

**Assessing the Taxonomic Status of *Mazama simplicicornis argentina* Lönnberg, 1919  
(Artiodactyla: Cervidae)**

Eluzai Dinai Pinto Sandoval<sup>1</sup>, Gabrielle Queiroz Vacari<sup>1</sup>, Juan Pablo Juliá<sup>2</sup>, Susana González<sup>3</sup>,  
Miluse Vozdova<sup>4</sup>, Halina Cernohorska<sup>4</sup>, Svatava Kubickova<sup>4</sup>, Daniela C. Kalthoff<sup>5</sup>, and José  
Mauricio Barbanti Duarte<sup>1,\*</sup>

<sup>1</sup>Núcleo de Pesquisa e Conservação de Cervídeos (NUPECCE), Faculdade de Ciências Agrárias e  
Veterinárias da Universidade Estadual Paulista (UNESP), Jaboticabal-SP, Brazil.

\*Correspondence: E-mail: mauricio.barbanti@unesp.br (Duarte).

E-mail: eluzaidinai@gmail.com (Sandoval); gabrielle\_g12@hotmail.com (Vacari)

<sup>2</sup>Facultad de Ciencias Naturales. Universidad Nacional de Tucumán, Argentina. E-mail:  
jupaju@yahoo.es (Juliá)

<sup>3</sup>Departamento de Biodiversidad y Genética, Instituto de Investigaciones Biológicas Clemente  
Estable, Montevideo, Uruguay. E-mail: sugonza9@yahoo.com (González)

<sup>4</sup>Veterinary Research Institute, Brno, Czech Republic. E-mail: miluse.vozdova@vri.cz (Vozdova);  
halina.cernohorska@vri.cz (Cernohorska); svatava.kubickova@vri.cz (Kubickova)

<sup>5</sup>Swedish Museum of Natural History, Frescativägen 40, Stockholm, Sweden. E-mail:  
daniela.kalthoff@nrm.se (Kalthoff)

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*Mazama simplicicornis argentina* is the name given to the description of a gray brocket collected in 1919 by Lönnberg in the central Chaco of Argentina. Subsequent authors, based on morphological similarities, considered this name as a synonym of the species *Subulo gouazoubira* Fischer, 1814 from Paraguay. In the absence of genetic analyses to compare the Argentinian and Paraguayan gray brockets, we aimed to clarify the taxonomy of *M. simplicicornis argentina* through an integrative assessment using morphological, cytogenetical, and molecular data from its holotype and a current topotype. Qualitative skull features and cranio-morphometric results of *M. simplicicornis argentina* showed a great similarity with the *S. gouazoubira* neotype characters. The diploid chromosome number of *M. simplicicornis argentina* topotype correspond to the karyotypical pattern of *S. gouazoubira* with  $2n = 70$  and  $FN = 70$ , showing a great similarity in all classic and molecular cytogenetic results and evidencing the homologies between karyotypes. The phylogenetic analysis of mitochondrial genes used in this study (concatenated partial *ND5* and *Cytb* gene) allocated the *M.*

*simplicicornis argentina* specimens in the monophyletic clade of *S. gouazoubira* with 100% of branch value. These results evidenced that there is no discontinuity between the Argentinian and Paraguayan gray brockets, therefore, the individuals originally described as *M. simplicicornis argentina* should be recognize as *S. gouazoubira* species.

**Key words:** Animal cytogenetic, Cervidae, Gray brocket, Mitochondrial DNA, Morphology.

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## BACKGROUND

The gray brocket deer *Subulo gouazoubira* (G. Fischer 1814) is a small to medium-sized deer that is distributed from south of the Amazon region to Uruguay and the north of Argentina (Black-Décima et al. 2010). The species was first described by Azara (1801) who diagnosed individuals from Paraguay and named them Gouazoubira, a name of Guaraní origin, that was later invalidated for not following the Principle of Binomial Nomenclature (ICZN, 1999). Based on Azara's observations, the species was named *Cervus gouazoupira* by G. Fischer (1814), subsequently transferred to the genus *Mazama* by Rafinesque (1817) considering the morphological similarities with the species that compose the genus. Rather recently, the spelling *gouazoubira* has been defined as the appropriate citation for the species (ICZN, 2001, Opinion, 1985), and the name *Mazama gouazoubira* was adopted by many authors for this taxon (Cabrera 1960; Rossi 2000; Black-Décima et al. 2010). However, later studies identified these similarities as adaptive convergence, and thus contradicting the monophyly of the genus *Mazama* (Gilbert et al. 2006; Duarte et al. 2008; Gutiérrez et al. 2017; Heckeberg et al. 2020).

Authors had discussed this polyphyletic condition of the genus *Mazama*, in which the species are recovered in two subtribes, the Blastocerina and Odocoileina (Heckeberg et al. 2020). In this sense, the Azara's individuals are recovered within the Blastocerina subtribe, separated from *M. americana* (Erxleben, 1777), the type species of the genus *Mazama*, that is recovered within Odocoileina subtribe (Gutiérrez et al. 2017; Heckeberg et al. 2020; Bernegossi et al. 2022). The recent study of complete mitochondrial phylogeny, confirmed the relation cited above and reviewed the availability of the name-group *Subulo* Smith, 1827, to allocated the gray brocket deer, renaming *Subulo gouazoubira* (G. Fischer 1814) (Bernegossi et al. 2022). This nomenclature rearrangement

was proposed as a starting point to assist in the taxonomic clarification of Neotropical deer, allowing the comparison with other taxa described in the past that were categorized as synonym or subspecies by many authors (Bernegossi et al. 2022).

This is the case of *Mazama simplicicornis*, an objective synonym attributed to Azara's (1801) gray brockets by Illiger (1815), which was considered the valid name by many authors (Smith 1827; Lesson 1842; Gray 1850; Pelzeln 1883; Allen 1915; Lydekker 1915; Lönnberg 1919; Miranda-Ribeiro 1919). Lönnberg (1919) considered that two subspecies were present in Argentina, *M. simplicicornis gouazoubira* (G. Fischer 1814) and a new one, which he named *M. simplicicornis argentina* based on an adult female collected in the Argentine Chaco. Lönnberg (1919) noticed that in comparison with *Mazama simplicicornis* from Paraguay, his new subspecies from Argentina had smaller skull and body dimensions, with the presence of a white spot below the nostril and behind the rhinarium considered absent in the individual from Paraguay (Lönnberg 1919).

During the 20<sup>th</sup> century, all the gray forms of brockets were considered to belong to *M. gouazoubira*, and for this reason, both subspecies of *Mazama simplicicornis* were synonymized with the *M. gouazoubira* (Cabrera 1960). Thus, Lönnberg's gray brocket was renamed *M. gouazoubira argentina* and identified as a subspecies restricted to the locality Rio de Oro in the Central Chaco Region of Argentina. On the other hand, *M. gouazoubira gouazoubira* is distributed in the southern of Brazil, the Pantanal, Paraguay, Uruguay and northern Argentina (in Tucumán, Santiago del Estero and Entre Rios Provinces) (Avila-Pires 1959; Pinder and Leeuwenberg 1997; Richard and Juliá 2001; Caraballo 2009; Periago and Leynaud 2009).

The definition at a sub-specific level has been discussed by several authors with divergent positions, in which some defended the absent of morphological differences between *S. gouazoubira* subspecies, and that Azara's description is the only gray brocket present in Argentina (Cabrera 1960; Czernay 1987). In this context, cytogenetic data is an important tool to clarify the taxonomic status, under a biological species concept. This was demonstrated in studies analyzing subspecies of *Mazama americana* and whose karyotypic divergences confirmed the validation of *M. jucunda* Thomas, 1913; *M. temama* (Kerr 1792) and *M. rufa* (Illiger 1815) (Jorge and Bernishke 1977; Duarte and Jorge 2003; Peres et al. 2021; Sandoval et al. 2022).

The revalidation of new species of *Mazama* confirms the importance of reviewing each taxon described and also raises doubts about the identity of other descriptions of species or subspecies that were considered synonyms of the former *M. gouazoubira*, now named *S. gouazoubira*. Thus, the goal of this study is to reassess the taxonomic status of Lönnberg's (1919) *Mazama simplicicornis argentina* and clarify its relationship to the gray brockets of Paraguay. This will be realized with the assistance of morphometric as well as molecular (mtDNA) and cytogenetic

methods. Lönnberg's type specimen (female) and the topotype (male), both from Chaco Central, Argentina, are central in our analysis.

## MATERIALS AND METHODS

### Specimens and samples

We collected an adult male topotype in Presidencia de la Plaza locality, in the Chaco Province of Argentina. The specimen received an identification number NUPECCE (T450) and catalog number (NPC172) at the Deer Research and Conservation Center (NUPECCE) museum where it remains deposited. In addition, data and tissue samples of the *M. simplicicornis argentina* holotype (adult female) were collected in the Swedish Museum of Natural History at Stockholm (NRM-MA620394) for craniometrical and molecular analysis.

### Morphological characterization

After collecting, the topotype was photographed, the skin was removed and treated with a tanning solution to preserve it. Aspects of general coat color, color of the neck, dorsal line of the body, tail, ventral region of the body, front and hind feet, pigmentation patterns in the hair of different regions of the body, length of hair in different regions of the body, occurrence of strips of anteverted hair and rounded hair tufts in the tarsal region were examined. Additionally, the facial color pattern was analyzed (HersHKovitz 1982).

Different angles of the skulls were photographed to complement the documentation and description of the specimens. Following the criteria of von den Driesch (1976), we took 26 skull measurements presented in the collected topotype and holotype of *M. simplicicornis argentina*, and also, we measured adult individuals of *S. gouazoubira*, *Mazama nemorivaga*, *Mazama americana* and *Mazama rufa* species from the NUPECCE database (Table S2). The following were measured: total length, condylobasal length, basal length, short skull length, premolar 1 – prosthion, viscerocranium length, greatest length of the nasals, short lateral facial length, oral palatal length, lateral length of the premaxilla, length of the cheektooth row, length of the molar row, length of the premolar row, greatest inner length of the orbit, greatest inner height of the orbit, greatest mastoid breadth, greatest breadth of the occipital condyles, greatest breadth at the bases of the paraoccipital, greatest breadth of the foramen magnum, Least frontal breadth, greatest breadth across the orbits,

least breadth between the orbits, zygomatic breadth, greatest breadth across the nasals, greatest breadth across the premaxillae, Basion – The highest point of the superior nuchal crest.

We used the “Paleontological Statistics” PAST program (Hammer et al. 2001) to performed a cluster analysis by the Unweighted Pair Group Method with Arithmetic mean method (UPGMA) based on Euclidian distance of 26 skull measurements. The dataset was formed by male and female individuals to include both the topotype (male) and the holotype (female) of *M. simplicicornis argentina*.

### **Cytogenetic analysis**

After collecting the topotype, 5 × 2 cm skin fragments from the inguinal region were collected and preserved in liquid nitrogen as described by Duarte et al. (2021). Then, the chromosomes were obtained by fibroblast *in vitro* culture according to Verma and Babu (1995). The chromosomal preparations were subjected to conventional Giemsa staining, G-banding (Seabright 1971, modified), C-banding (Sumner 1972), and Ag-NOR staining (Howell and Black 1980). Chromosomes were numbered according to the *S. gouazoubira* karyotype described in Bernegossi et al. 2022 showing correspondence to cattle (*Bos taurus*, BTA) chromosomes.

Fluorescence *in situ* hybridization using BAC probes was performed to characterized the homologies between the karyotype of *Mazama simplicicornis argentina* topotype and the chromosomes of *S. gouazoubira* (2n = 70 and NF = 70). BAC clones were selected from the CHORI-240 cattle library based on the NCBI ARS-UCD1.2 Assembly data was obtained from BACPAC Genomics, Emeryville, CA, USA (Table S3). For DNA extraction, we used a protocol adapted from the method included in Wizard<sup>®</sup> Plus SV Minipreps DNA Purification Systems. BAC DNA was labeled with Green-DdUTP (Abbott, IL, USA), biotin 16-dUTP or digoxigenin-11-dUTP (Roche, Mannheim, Germany) using BioPrime<sup>®</sup> Array CGH Genomic Labeling (Invitrogen, Carlsbad, CA, USA). FISH was performed as described in Vozdova et al. (2019). A Zeiss Axio Image Z2 (Carl Zeiss Microimaging GmbH, Jena, Germany) fluorescence microscope, equipped with appropriate fluorescence filters for the visualization of FISH results, was used.

### **DNA extraction, amplification and sequencing**

The genomic DNA was extracted from liver of the topotype using a modified protocol based on the methodology described by Sambrook et al. (1989), and from nasal bone of the holotype using a modified protocol based on the methodology described by Medrano et al. (1990) with

optimizations performed as in González (1997). The samples were quantified by spectrophotometry and analyzed by agarose gel analysis, and then diluted in a solution for use.

For the topotype, the amplification was performed using the protocol described by Peres et al. (2021) of the following partial genes: cytochrome b (*Cytb* 480bp and 660 bp) (Hassanin et al. 1998; Duarte et al. 2008) and NAD5 Dehydrogenase subunit 5 (*ND5* 691 bp and 688 bp) (Caparroz et al. 2015).

For the holotype, the amplification was performed with real time PCR using a Rotor Gene (Qiagen) and the following partial genes: cytochrome *b* (*Cytb* 224pb) (González et al. 2009), NAD5 Dehydrogenase subunit 5 (*ND5* 224pb) and NAD5 (*ND5* 251pb) (Leandro 2019). The reaction was composed by 5 µL of SensiFAST™ HRM Kit (1X), 0.6 µL of each primer (10 µM), 5 µL of DNA (10 ng/µL) and 3.8 µL of water. For DNA amplification the cycles were 95°C for 2 minutes / 95°C for 5 seconds / 54°C for 10 seconds (20 cycles) / 53°C for 10 seconds (20 cycles) / 52°C for 10 seconds (20 cycles) / 72°C for 20 seconds.

Purification of the PCR products was performed with the Wizard SV gel and PCR Clean-Up System kit (Promega™), followed by sequencing using the ABI BigDye Terminator kit (Applied Biosystems) in an ABI 3130xl automatic sequencer (Applied Biosystems).

## Phylogenetic analysis

We generated mtDNA sequences of the holotype (*Cytb* 224 bp partial gene, *ND5* 224bp partial gene and *ND5* 249 bp partial gene), and topotype (*Cytb* 480bp partial gene, *ND5* 691bp partial gene and *ND5* 688 bp partial gene) (Table 1). We obtained available sequences in GenBank from Neotropical deer species *M. americana* sensu lato, *M. rufa*, *M. nemorivaga*, *M. nana*, *M. pandora*, *M. jucunda*, *M. temama*, *S. gouazoubira*, *H. antisensis*, *B. dichotomus* and *O. bezoarticus* to complement the matrix for phylogenetic analysis using the species *A. alces* as outgroup. The concatenated nucleotide sequences were aligned using the Clustal X program (Thompson et al. 1997), and manually handled replacing the ends with BioEdit “N” (Hall 1999). The evolutionary model was generated with Modeltest 3.7 (Posada and Crandall 1998).

Bayesian inference (BI) analyses were performed using the MR BAYES 3 software (Huelsenbeck and Ronquist 2001), with 10,000,000 generations until obtaining a variance of < 0.01, adopting a 25% burn-in discard. To estimate the posterior probability, the “Markov Chain Monte Carlo” (MCMC) method was used with nchains = 4, nruns = 2 and burninfrac = 0.25 for all genes. The tree obtained was edited with the FigTree v.1.4.0 software (Rambaut 2012).

**Table 1.** Mitochondrial gene sequences used for phylogenetic analysis of *Mazama simplicicornis argentina* Lönnberg, 1919, topotype and other neotropical cervids

Species	Cytb	ND5	Origin	Description
<i>Mazama simplicicornis argentina</i> (NRM-MA620394)	OP627522	OP627524 OP627525	Chaco Province, Argentina. Lönnberg 1919.	This study
<i>Mazama simplicicornis argentina</i> (T450)	OP627521	OP627523	Chaco Province, Presidencia de la Plaza. Argentina	This study
<i>Subulo gouazoubira</i> (T377)	MZ350858	MZ350858	Puerto Galileo, Paraguay	Bernegossi et al. 2022
<i>Subulo gouazoubira</i> (T389)	MZ350866	MZ350866	Puerto Arecutacua, Paraguay	Bernegossi et al. 2022
<i>Subulo gouazoubira</i> (T082)	MZ350862	MZ350862	Camobi-RS, Brazil	Bernegossi et al. 2022
<i>Subulo gouazoubira</i>	KJ772514	KJ772514	Pantanal, Brazil	Caparroz et al. 2015
<i>Subulo gouazoubira</i> (MRGsp2)	JN632658	JN632658	Colombia	Hassanin et al. 2012
<i>Mazama nemorivaga</i> (T24)	MZ350861	MZ350861	Porto Velho-RO, Brazil	Bernegossi et al. 2022
<i>Mazama nemorivaga</i> (T346)	MZ350867	MZ350867	Lupar-PA, Brazil	Bernegossi et al. 2022
<i>Mazama nemorivaga</i>	JN632659	JN632659	Peru	Hassanin et al. 2012
<i>Mazama nemorivaga</i>	JN632660	JN632660	French Guiana	Hassanin et al. 2012
<i>Mazama americana</i> (MAZ9472)	JN632656	JN632656	French Guiana	Hassanin et al. 2012
<i>Mazama americana</i> (T358)	MZ350857	MZ350857	Cayenne, French Guiana	Bernegossi et al. 2022
<i>Mazama americana</i> (T255)	MN726909	MZ488890	Juina, Brazil	Rincon-Cifuentes et al. 2020/ Peres et al. 2021
<i>Mazama americana</i> (T253)	MZ350856	MZ350856	Juina, Brazil	Bernegossi et al. 2022
<i>Mazama rufa</i> (T385)	MZ488852	MZ488894	Foz de Iguacu, Paraná, Brazil	Peres et al. 2021
<i>Mazama nana</i> (T107)	MZ350863	MZ350863	Paraguay	Bernegossi et al. 2022
<i>Mazama jucunda</i> (T071)	DQ789231	MZ488899	Paraná, Brazil	Duarte et al. 2008/ Peres et al. 2021
<i>Mazama jucunda</i> (T215)	MZ350859	MZ350859	P. E. Intervalles-SP, Brazil	Bernegossi et al. 2022
<i>Mazama temama</i> (T366)	MZ350864	MZ350864	Veracruz, México	Bernegossi et al. 2022
<i>Hippocamelus antisensis</i>	JN632646	JN632646	Argentina	Hassanin et al. 2012
<i>Alces alces</i>	MF784602	MF784602	Poland	Swiloska et al. 2020
<i>Ozotoceros. Bezoarticus</i> (FNMA40)	MZ350860	MZ350860	Brazil	Bernegossi et al. 2022
<i>Blastocerus dichotomus</i> (CV21)	OM543539	OM543540	Brazil	Sandoval et al. 2022

## RESULTS

The *Mazama simplicicornis argentina* Lönnberg, 1919, topotype shows a general gray color of body, tinged yellowish laterally, with presence of mental and nasal whitish patch, a brown pale mandibular band and a dark brown rostral stripe toward the antler button. The supra and infraorbital band has a pale yellow with brown-speckled hair, with presence of a white superciliary spot, and a frontal hair tuft. The specimen also shows big ears with inner border white, yellow hair laterally on the lower border, and white posterobasal auricular patch. The color of the dorsal neck to the anterior region of the body has gray-brown color and the posterior region has a dark brown color speckled with golden hairs. Flanks showed a pale brown color where the coat is longer with the presence of the yellowish hair. The perianal region and lower surface of the tail has a white color, the external

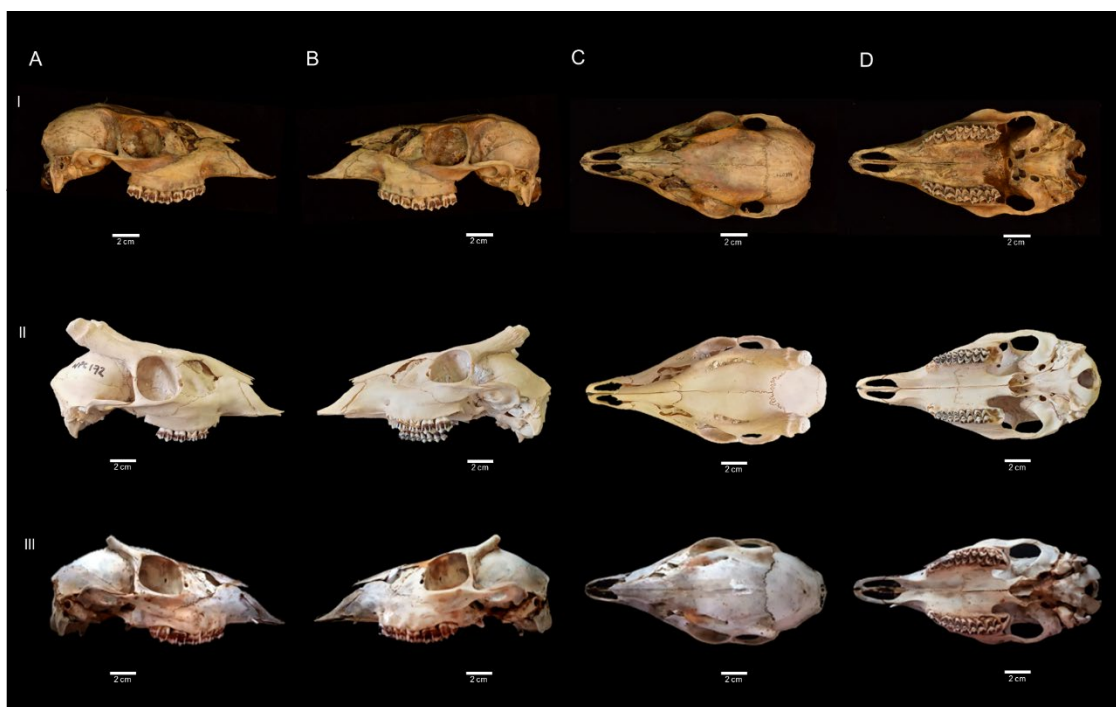
proximal area of the members of the same color as the dorsum of the neck and the distal part shows a dark golden color (Fig. 1).



**Fig. 1.** Lateral view of the adult male topotype of *Mazama simplicicornis argentina* (Lönnerberg 1919) collected in the Chaco Province of Argentina.

The skull of the topotype and holotype showed a flat lacrimal fossa, two lacrimal foramina externally at the orbital border, separated from each other, with an extended vomerine septum, and a small, flat tympanic bulla. In addition, the topotype's skull presented a short, thick, inclined pedicles and a preorbital region with inverted triangular shape (Fig. 2). All these characters were in accordance with the skull described for *S. gouazoubira* neotype, excepting the pedicles that in this last specimen was slender, but also short and inclined.

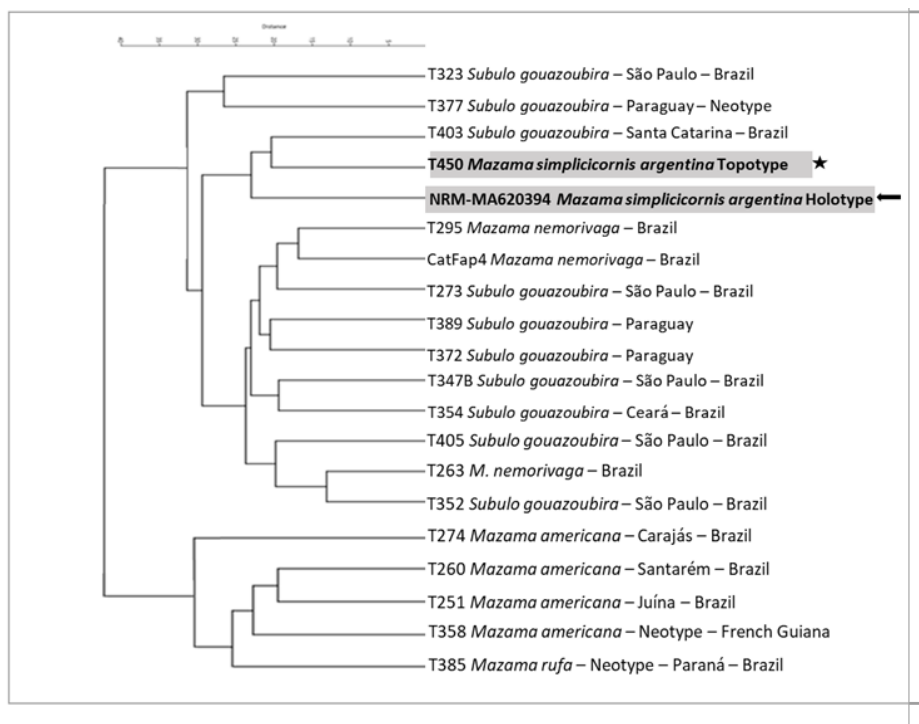




**Fig. 2.** Skull views of *Mazama simplicicornis argentina* (I) Holotype and (II) Topotype, compared with (III) the skull of *Subulo gouazoubira* neotype (Bernegossi et al. 2022, adapted). A = right lateral view, B = left lateral view, C = dorsal view and D = ventral view.

The skull measures of these specimens were very similar within each other. The only difference was the length of nasal that in *M. simplicicornis argentina* topotype showed a greater size than the holotype described by Lönnberg (1919) and also, the *S. gouazoubira* (G. Fischer 1814) neotype.

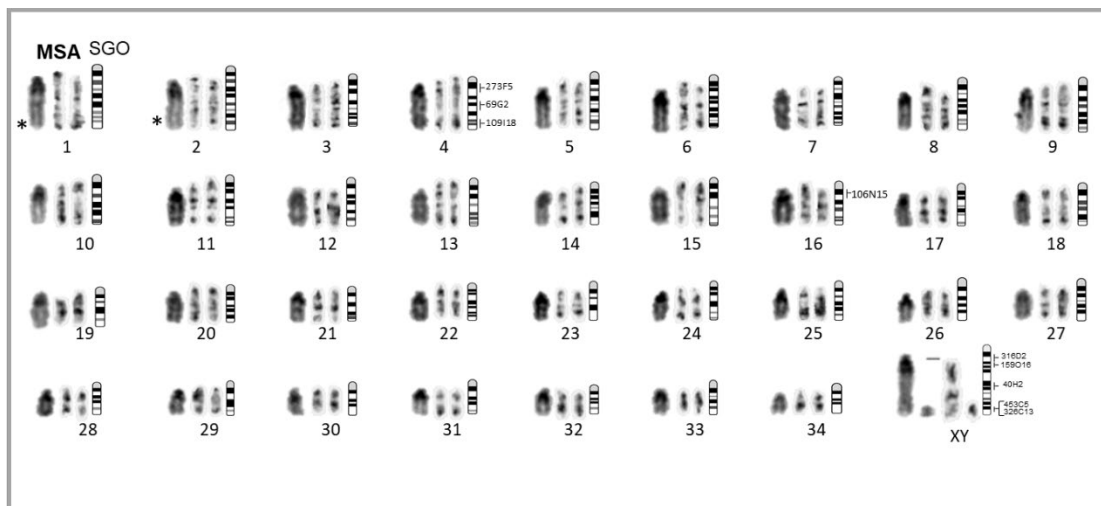
The cluster analysis of 26 craniometrical characters (Fig. 3) shows that the two individuals of *M. simplicicornis argentina* fall within the group of small brockets with *S. gouazoubira* and *M. nemorivaga* separated from a second group with *M. americana* and *M. rufa*. All *S. gouazoubira* specimens from different origins in Brazil, Paraguay and Argentina, formed a mixed group, in which both topotype and holotype were grouped in a subgroup with one individual *S. gouazoubira* from Santa Catarina state in Brazil.



**Fig. 3.** Cluster analysis of skull measurements of *Mazama simplicicornis argentina* (MSA) (Lönnberg 1919) compared with *Subulo gouazoubira*, *Mazama nemorivaga*, *Mazama americana* and *Mazama rufa*; star = collected adult male topotype; arrow = adult female holotype.

### Cytogenetic description

The cytogenetic analysis performed in the *M. simplicicornis argentina* (MSA) topotype revealed a karyotype with diploid number  $2n = 70$ , XY, and absence of B chromosomes (Fig. 4). All autosomes and the X chromosome were acrocentric, showing correspondence in G-banding patterns and FISH results to *S. gouazoubira* chromosomes (SGO) (Fig. 4, Table S4). The C-banding revealed heterochromatic positions in the centromeric regions of each chromosome. The NORs were detected in the telomeric regions of /MSA1 and MSA2 pairs. The BAC probe derived from the centromeric region of BTA1 (BAC106N15) hybridized in the centromeric region of chromosome MSA16, while the BAC probes derived from the proximal (BAC69G2), distal (BAC109I18) and telomeric (BAC273F5) regions of BTA1 hybridized to the chromosome MSA4 (Fig. 4). BAC clones derived from the BTA X hybridized in the proximal (BAC316D2X and BAC159O16) and middle (BAC 40H2) regions, and distally in the PAR region (BACs 453C5, 326C13) of the MSAX chromosome showing correspondence to the SGOX chromosome (Fig. 4 and Fig. S1).

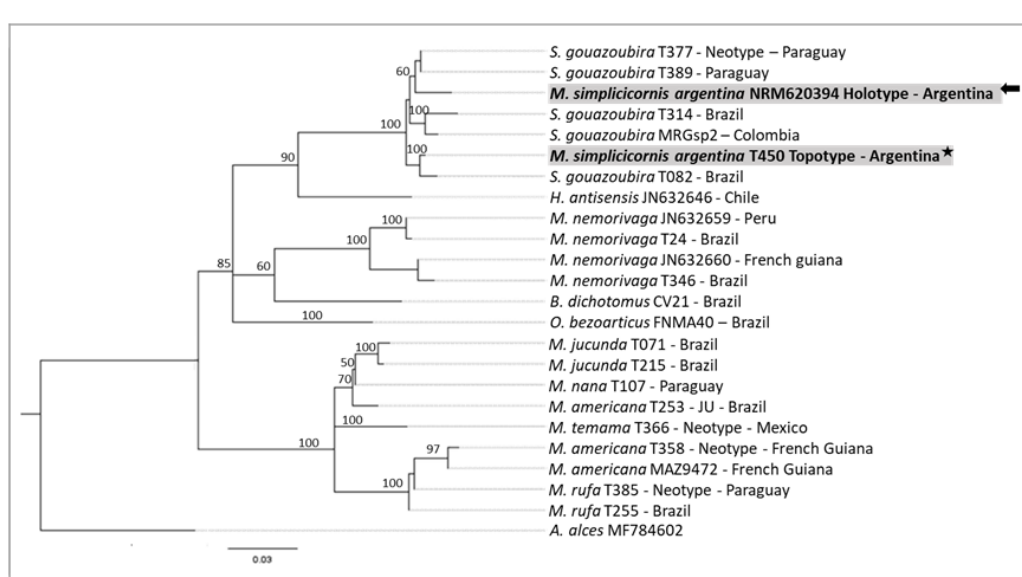


**Fig. 4.** Karyotype of a male topotype of *Mazama simplicicornis argentina* (MSA) (Lönnerberg 1919) collected in the Chaco Province of Argentina. From left to the right: chromosomes under C-banding, G-banding and ideogram of *Subulo gouazoubira* (SGO) showing correspondence to MSA chromosomes (adapted from Bernegossi et al. 2022). The NOR positions are marked with asterisks. FISH results using BAC probes derived from BTA1 and BTAX are presented on the right of the SGO4.

### Phylogenetic analysis

The Bayesian Inference tree of concatenated fragments of *Cytb* and *ND5* showed two great clades. One, formed by the Odocoileina subtribe that recovered the species *M. jucunda*, *M. nana*, *M. temama*, *M. rufa* and the paraphyletic *M. americana* and the other formed by the Blastocerina subtribe, with the species *S. gouazoubira*, *M. nemorivaga*, *H. antisensis*, *B. dichotomus* and *O. bezoarticus* (Fig. 5). Both holotype and topotype of *M. simplicicornis argentina* were recovered in the Blastocerina subtribe, within the *S. gouazoubira* clade (100% branch value) with *H. antisensis* as sister taxon (90% branch value). The holotype was recovered in a subclade with individuals *S. gouazoubira* from Paraguay and the topotype was recovered in a subclade with one individual from Brazil.

In the same Blastocerina subtribe were allocated the *M. nemorivaga* with *B. dichotomus* as sister taxa. The species *O. bezoarticus* was showed as the first group that diverged in the Blastocerina subtribe. (Fig. 5).



**Fig. 5.** Bayesian Inference of concatenated mitochondrial genes *Cytb* (partial 224bp) and *ND5* (partial 224bp and partial 249bp) of neotropical deer species including *Mazama simplicicornis argentina* topotype (star) and holotype (arrow). Outgroup= *A. alces*.

## DISCUSSION

Qualitative comparison between *M. simplicicornis argentina* individuals and the *S. gouazoubira* neotype showed a great morphological similarity of all characters. The color pattern of external body features of amended description of *S. gouazoubira* neotype were present in *M. simplicicornis argentina* topotype. The topotype shared the white spot behind the rhinarium and below the nostril with the holotype that Lönnberg (1919) considered absent in the animal from Paraguay, however, this character has been reported as an intra-specific variation within *S. gouazoubira* populations (Allen 1915; Rossi 2000). As there is no study that correlates the rostral spot with geographic delimitation, this feature should not be considered as diagnostic of Lönnberg’s Argentinian gray brocket *M. simplicicornis argentina*.

Lönnberg (1919) also noticed a size difference between the *M. simplicicornis argentina* holotype and the Azara’s (1801) gray brocket from Paraguay, however, all morphological measurement in the topotype of *M. simplicicornis argentina* were in the pattern described for *S. gouazoubira* species (González et al. 2018; Bernegossi et al. 2022). This size variation observed by Lönnberg (1919) could be also associated to the wide intraspecific variability that has been reported among *S. gouazoubira* populations (Duarte and Jorge 1998; Rossi 2000; Black-Décima et al. 2010). In addition, the cluster analysis of skull measurement features revealed that the *M. simplicicornis argentina* topotype is allocated in the same small brocket group than *S. gouazoubira* individuals. It

is important to note that in the same group were recovered the *M. nemorivaga* individuals, contrasting with other studies that showed this last species morphometrically different from *S. gouazoubira*, related to smaller skull measurements (González et al. 2018; Rossi 2000). The results divergence may be related with sampling number difference of *M. nemorivaga* that were assessed in each analysis. Qualitative characters such as larger, rounded ears, smaller eyes, smaller orbital cavities, and wider auditory bulla have been cited as efficient to recognize *S. gouazoubira* from *M. nemorivaga* with larger orbital cavities, and narrow auditory bulla (Rossi 2000), and may be taken into consideration to distinguish these two well-established species. Although the skull measurement distance failed to separate the *M. simplicicornis argentina* topotype from *M. nemorivaga*, the similarity of qualitative characters with *S. gouazoubira* is the main morphological correspondence between *M. simplicicornis argentina* and *S. gouazoubira*.

This study contributed with the first cytogenetic description of a gray brocket from Chaco Region in Argentina. diploid number of *M. simplicicornis argentina* topotype corresponds to the *S. gouazoubira* pattern with  $2n = 70$  and  $FN = 70$  (Neitzel 1987; Tomazella 2016; Valeri et al. 2018; Bernegossi et al. 2022). The descriptions of individuals with  $2n = 69$  and  $FN = 70$ , as in the case of the *S. gouazoubira* neotype, were associated with the occurrence of intraspecific chromosomal polymorphism commonly described for the species characterized by heterozygous centric fusion arrangement (Valeri et al. 2018; Bernegossi et al. 2022). However, we did not observe this or any other translocation in any of the analyzed metaphases of *M. simplicicornis argentina* topotype. In addition, the B chromosome were not observed in the metaphases of the topotype, that even are commonly reported in *S. gouazoubira* individuals, the descriptions had showed an intra and inter individual variation of B chromosome numbers from 0 to 5 (Tomazella 2016). Our comparative FISH results with the bovine BAC clones revealed X chromosome structure similar to the acrocentric variant present in *S. gouazoubira* (Bernegossi et al. 2022) and other species from subfamily Cervinae (Frohlich et al. 2017). Also, the FISH positions of the BAC clones for BTA1 corresponded to their localization in *S. gouazoubira* karyotype, with the BTA1 centromeric region in one separated acrocentric chromosome (MSA16, SGO16) and the proximal, distal and telomeric part of BTA1 in another chromosome (MSA4, SGO4) (Bernegossi et al. 2022).

Therefore, the great similarity of Ag-Nor staining, C- and G-banding with the *S. gouazoubira* described ideogram, and also the identical BAC clone's hybridization in *M. simplicicornis argentina* individual and *S. gouazoubira* are evidence of the homologies between karyotypes of these species (Bernegossi et al. 2022). We inferred that there is no chromosomal difference that could represent a reproductive barrier between Argentina and Paraguay gray brocket as occurring between other *Mazama* species (Galindo et al. 2021).

The phylogenetic analysis of mitochondrial markers used in this study (concatenated partial *ND5* and *Cytb* gene) allocated the *M. simplicicornis argentina* topotype and holotype in the monophyletic clade of *S. gouazoubira* with 100% of branch value. The recovery of individuals *M. simplicicornis argentina* in separated subclades associated with *S. gouazoubira* from different origins apparently indicates an absence of genetic structure, however, the low branch values do not support this recognition.

The phylogenetic results also confirmed *S. gouazoubira* clade within Blastocerina subtribe as previously described (Gutiérrez et al. 2017; Heckeberg et al. 2020), confirming the species as sister taxa of *Hippocamelus antisensis*, separated from the *M. nemorivaga*, *Ozotoceros bezoarticus* and *Blastocerus dichotomus* species.

Finally, our morphologic, cytogenetic and phylogenetic analyses do not support *M. simplicicornis argentina* as a valid species or subspecies. Furthermore, we have no detected differentiation between the *M. simplicicornis argentina* holotype, topotype and *S. gouazoubira* neotype, and the other *S. gouazoubira* specimens. In conclusion, our analysis supports that *M. simplicicornis argentina* should be considered as junior synonym of *Subulo gouazoubira*. This confirmation, in addition to reveal genetic data of gray brocket individuals from Chaco Argentina, contributes with the taxonomic clarification of the Lönnberg's (1919) description in the context of resolution of the genus *Mazama*.

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**Authors' contributions:** EDPS contributed with the writing and performed all morphological and genetic analyzes of the specimens. GQV and SG contributed with molecular genetics analysis of the holotype. DK contributed with sampling and morphometric analysis of the holotype. MV, HC and SK contributed with cytogenetic analysis of the topotype, JPJ contributed with topotype's collection and analysis of this study and JMBD organized the research conception and the article design and conducted the final revision of the article.

**Availability of data and materials:** The datasets presented in this study can be found in GenBank. Sequences obtained from tissue samples were recorded with the accession numbers OP627521 to

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## Supplementary materials

**Fig. S1.** FISH results using BAC clones derived from cattle on *Mazama simplicicornis argentina* (MSA) chromosomes. (A) X chromosome labelled with BAC40H2 (red) and BACs 453C5 and 326C13 (green). (B) X chromosome labelled with BAC159O16 (red). (C) Hybridization with the BAC clones derived from the BTA1 (see the results). (download)

**Table S1.** External body measurements (in mm) of a male *Mazama simplicicornis argentina* (Lönnerberg, 1919) topotype T450. (download)

**Table S2.** Skull measurement of a male topotype of *Mazama simplicicornis argentina* Lönnerberg, 1919, and other neotropical cervids. (download)

**Table S3.** BAC clones 1 and X from the CHORI-240 cattle (BTA) library tested in *Mazama simplicicornis argentina* Lönnerberg, 1919. (download)

**Table S4.** Homologies between *Mazama simplicicornis argentina* (MSA), *S. gouazoubira* (SGO) chromosomes (Bernegossi et al. 2022), and cattle BTA chromosomes. (download)