited in GenBank with the accession numbers PP537895 (CytB) and PP528439 (COI).

For the molecular species identification, the obtained sequences from both genes were subjected to comparative alignments using BLAST (basic Local Alignment Search Tool) available in GenBank (www.ncbi.nlm.nih.gov/genbank). Also, with the concatenated mtDNA (COI: 638 base pairs; CytB: 689 base pairs), we analyzed the genetic distance between species, examining the mean interspecific Kimura two-parameter (K2P) distance calculated for each candidate species using MEGA 6.04 (Tamura et al. 2021). We compared the sequence of our sample with sequences of the most likely species (*D. novemcinctus*, *D. s. septemcinctus*, *D. s. hybridus*) obtained from the GenBank database (GenBank Accession Number: COI: NC_028565.1, NC_028569.1, NC_001821.1, MH710606.1, MH710612.1, MH710614.1, MH710615.1, MH710616.1, MH710620.1, MH710621.1, Y11832.1; CytB: NC_028565.1, NC_028569.1, NC_001821.1, MH710623.1, MH710629.1, MH710631.1, MH710633.1, MH710634.1, MH710637.1, MH710638.1, Y11832.1).

Finally, we carried out a phylogenetic analysis to identify in which clade our sequence fitted into. We used the Bayesian inference (BI) method to reconstruct the most likely tree according to its topology (Knowles & Kubato 2010). An ultrametric topology was built using the BI method implemented in BEAST v.2.2.1 (Bouckaert et al. 2014), with a nucleotide substitution model based on the Bayesian information criterion (BIC), determined by JModeltest v.2.1.10 (HKY + I) (Darriba et al. 2012); strict molecular clock (Drummond & Bouckaert 2015); and Yule model for the prior of the tree. Three independent runs of the Markov chains were carried out, each with 100 million iterations, and the trees and parameters were saved every 10,000 generations. The combination of the tree and log files was performed with LogCombiner v.1.8 (Drummond et al. 2012). The convergence of the chains and the ESS values for the estimated parameters were checked in Tracer v.1.7 (Rambaut et al. 2014), considering values of effective sample size (ESS) of 200 or more for all parameters. The tree was summarized with TreeAnnotator v.1.8 (Rambaut & Drummond 2012), and later visualized in FigTree v.1.4. (http://tree.bio.ed.ac.uk/).

Results

A total of eight species of Xenarthra was recorded through 596 independent camera trap records in the PNMP. These records belonged to six armadillo species: *Priodontes maximus* (RF = 29.53%), *Cabassous tatouay* (RF = 3.52%), *Cabassous squamicaudis* (RF = 1.51%), *Euphractus sexcinctus* (RF = 9.06%), *D. novemcinctus* (RF = 4.03%), and *D. septemcinctus* (RF = 0.17%); and two anteater species: *Tamandua tetradactyla* (RF = 20.81%), and *Myrmecophaga tridactyla* (RF = 31.38%) (Supplementary Table S1). By the second month of camera trap monitoring, seven Xenarthra species had already been recorded and the rarefaction curve stabilized (Figure 2). Only in the 12th month of monitoring, we obtained the increment of a new species reaching 89.2% of the xenarthran richness estimated (S est.= 8.96 ± 0, 96) (Figure 2). An individual of *D. septemcinctus* was recorded on March 2, 2023, at 11:53 a.m. using a giant armadillo feeding hole in PNMP (20°21’19.4”S, 52°40’56.5”W), in an area of savannah field vegetation (Figure 3).

In the roadkill monitoring, we identified over 1,000 *D. novemcinctus* carcasses but only one carcass suspected of being from *D. septemcinctus* (Supplementary Table S2). The suspected *D. septemcinctus* was found on January 2nd, 2020 (20°28’00.1”S, 52°57’42.1”W) (Figure 1, Figure 4). This locality was 31.5 km apart from the camera trapping detection (Figure 1). The carcass was deposited at the museum of Universidade Federal do Mato Grosso do Sul (UFMS) and received the number ZUFMS-MNV00293. The tissue sample from the carcass received the deposit number MAM1131 at the Laboratory of Molecular Biodiversity and Conservation, Universidade Federal de São Carlos (UFSCar), Brazil.

In both above mentioned records (camera trap and roadkill), the individuals were morphologically identified as *D. septemcinctus* due to the external characteristics, such as the presence of six movable bands and a relatively short tail, in relation to its head-body length. These two characteristics can distinguish *D. septemcinctus* from *D. novemcinctus* (Hamlett 1939, Wetzel et al. 2008, Feijó et al. 2018). For the camera trap record, we can observe that the dorsal profile of the skull is straight in a lateral view which is congruent with *D. septemcinctus* (Figure 3a and 3b).

Based on the molecular data, the comparison of the mitochondrial sequences (COI, CytB) obtained from the tissue sample of the road-killed animal (MAM1131) with GenBank sequences resulted in a high similarity with both subspecies, *D. s. septemcinctus* and *D. s. hybridus*, and for both COI and CytB genes (higher than 99%). On the other hand, we found lower similarity with available sequences of *D. novemcinctus* (COI: 94.65%; CytB: 94.45%). Using Kimura two-parameter (K2P), higher genetic distance was observed between the specimen and *D. novemcinctus* than between the specimen and *D. s. septemcinctus* or *D. s. hybridus* (Table 1). The phylogenetic approach grouped the specimen (MAM1131) within the clade formed by *D. s. septemcinctus* and *D. s. hybridus* (Figure 5), not allowing to distinguish the road-killed individual between the two subspecies. All these results reinforce that MAM1131 represents *D. septemcinctus*, and refutes the possibility that the specimen belongs to the species *D. novemcinctus.*

Table 1. Pairwise genetic distance estimates using the Kimura two-parameter (K2P). The genetic distance was estimated between the road killed specimen of *Dasypus* (MAM1131) and the available sequences for *D. s. septemcinctus*, *D. s. hybridus* and *Dasypus novemcinctus*.

	Pairwise distance (%)			
Species	**Specimen (MAM1131)**	***D. s. hybridus***	***D. s. septemcinctus***	***Dasypus novemcinctus***
Specimen (MAM1131)	–			
D. s. hybridus	0.532	–		
D. s. septemcinctus	0.647	0.825	–	
Dasypus novemcinctus	6.838	6.995	7.118	–

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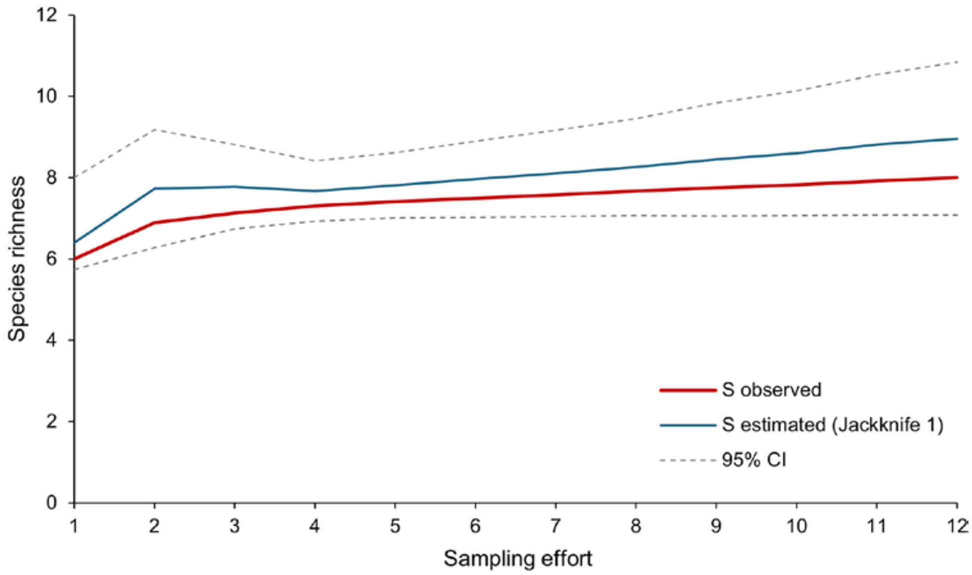

Figure 2. The rarefaction curve of Xenarthra species richness recorded using camera traps between April 2022 and March 2023 in Parque Natural Municipal do Pombo (PNMP), Mato Grosso do Sul, Brazil. The sampling effort is represented in months and the Jackknife 1 estimator was used to calculate the estimated richness.

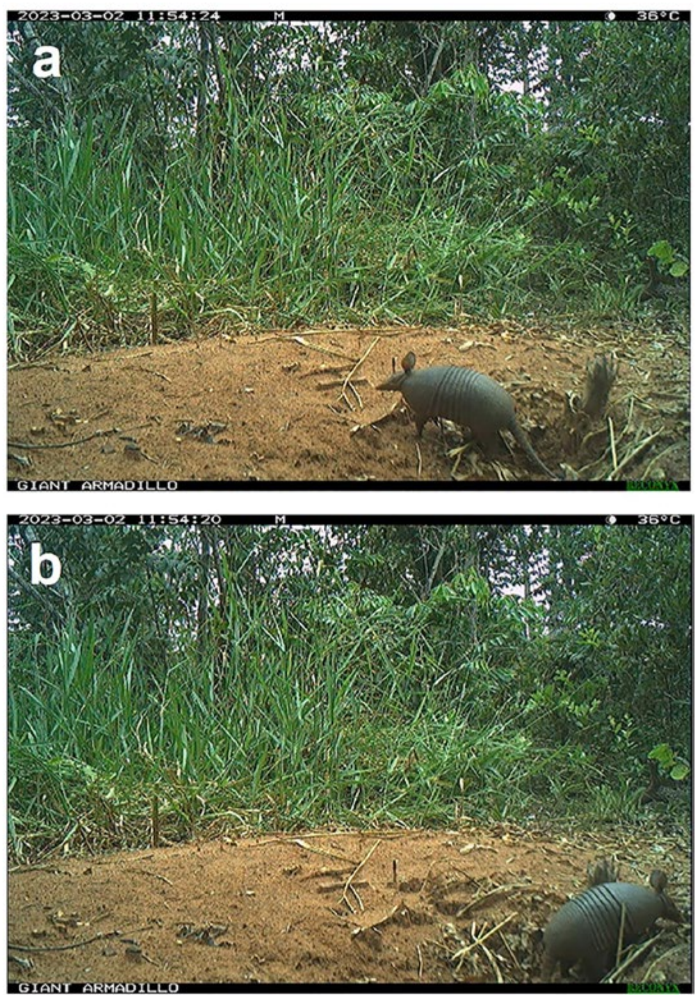

Figure 3. Camera trap record of a *Dasypus septemcinctus* individual using a giant armadillo feeding hole in Parque Natural Municipal do Pombo (PNMP), Mato Grosso do Sul state, Brazil.

Figure 4. Road-killed carcass identified as *Dasypus septemcinctus* collected in a paved road of Mato Grosso do Sul state, Brazil.

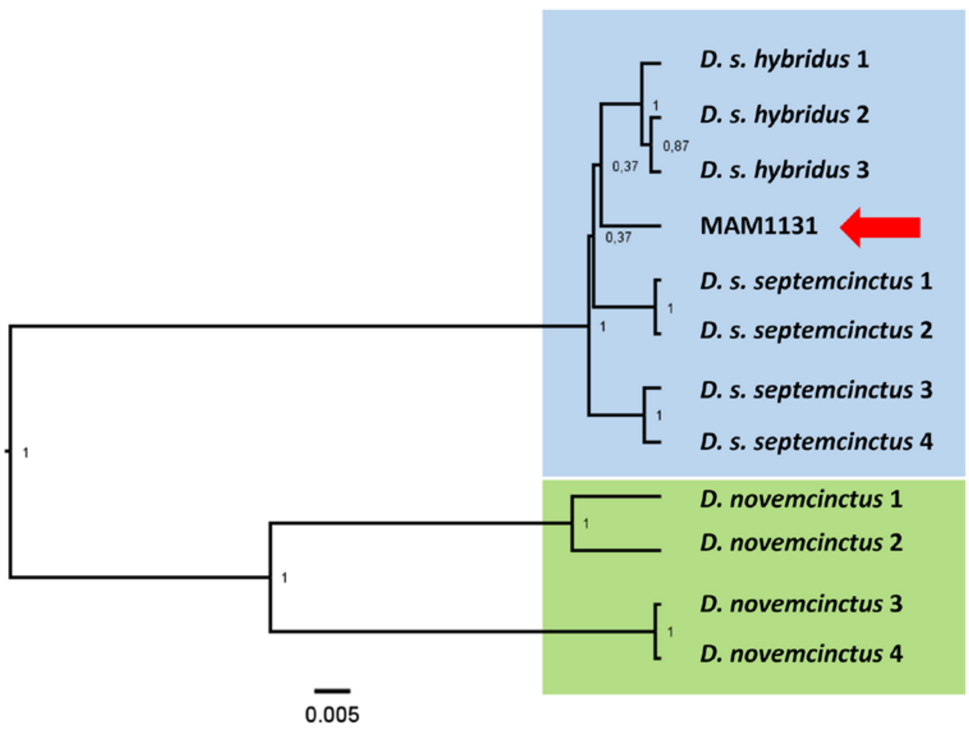

Figure 5. Topology derived by Bayesian Inference, using combined COI and CytB markers, to identify in which haplogroup the target specimen fitted. The colored branches indicate the best supported haplogroups representing *Dasypus septemcinctus* (blue) and *Dasypus novemcinctus* (green) species.

Discussion

In this study, we confirmed the presence of *D. septemcinctus* in Mato Grosso do Sul State (MS). This is the first reliable record confirmed through molecular analysis, although MS is in the middle of the predicted distribution range of *D. septemcinctus* (Anacleto et al. 2014). While confirming the presence of the species, this study also underlines its very low detection rate in the region, highlighting the importance of further studies to understand environmental factors influencing the distribution range and densities of *D. septemcinctus*.

Despite extensive camera trapping and roadkill monitoring efforts, only two records of *D. septemcinctus* have been obtained, both in the northeast region of MS (Figure 1). Although MS is one of the hotspot areas for Xenarthra studies, i.e., an area that requires more effort of data collection (Feijó et al 2022), the few records of *D. septemcinctus* in the state suggest its low natural presence in the region. Further efforts need to be made to confirm the presence of the species in other localities of the state. Several mammal studies have been carried out in MS in the last decades (Porfirio et al. 2014, Ascensão et al. 2017, Tomas et al. 2021, Assis et al. 2022), and the non-detection of *D. septemcinctus* raised questions about its distribution range. It could be suggested that this region is a natural distribution gap or that due to recent habitat modifications in the Cerrado the species could have been locally extirpated, finally we cannot discard the possibility of a recent spatial or populational expansion. Except for *D. septemcinctus*, all other Xenarthra species are frequently recorded by camera traps in the Parque Natural Municipal do Pombo (PNMP) and in roadkill monitoring (Ascensão et al. 2021). Thus, the low detection rate of *D. septemcinctus* may suggest that this species naturally occurs at low density in the region, or maybe it has a particular behavior resulting in low detection by camera traps and avoids crossing paved roads.

Additional factors may have contributed to the low detection of *D. septemcinctus*. The sampling design in this study was biased to detect giant armadillos. Cameras were usually placed in front of a giant armadillo burrow or excavation or termite mounds (Massocato & Desbiez 2019). However, all other species of xenarthrans were registered in the first two months of camera trapping. A particular behavior or habitat preference may reduce *D. septemcinctus* detection. Several studies have noted the difficulty in registering *D. septemcinctus* in camera traps even in areas where its presence is highly probable or even certain (Santos-Filho & Silva 2002, Sanderson & Silveira 2003, Trolle & Kéry 2005, Ciochete 2007, Juarez 2008, Schittini 2009, Oliveira 2010, Zimbres 2010). Also, it is possible that more *D. septemcinctus* specimens were registered but improperly classified in both camera trap and roadkill monitoring as this species is often mistaken for young *D. novemcinctus* (Silva & Henriques 2009). Several camera trap records in our study were listed as *Dasypus* sp. Identification through photo records can be challenging for a nocturnal animal which can appear blurry. Often only a portion of the armadillo is recorded, finally when an animal is moving rapidly key details necessary to distinguish between *D. septemcinctus* and *D. novemcinctus* cannot be observed. In these cases, the record is classified as *Dasypus* sp. As for road-killed animals, the use of molecular data can be decisive to the identification of species (Barragán-Ruiz et al. 2023). The carcasses of small mammals such as *D. novemcinctus* or *D. septemcinctus* can become almost unrecognizable when vehicles have repeatedly run over the carcass.

In this study, the molecular data corroborated morphological evaluation and removed any doubts about the identification of the specimen belonging to *D. septemcinctus*. Importantly, the comparison of the obtained molecular data with those available in databases reinforce that *D. s. hybridus* and *D. s. septemcinctus* form only one clade, as suggested by Feijó et al. (2019). Neither COI nor CytB were able to decisively discriminate between *D. s. hybridus* and *D. s. septemcinctus*, suggesting that other mitochondrial or nuclear markers still need to be tested if this question is targeted. An effort to gather genetic samples from this region will allow the evaluation of the genetic variability of this population, also bringing light to the taxonomy of these two subspecies and their distribution. According to Feijó et al. (2018), the southern limits of *D. s. septemcinctus* are unclear, but it is proposed that it can overlap with *D. s. hybridus* in the state of Rio Grande do Sul (Feijó et al. 2018). *D. s. septemcinctus* is supposedly restricted to the Atlantic Forest, whereas *D. s. hybridus* would be limited to open areas (Abba et al. 2014, Anacleto et al. 2014). However, *D. s. septemcinctus* may also inhabits southern portions of the Amazon, savannas (Cerrado), and dry forests (Caatinga) (Anacleto et al. 2014). Since we were not able to distinguish our record as one of both subspecies, the studied area may be near of the limits of their distributions and deserves to be better investigated to improve the distribution limit knowledge and understand the molecular integrity of *D. s. septemcinctus* and *D. s. hybridus*, as also pointed by Feijó et al. (2018).

The confirmation of *D. septemcinctus* occurrence in the state of MS, Brazil, brings important insights to the knowledge of this widely distributed but poorly studied species. Camera trap images in this study were taken near noon. However, previous studies in the Cerrado observed the species during the day between 6h e 14h (Bonato 2002) or 10h e 17h (Silva & Henriques 2009). With a total of eight species of Xenarthra recorded, this study also highlights the importance of PNMP for Xenarthra conservation in the Cerrado biome. The Cerrado savanna in Brazil is one of the world's largest biodiversity hotspots but has one of the lowest levels of protection (1.6% strictly protected) and receives very little conservation attention or funding (Klink & Machado 2005, Strassburg et al. 2017, Green et al. 2019). In Mato Grosso do Sul, only 16% (58,459 km^2) of native Cerrado remains, divided into small fragments (average ~9ha) surrounded by intensive agriculture (Reynolds et.al. 2016). The PNMP is a poorly known municipal park, and the only protected area in the Cerrado of MS where giant armadillos are found (Ferraz et al. 2021). Mato Grosso do Sul state includes part of the Xenarthra diversity hotspot, within an intensely human-modified landscape (Feijó et al. 2022), which reinforces the need to continue studying xenarthrans in this region. This study shows that, in addition to giant armadillos, the park is key to the survival of other species of xenarthrans and efforts must be made to increase its level of protection, funding as well as its biodiversity value to the general public. Further studies, seeking other occurrence localities of *D. septemcinctus* in this region will clarify the environmental predictors of its distribution. Moreover, an effort to gather genetic samples from this region will allow the evaluation of the genetic variability of this population, also bringing light to the taxonomy of these two subspecies.

Supplementary Material

The following online material is available for this article:

Table S1 – Camera trap registers on xenarthran species in the Parque Natural Municipal do Pombo (PNMP), between April 2022 and March 2023.

Table S2 – Roadkill registers on *Dasypus* species in federal roads BR-262 and BR-267, and the state roads MS-040 and MS-338.

Acknowledgments

The camera trap data from this study was collected as part of the Giant Armadillo Conservation Program while the carcass was found during road surveys done as part of the Anteaters & Highways

Program. Both programs received multiple grants listed at https://www.icasconservation.org.br/. We thank Nina Attias for reviewing a previous version of this manuscript. PMGJ thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 303524/2019-7).

Associate Editor

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data availability

The sequences generated in this study are deposited in the GenBank repository with the accession numbers PP537895 and PP528439. The camera trap raw files and road-kill records are available in the supplementary materials.

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Received: 16/04/2024
Accepted: 24/11/2024
Published online: 10/01/2025