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New record in Uruguay of the marsh deer (*Blastocerus dichotomus* Illiger, 1815) redefines its southern geographic distribution area

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Introduction

The Neotropical biogeographical region stands as one of the richest in deer diversity, with the marsh deer (*Blastocerus dichotomus*, Illiger, 1815) emerging as the largest contemporary species (González and Duarte, 2020). Historically, this species roamed across various wetland habitats throughout Argentina, Bolivia, Brazil, Paraguay, Peru, and Uruguay (Weber and González, 2003). However, extensive habitat loss has led to a drastic reduction in its distribution, with current populations being small and isolated (Pinder and Grosse, 1991; Tomas et al., 1997). Although marsh deer once inhabited northeastern Brazil during the Pleistocene (Pinder and Grosse, 1991; Magalhaes et al., 1992), no paleontological or archaeological evidence exists for their presence in the Holocene north of latitude 10° S. Today, relictual populations persist in key areas such as the Pantanal in Brazil, and the Iberá and Paraná Delta in Argentina (Pinder and Grosse, 1991; Beccaceci, 1994; Mauro et al., 1998; Varela, 2003).

Currently, in Uruguay, the marsh deer is considered extinct in the wild, marking the southernmost limit of its range and the distribution boundary for several species of tropical flora and fauna, fostering the assembly of diverse ecotypes (González and Lessa, 2014). Historical records place the species in the departments of Rocha and Treinta y Tres within the wetlands of the "Bañados del Este," and along the Uruguay River to the west (González, 1994). The last documented sighting dates to the capture of a specimen in 1958 in the marshy area known as "Los Indios" (Arredondo, 1959). Additionally, in 1991, an individual was poached in the Department of Soriano, though it likely originated from the Tigre Delta area in Argentina rather than representing a stable population in Uruguay (González, 1994). Evidence suggests that populations existing north of the Cuareim River in Brazil, near the Uruguayan border, may extend into the Artigas Department (Autuori, 1972).

In Argentina several contributions have recognized the presence of *Blastocerus* during the Platan Stage/Age, spanning from 8000 years BP to the present, as documented by Menegaz (2000); Alcaraz (2010), and Cione and Tonni (1999), Cione and Tonni (2005). Additionally, references to cf. Blastocerus have been made in the Argentine Pampean region during the Late Pleistocene (Chimento et al., 2019; Vezzosi et al., 2019). Concerning the archaeological record, numerous studies have documented the presence of marsh deer in Argentina, contributing significantly to our understanding of the species' historical distribution within the region (Politis et al., 2011; Rodriguez and Bonomo, 2021).

However in Uruguay the presence was cited in archaeological sites (Moreno, 2016). During a drought in 1995, the retraction of the edge of Negra Lagoon (33° 95' 83" S, 53° 38' 41" W) led to the recovery of two partial male skeletons, though the fossilization process precluded material isolation for dating and genetic analysis (Roibal et al., 1996). In this report, we document the discovery of a complete male skull recovered in 2010 from the coastline of the Solís Grande stream, situated on the border of the Canelones and Maldonado departments in Uruguay (Figure 1). Our objective was to estimate the specimen's age through radiocarbon dating, extract DNA, and analyze with mitochondrial marker to compare them with haplotypes described in Argentina and Brazil.

Methods

Sample collection

In the summer of 2010, during a drought along the coastline of the Solís Grande stream (34° 44' 14.33" S; 55° 27' 3.68" W), bordering the departments of Canelones and Maldonado in Uruguay, a marsh deer skull was observed and manually extracted. The sedimentary deposits are from the Quaternary period, composed of very fine sand and Holocene mud, specifically located on the river margin with variable depth (Spoturno et al., 2012). The substrate is characterized by elongated bays, each with moderate concavity and asymmetry towards the east, filled by beach deposits and wind accumulations. The skull was photographed and measured, confirming it as an adult male marsh deer (see Supplementary Figures 1–3; Supplementary Tables 1–3).

Radiocarbon dating

Approximately 1 g of turbinate bone was extracted and sent to the Accelerator Mass Spectrometry Laboratory at the University of Arizona for radiocarbon dating. Radiocarbon ages were calculated as weighted averages of combined machine runs, with a small sample correction applied to samples with a carbon mass less than 0.50 mg.

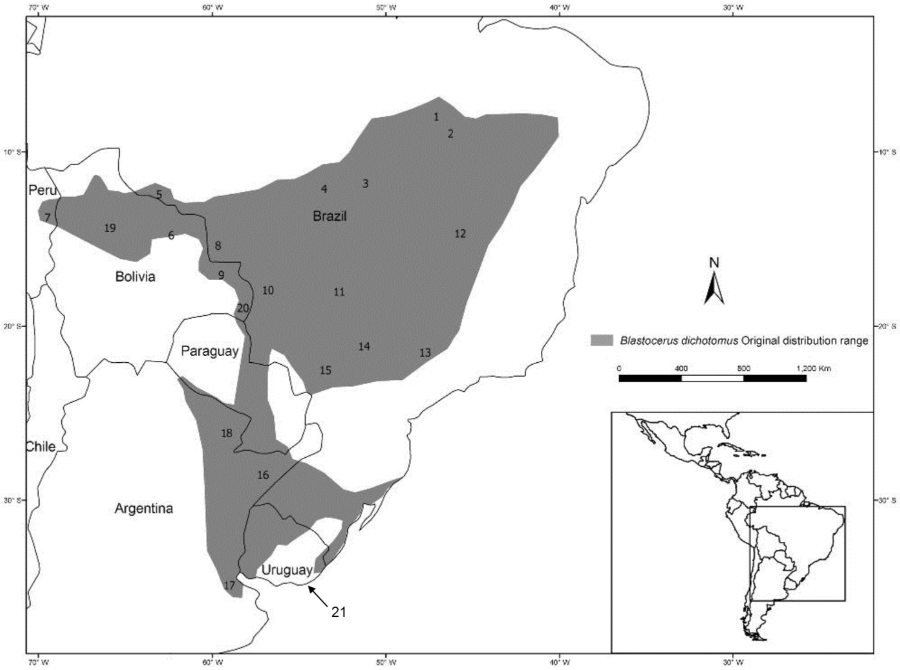


FIGURE 1
Geographic range of *Blastocerus dichotomus* and current known populations. 1 – Krahô Indian Land, Brazil; 2 – Southern Maranhão state, Brazil; 3 – floodplains of the Araguaia and Mortes rivers, Brazil; 4 – Xingu National Park, Brazil; 5 – Guaporé river floodplains in Bolivia and Brazil; 6 – Park Noel Kempff, Bolivia; 7 – Pampas del Heath floodplains, Peru; 8 – upper Guaporé river floodplains, Brazil; 9 – San Matias region, Bolivia; 10 - Pantanal wetland, Brazil; 11 – Emas National Park and Correntes river, Brazil; 12 – Grande Sertão Veredas National Park and headwaters of tributaries of São Francisco River near to the Serra Geral de Goiás/Chapadão and Eastern Bahia, Brazil; 13 – Jataí Ecological Station, Brazil; 14 – tributaries of the Porto Primavera Dam, Brazil; 15 – Floodplains of the Paraná river, Brazil; 16 – Esteros de Iberá/Esteros Santa Lucía, Riachuelo, Miriñay y Batel/Cuenca del Aguapey, Corrientes, Argentina; 17 – Delta del Paraná, Argentina; 18 – Wetlands of Eastern Formosa and Chaco, Argentina; 19 – Department of Beni, Bolivia; 20 – Otuquis National Park, Bolivia; 21 New record Department of Maldonado, Uruguay. Map based on Piovezan et al. (2010).

DNA extraction and PCR amplification

Genomic DNA was extracted from tissue samples (50 mg) following González et al. (1998) protocol. We used two set of primers to amplify a partial fragment of the D-loop 364 bp. DNA was amplified using two primers designer with Primer 3 software specifically for marsh deer (BD1F Primer 1 → 5’ACCAATCTCCTAAGACTCAAGG -3’ and BD1R Primer 2 → 5’TGGCTATTGAGTACAAAACTGT-3’ and DLFHB F 5’GCGGCATGGTAATTAAGCTC 3’, DLRHB R 5’CATTACATTATATGCCCCATGC 3’).

The PCR reactions were set up in a type II A laminar flow chamber with UV light in a room separate from that with the real time PCR thermal using negative controls.

PCRs were performed in 10 µL reactions with 1× Sensimix Evagreen PCR Buffer containing a hot-start DNA polymerase (Bioline) for high specificity and sensitivity PCR, 0.3 pM of each primer, and 0.7–1.5 µL of DNA extract. Two replicates were prepared for each sample and PCR negative controls were included for each set of reactions. PCR was done in a Corbett 6000^{TM} (Rotor Gene) thermal cycler using the following conditions: an initial denaturation at 95°C for 10 min, followed by 45 cycles of 15 s at 95°C, 15 s at 55°C and 15 s at 72°C. The final extension was 10 min at 72°C. The HRM step included a 65–95°C ramp increasing at 0.1°C per step with a 2 s pause at each step.

PCR products were purified with DNAClean and Concentrator^{TM} (Zymo Research^{TM}) kit and diluted to an equal final concentration using a Nanodrop 1000^{TM} Spectrophotometer (Thermo Fisher Scientific). The amplicons were sent to sequence by the Sanger method to Macrogen service System Sequencer (MACROGEN Inc., Korea).

Bioinformatic analysis

Sequences were aligned and edited using MEGA11 (Tamura et al., 2021) and compared with the nucleotide database available in the National Centre for Biotechnology Information (NCBI) using the BLAST utility. Diversity indices such as haplotype and nucleotide diversities were calculated, and the number of polymorphic sites determined. Evolutionary genetic distances among haplotypes were determined using the Kimura 2-Parameter distance, with nucleotide sequence data analyzed using Maximum Parsimony (MP) and Neighbor Joining (NJ) methods (Kimura, 1980; Bandelt et al., 1999; Rozas et al., 2017).

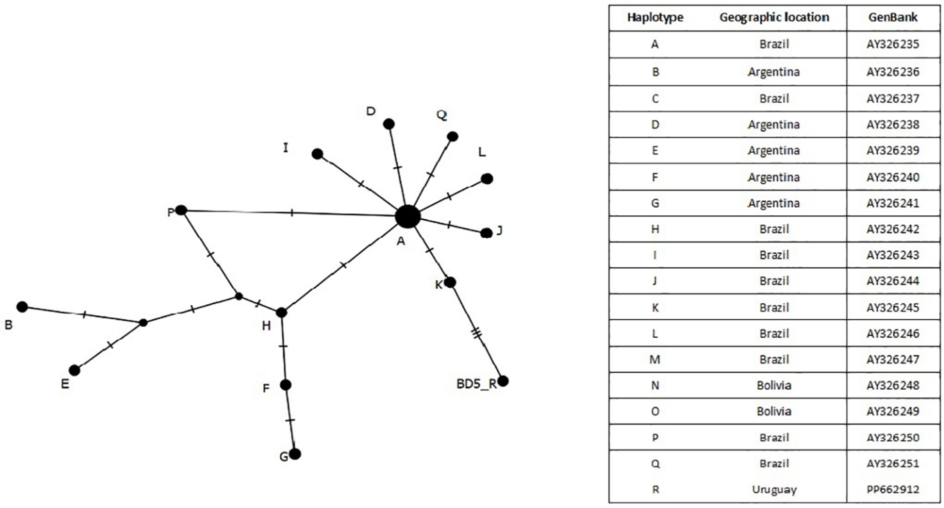
Haplotype networks

To evaluate the evolutionary relationships among haplotypes and their geographic distribution, a haplotype network of D-loop sequences was constructed using the median-joining network approach implemented on PopART 1.7 (Márquez et al., 2006; Leigh and Bryant, 2015). We downloaded all the available D-loop sequences deposited in GenBank for the marsh deer, representing 17 haplotypes (AY326235–AY326251).

Preliminary analysis and conservation implications

Radiocarbon dating yielded a calibrated age range of 294 years to the present with a 95% probability, confirming the presence of marsh deer in Uruguay over the last three centuries and extending the species’ geographic range, providing valuable insights into its historical distribution.

Furthermore, the analysis of a 364 bp D-loop fragment and comparison with haplotypes from six South American localities including Argentina, Bolivia, and Brazil revealed significant genetic diversity and past population dynamics of marsh deer in the region (Figure 2). The R haplotype displayed a clear connection



Haplotype	Geographic location	GenBank
A	Brazil	AY326235
B	Argentina	AY326236
C	Brazil	AY326237
D	Argentina	AY326238
E	Argentina	AY326239
F	Argentina	AY326240
G	Argentina	AY326241
H	Brazil	AY326242
I	Brazil	AY326243
J	Brazil	AY326244
K	Brazil	AY326245
L	Brazil	AY326246
M	Brazil	AY326247
N	Bolivia	AY326248
O	Bolivia	AY326249
P	Brazil	AY326250
Q	Brazil	AY326251
R	Uruguay	PP662912

FIGURE 2
Minimum Spanning Network *D-loop* haplotypes connection using the median-joining network approach implemented on PopART 1.7 (Leigh and Bryant, 2015). The new record is BD5 belonging to haplotype R submitted to GenBank, PP662912.

to Brazilian haplotypes, indicating a population expansion from the ancestral A haplotype, which underwent four mutations in the haplotype network.

Identification of a new haplotype in this skull sample provides compelling evidence for the presence of marsh deer populations in southeast Uruguay. These findings underscore the critical need for habitat restoration as a clear mandate for planning future rewilding strategies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

SG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MA: Methodology, Writing – original draft. FM: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1424867/full#supplementary-material

References

Alcaraz, M. A. (2010). *Sistemática de los cérvidos (Mammalia, Artiodactyla) del Pleistoceno de las áreas extraandinas de Argentina* (La Plata (Ar: Universidad Nacional de la Plata).

Arredondo, H. (1959). *Notas zoológicas Uruguayas* (Montevideo: Imprenta LIGU), 182 pp.

Autuori, M. P. (1972). Biological data and growth of the first horns of the marsh deer, Blastocerus dichotomus (Illiger 1811) (Cervidae). *Zool. Garten* 42, 225–235.

Bandelt, H. J., Forster, P., and Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48. doi: 10.1093/oxfordjournals.molbev.a026036

Beccaceci, M. D. (1994). A census of marsh deer in Iberá Natural Reserve, its Argentine stronghold. *Oryx* 29, 131–134. doi: 10.1017/S0030605300028441

Chimento, N., Zuccari, J. I., Machetto, J. M., and Berbach, L. (2019). Nuevos restos de ciervos (Cervidae, Mammalia) para el Pleistoceno de la Región Pampeana (Argentina): consideraciones paleobiogeográficas y paleoecológicas. *Rev. Bras. Paleontologia* 22, 67–85. doi: 10.4072/rbp

Cione, A. L., and Tonni, E. P. (1999). "Biostratigraphy and chronological scale of uppermost Cenozoic in the Pampean Area, Argentina," in *Quaternary Vertebrate Paleontology in South America. Quaternary of South America & Antarctic Peninsula 12.* Eds. E. P. Tonni and A. L. Cione (A. A. Balkema Publishers, Rotterdam), 23–51.

Cione, A. L., and Tonni, E. P. (2005). Bioestratigrafía basada en mamíferos del Cenozoico superior de la provincia de Buenos Aires, Argentina. *Geología y recursos minerales la provincia Buenos Aires* 11, 183–200.

González, S. (1994). *Marsh deer in Uruguay. Population and Habitat Viability Assessment, in Workshop for the Marsh Deer (Blastocerus dichotomus)* Vol. Section 4. Ed. IUCN/CBSG, (Apple Valley, Minnesota: CBSG publication), 1–6.

González, S., and Duarte, J. M. B. (2020). Speciation, evolutionary history, and conservation trends of Neotropical deer. *Mastozoologia Neotropical* 27, 37–47. doi: 10.31687/saremMN_SI.20.27.1.05

González, E. M., and Lessa, E. P. (2014). *Historia de la mastozoología en Uruguay, in Historia de la mastozoología en Latinoamérica, las Guayanas y el Caribe.* Eds. J. Ortega, J. L. Martínez and D. G. Tirira (Quito y México DF: Editorial Murciélago Blanco y Asociación Ecuatoriana de Mastozoología), 381–404.

González, S., Maldonado, J. E., Leonard, J. A., Vilà, C., Barbanti Duarte, J. M., Merino, M., et al. (1998). Conservation genetics of the endangered Pampas deer (Ozotoceros bezoarticus). *Mol. Ecol.* 7, 47–56. doi: 10.1046/j.1365-294x.1998.00303.x

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120. doi: 10.1007/BF01731581

Leigh, J. W., and Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116. doi: 10.1111/2041-210X.12410

Magalhaes, R. M., Mello, M. G., and Bergvist, L. P. (1992). Pleistocene cervid from the northeastern region of Brazil. *Anais da Academia Bras. Cienc.* 64, 149–168.

Márquez, A., Maldonado, J. E., González, S., Beccaceci, M. D., Garcia, J. E., and Duarte, J. M. B. (2006). Phylogeography and Pleistocene demographic history of the endangered marsh deer (Blastocerus dichotomus) from the Río de la Plata Basin. *Conserv. Genet.* 7, 563–575. doi: 10.1007/s10592-005-9067-8

Mauro, R. A., Mourão, G. M., Coutinho, M. E., Silva, M. P., and Magnusson, W. E. (1998). Abundance and distribution of marsh deer Blastocerus dichotomus (Artyodactila: Cervidae) in the Pantanal, Brazil. *Rev. Ecología Latino Americana* 5, 13–20.

Menegaz, A. N. (2000). *Los Camèlidos y Cèrvidos del Cuaternario del Sector Bonaerense de la Regiòn Pampeana. [dissertation thesis]* (La Plata (Ar: Universidad Nacional de la Plata).

Moreno, F. (2016). La gestión animal en la prehistoria del este de Uruguay: de la economía de amplio espectro al control de animales salvajes. *Tessituras* 4, 161–187.

Pinder, L., and Grosse, A. P. (1991). Blastocerus dichotomus. *Mamm. Species* 380, 1–4. doi: 10.2307/3504311

Piovezan, U., Tiepolo, L. M., Tomas, W. M., Duarte, J. B., Varela, D., and Marinho-Filho, J. S. (2010). “Marsh deer *Blastocerus dichotomus* (Illiger, 1815)", in *Neotropical Cervidology: biology and medicine of Latin American deer*, ed. J. B. Duarte and S. González (Jaboticabal: FUNEP/IUCN), 66–76.

Politis, G. G., Prates, L., Merino, M. L., and Tognelli, M. F. (2011). Distribution parameters of guanaco (Lama guanicoe), pampas deer (Ozotoceros bezoarticus) and marsh deer (Blastocerus dichotomus) in Central Argentina: Archaeological and paleoenvironmental implications. *J. Archaeological Sci.* 38, 1405–1416. doi: 10.1016/j.jas.2011.01.013

Rodriguez, J. M., and Bonomo, M. (2021). Nuevos análisis zooarqueológicos y tafonómicos del sitio Cerro Tapera Zázquez (Parque Nacional Pre-delta, Argentina). *COMECHINGONIA* 25, 81–100. doi: 10.37603/2250.7728.v25.n1.30411

Roibal, D., González, S., and Ubilla, M. (1996). “Restos de ciervo de los pantanos en El Potrerillo de Santa Teresa,” in *IV Jornadas de Zoología del Uruguay* (Sociedad Zoológica del Uruguay, Montevideo), 35.

Rozas, J., Ferrer-Mata, A., Sánchez-Del Barrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., et al. (2017). DnaSP 6: DNA sequence polymorphism analysis of large datasets mol. *Biol. Evol.* 34, 3299–3302. doi: 10.1093/molbev/msx248

Spoturno, J. J., Oyhantçabal, P. B., and Loureiro, J. (2012). *Mapa Geològico del Departamento de Maldonado escala 1/100 000* (Uruguay: Informe Ministerio de Industria y Energia).

Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027. doi: 10.1093/molbev/msab120

Tomas, W. M., Beccaceci, M. D., and Pinder, L. (1997). “Cervo-do-pantanal (Blastocerus dichotomus),” in *Biologia e conservação de cervídeos sul-americanos*. Ed. J. M. B. Duarte (FUNEP, Jaboticabal), 24–40.

Varela, D. (2003). *Distribución, abundancia y conservación del ciervo de los pantanos (Blastocerus dichotomus) en el Bajo Delta del río Paraná, Provincia de Buenos Aires, Argentina. Licentiate thesis* (Buenos Aires: University of Buenos Aires).

Vezzosi, R. I., Brunetto, E., and Zalazar, M. C. (2019). El registro fósil de vertebrados del Cuaternario de Santa Fe (Argentina): Primera Actualización. *Opera Lilloana* 52, 638–670.

Weber, M., and González, S. (2003). Latin American deer diversity and conservation: a review of status and distribution. *Ecoscience* 10, 443–454. doi: 10.1080/11956860.2003.11682792