**UNIVERSIDADE ESTADUAL PAULISTA**
**"JÚLIO DE MESQUITA FILHO"**
**FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS**

# O USO DE TOPÓTIPOS ATUAIS PARA AUXLIAR NA RESOLUÇÃO TAXONÔMICA DO GÊNERO *Mazama* RAFINESQUE, 1817, NA AMÉRICA LATINA

**Eluzai Dinai Pinto Sandoval**
Médica Veterinária

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**Discente: Eluzai Dinai Pinto Sandoval**

**Orientador: Prof. Dr. José Maurício Barbanti Duarte**

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Genética e Melhoramento Animal.

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## IMPACTO POTENCIAL DESTA PESQUISA

O esclarecimento genético de cada táxon descrito por meio da análise de topótipos recentes tem sido citado como uma estratégia eficiente para esclarecer a complexa taxonomia das espécies de cervídeos neotropicais do gênero *Mazama*. Este estudo analisou a identidade genética e morfológica de espécimes coletados recentemente na Venezuela, México e Argentina contribuindo com o conhecimento sobre a identificação taxonômica da espécie de cervídeo que habita nos países previamente citados. Acessando às sequências de mitogenoma completo de espécimes coletados nas localidades tipo, foi possível discutir a posição filogenética de cada táxon e também, realizar a primeira descrição cariotípica de *Mazama americana citus* Osgood (1912), *Odocoileus pandora* Merriam (1901), *Mazama rufa toba* (Lönnberg, 1919) e *Mazama simplicicornis* argentina (Lönnberg, 1919). Dessa forma, o estudo impacta potencialmente o conhecimento científico sobre as espécies de cervídeos que habitam nesses países da América Latina, contribuindo assim com a discussão da validade de cada espécie analisada como um primeiro passo para atualizar a taxonomia alfa e beta dos cervídeos neotropicais. O esclarecimento da complexa taxonomia do gênero *Mazama* deve ser seguido pela discussão do status populacional de cada espécie com o objetivo de desenvolver estratégias locais e internacionais de conservação. Este estudo também reforça a necessidade de uma taxonomia integrativa baseada na coleta de espécimes atuais de cervídeos neotropicais.

## POTENTIAL IMPACT OF THIS RESEARCH

The genetic clarification of each taxon described through the analysis of recent topotypes have been cited as an efficient strategy to clarify the complex taxonomy of the Neotropical deer species of the genus *Mazama*. This research analyzed the genetic and morphological identity of recently collected specimens from Venezuela, Mexico and Argentina contributing with knowledge about the taxonomic identify of the

brocket species that inhabits in the previous countries. Assessing mitogenome sequences of specimen from type locality, discussing the phylogenetic position of each taxon and describing the first karyotypes of *Mazama americana citus* Osgood (1912), *Odocoileus pandora* Merriam (1901), *Mazama rufa toba* (Lönnberg, 1919) and *Mazama simplicicornis argentina* (Lönnberg, 1919), potentially impacts the scientific knowledge about the deer species inhabiting Latin America. In addition, this study contributes with the discussion of the validity of each analyzed species as a first step to update the alfa and beta taxonomy of Neotropical deer. The clarification of the complex taxonomy of the genus *Mazama* should be follow by the discussion of populational status of each species aiming to develop local and international conservation strategies. This study also reinforces the need for an integrative taxonomy based on the collection of current specimens of Neotropical cervids.

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## CERTIFICADO DE APROVAÇÃO

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## DADOS CURRICULARES DO AUTOR

**Eluzai Dinai Pinto Sandoval –** natural de Venezuela, Médica Veterinária formada em 2012 pela Universidade Central da Venezuela. Em 2012 cumpriu estágio curricular em Bioparque Temaikén, Argentina. Durante o curso de graduação foi bolsista da Fundación Gran Mariscal de Ayacucho (FUNDAYACUCHO). Trabalhou como veterinária da Unidade de Saúde e Bem-estar Animal do Parque Zoológico El Pinar da Coorporativa de Serviços do Governo do Distrito Capital, Venezuela, durante o período fevereiro 2014-dezembro 2015. Participou do Treinamento técnico do Núcleo de Pesquisa e Conservação de Cervídeos (NUPECCE) em 2016. Obteve Bolsa de Apoio técnico pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) durante o período de novembro 2016-fevereiro 2017 pelo Museu Cervidológico do NUPECCE. Recebeu o título de mestre em Genética e Melhoramento Animal da Unesp (Faculdade de Ciências Agrárias e Veterinárias - Câmpus Jaboticabal) sob orientação do Prof. Dr. José Maurício Barbanti Duarte com financiamento pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Obteve bolsa de Treinamento técnico (TT3) pela Fundação de Apoio à Pesquisa do Estado de São Paulo (Fapesp) no período abril 2019- julho 2019, associada ao projeto temático do NUPECCE. Em agosto 2019 ingressou no doutorado no Programa de Genética e Melhoramento Animal da Unesp (Faculdade de Ciências Agrárias e Veterinárias - Câmpus Jaboticabal) sob orientação do Prof. Dr. José Maurício Barbanti Duarte. A aluna obteve financiamento durante o doutorado pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Desenvolveu parte da pesquisa no Veterinary Research Institute em Brno, República Tcheca.

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um presente que Deus me deu,
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luthier, filho de Deus, servo,
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**SUMÁRIO**

# O USO DE TOPÓTIPOS ATUAIS PARA AUXILIAR NA RESOLUÇÃO TAXONÔMICA DO GÊNERO *Mazama* RAFINESQUE, 1817, NA AMÉRICA LATINA

**RESUMO** – Os estudos taxonômicos dos cervídeos neotropicais do gênero *Mazama* tem mostrado a similaridade morfológica e alta diversidade cariotípica entre as espécies, sugerindo que muitos nomes considerados hoje sinônimos de outras espécies poderiam ser validados a partir das técnicas citogenéticas e genética molecular. Visando conhecer a identidade de vários táxons nominais designados para exemplares que foram descritos em países de América Latina, para os quais há ausência de pesquisa com respeito à sua taxonomia, o objetivo do trabalho foi complementar a descrição taxonômica de espécimes coletados no México, Argentina e Venezuela. Foi feita uma análise integrativa através da caracterização morfológica (biometria corporal, padrões de coloração e craniometria), citogenética (cariótipo convencional, biometria cromossômica, bandamento C, bandamento G, Coloração de Ag-NOR e FISH) e molecular (DNA mitocondrial) de topotipos atuais de *Mazama pandora, Mazama americana citus, Mazama rufa toba* e *Mazama simplicicornis argentina*. Como resultados, a filogenia mitocondrial recupera *M. pandora* como grupo-irmão de *Odocoileus*, e a investigação morfológica aponta para a existência de caracteres diagnósticos do táxon. Somado a exclusividade do cariótipo observado, os dados permitem a discussão de um gênero distinto de *Mazama* e *Odocoileus*. Para *M. americana citus,* os resultados moleculares apontam uma posição basal em Blastocerina, sendo essa linhagem distintamente relacionada com outros cervídeos neotropicais atualmente reconhecidos no gênero *Mazama*. O cariótipo único, a monofilia do clado e os caracteres exclusivos de tamanho e coloração corporal permitem reconhecer o nível específico do táxon. Para *Mazama rufa toba*, os dados moleculares, cariotípicos e morfológicos também são concordantes para o seu reconhecimento específico. A análise do genoma mitocondrial completo sustenta uma posição filogenética independente em Odocoileina para o espécime em relação a outros cervideos neotropicais e, em particular, à espécie *M. rufa*, já descrita para a região do Paraguai. Por outro lado, as características morfológicas e cariotípicas evidenciaram as homologias entre *M. simplicicornis argentina* e *Subulo gouazoubira*. Os resultados filogenéticos recuperaram ambos táxons no mesmo clado, evidenciando que *M. simplicicornis argentina* deve ser considerada como sinónimo junior de *Subulo goauzoubira*. Os topotipos analisados contribuíram com o entendimento da identidade taxonômica de exemplares descritos na América Latina e sua relação com as outras espécies de cervídeos neotropicais. O reconhecimento de espécies válidas e novos nomes genéricos evidenciados no presente estudo, sustenta a necessidade de continuar a revisão aprofundada do gênero *Mazama*, e constitui o passo inicial para estudos com abordagens que avaliem as populações e, estabelecem planos de conservação.

**Palavras-chave:** cervídeos neotropicais, citogenética animal, DNA mitocondrial, morfologia

# THE USE OF CURRENT TOPOTYPES TO ASSIST IN THE TAXONOMIC RESOLUTION OF THE GENUS *Mazama* RAFINESQUE, 1817, IN LATIN AMERICA

**ABSTRACT** – Taxonomic studies of Neotropical deer of the genus *Mazama* have shown great morphological similarity and high karyotypic diversity among the species, suggesting that many names considered today synonymous with other species could be validated using cytogenetic and molecular genetic techniques. Aiming to know the identity of several nominal taxa assigned to specimens that were described in Latin American countries, for which there is a lack of research regarding their taxonomy, the objective of this work was to complement the taxonomic description of topotypes collected in Mexico, Argentina and Venezuela. An integrative analysis was performed through morphological (body measurement, coloration body patterns and skull measurement), cytogenetic (conventional karyotype, chromosomal biometry, C-banding, G-banding, Ag-Nor staining and FISH) and molecular (mitochondrial DNA) characterization) of current topotypes of the *species M. pandora, M. cita, M. rufa toba* and *M. simplicicornis guoazoubira*. As results, mitochondrial phylogeny recovers *M. pandora* as sister taxon of *Odocoileus*, and the morphological assessment shows diagnostic characters in this taxon. This, together with the observed karyotype exclusivity, the data allows the discussion of positioning this taxon in different genera from *Odocoileus* and *Mazama*. Regarding *Mazama americana citus*, the results reveal a basal position within Blastocerina, a lineage not closely related with the other Neotropical cervids currently recognized as *Mazama*. The taxon *Mazama rufa toba*, molecular, karyotypical and morphological data agree with its specific recognition. Complete mitochondrial genome analysis evidence an independent phylogenetic position of the specime within Odocoileina, compared with the other Neotropical deer, in particular, with *Mazama rufa* species previously described in Paraguay. The morphological and karyotypic characteristics showed homologies between *M. simplicicornis argentina* and *Subulo gouazoubira*. The phylogenetic results recovered both taxa in the same monophyletic clade, showing that *M. simplicicornis argentina* should be considered as a junior synonym of *Subulo goauzoubira*. The topotypes analyzed here contributed to the understanding of the taxonomic identity of specimens described in Latin America and their relationship with other species of Neotropical deer. The recognition of valid species and new generic names evidenced in the present study, supports the need to continue further reviewing of the genus *Mazama*, and is the first step for studies with approaches that assess populations and establish conservation plans.

**Keywords:** animal cytogenetic, mitochondrial DNA, morphology, Neotropical cervids

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# CAPÍTULO 1 – Considerações gerais

## 1. INTRODUÇÃO

O gênero *Mazama* Rafinesque, 1817 agrupa cervídeos de pequeno a médio porte que possuem chifres simples e não ramificados e ocorrem desde o nordeste mexicano até a região central da Argentina (Allen, 1915; Eisenberg, 1987).

A descrição das espécies de *Mazama* foi baseada em detalhes da morfologia externa (Allen, 1915; Miranda-Ribeiro, 1919), gerando 42 táxons nominais em toda sua distribuição, dos quais somente 8 são reconhecidos atualmente como espécies válidas: *M. americana, M. rufa, M. nana, M. nemorivaga, M. jucunda, M. temama, M. rufina, e M. chunyi* (Duarte e González, 2010; Gutiérrez et al. 2017; Peres et al. 2021). Devido à convergência adaptativa, existe uma grande similaridade morfológica entre as espécies que compõem o gênero, portanto, a morfologia não é um bom critério taxonômico no grupo (Groves e Grubb 1987; Merino e Rossi 2010). Assim, as ferramentas de citogenética e de genética molecular, podem auxiliar na resolução de incertezas taxonômicas do gênero (Duarte e González, 2010; Heckeberg et al., 2016).

Especificamente a citogenética se apresenta como uma técnica extremamente valiosa para taxonomia de cervídeos do gênero *Mazama*. Exemplo disso são os estudos cariotípicos em *Mazama bororo* Duarte, 1996, sinônimo junior de *M. jucunda* (Thomas, 1913) que permitiram o reconhecimento do táxon como espécie válida a partir das diferenças com o cariótipo de *Mazama americana* (Erxleben, 1777). Também, as descrições citogenéticas de *M. temama* (Kerr, 1792) (Jorge and Bernishke, 1977) que evidenciaram cariótipos diferenciados de *Mazama americana* (Erxleben, 1777), de quem era subespécie, provando serem espécies separadas. Foi demonstrado também a existência de citótipos de *M. americana* no Brasil cujos híbridos apresentam problemas reprodutivos importantes, que podem variar de subfertilidade a infertilidade total (Abril et al. 2010; Cursino et al. 2014; Salviano et al. 2017; Galindo et al. 2021). A caracterização citogenética de um neótipo para *Mazama americana* coletado na localidade tipo da espécie em Guiana Francesa, permitiu a comparação com exemplares do citótipo do Paraná cujas divergências cariotípicas evidenciaram

mais de 15 rearranjos entre os cariótipos (Peres et al. 2021). Esses resultados permitiram a revalidação da espécie *Mazama rufa* (Illiger,1815), designando assim ao citótipo previamente mencionado e caracterizando a um neótipo para a espécie (Peres et al. 2021).

No entanto, a citogenética é uma técnica de uso restrito em veados, basicamente devido à dificuldade de capturar os animais para coleta do material, pois precisam de células vivas para obter as preparações cromossômicas. Isso também impede a sua aplicação em amostras de museus, fezes encontradas no campo, ou animais caçados quando o pesquisador não está acompanhando esta atividade. De qualquer forma, a citogenética deve ser considerada uma linha de base para a definição de espécies em *Mazama*.

Outra ferramenta importante no estudo taxonômico de *Mazama* é a filogenia mitocondrial, servindo como base para que algumas espécies do gênero fossem submetidas a alterações de nomenclatura. A evidencia da poliflia do gênero demonstrou que as espécies de *Mazama* divergem em duas subtribos (Duarte et al. 2008; Heckeberg et al. 2016; Heckeberg, 2020), das quais somente as espécies dentro da subtribo Odocoileina, intimamente relacionadas à *M. americana*, a espécie tipo do gênero, devem ser consideradas no gênero *Mazama*. Dessa forma, é necessária a designação de outros nomes genéricos para as espécies que são recuperadas na subtribo Blastocerina (Bernegossi et al. 2022). A espécie antigamente conhecida como *M. gouazoubira*, foi realocada dentro do recém validado gênero *Subulo*, no qual os autores propuseram a denominação *S. gouazoubira* (G. Fischer, 1814) (Bernegossi et al. 2022). Também, foi sugerido a disponibilidade do gênero *Passalites* para alocar à *M. nemorivaga* (Cuvier, 1817), outra espécie dentro de Blastocerina, por não possuir ancestral comum recente com a linhagem do gênero *Subulo* (Gutiérrez et al. 2017; Bernegossi et al. 2022). Da mesma forma, recentemente foi realizada a revalidação de *M. jucunda* por evidencias moleculares, reclassificando *M. bororo* como seu sinônimo júnior (Mantellato et al. 2022).

Nesse contexto, várias pesquisas tem sido desenvolvidas com o intuito de caracterizar taxonomicamente as espécies consideradas válidas do gênero *Mazama*, entretanto, ainda é necessário conhecer a identidade de vários táxons nominais designados para exemplares que foram descritos em outros países, para os quais há ausência de pesquisa com respeito à sua taxonomia. Muitos

nomes foram considerados sinônimos de outras espécies devido aos aspectos da morfologia externa e, hoje, frente às novas técnicas poderiam ser validados.

Exemplo disso, a espécie *Mazama pandora* Merriam, 1901*,* descrita no México, *Mazama americana citus* Osgood, 1912, descrita na Venezuela e, *Mazama rufa toba* e *Mazama simplicicorniis argentina*, estas últimas descritas na Argentina (Lönnberg, 1919). Cada um desses nomes foi considerado como espécie válida e diferenciada das outras pelo autor da sua descrição, baseado em características diagnósticas definidas de cada exemplar coletado.

Com isso, o presente estudo busca complementar a informação taxonômica de espécies descritas no México, Argentina e Venezuela, através de uma caracterização morfológica (biometria corporal, padrões de coloração, craniometria, caracterização de pós-crânio), citogenética (cariótipo convencional, biometria cromossômica, bandamento C, bandamento G, coloração AG-NOR) e molecular (quatro regiões mitocondriais) de topotipos atuais. São estes, *M. pandora, M. americana citus, M. rufa toba* e *M. simplicicornis argentina*, que desde uma visão que integre e compare os diversos padrões já descritos na literatura serão ou não validados. As localidades de coleta de cada espécime são indicadas na Figura 1. Os resultados poderão ajudar a elucidar a taxonomia dessas entidades para posteriormente conseguir avaliar as populações e estabelecer planos de conservação. Além disso, contribuirá com o projeto temático do Núcleo de Pesquisa e Conservação de Cervídeos (NUPECCE) com base na análise integrativa para resolução taxonômica do gênero *Mazama* na América latina, designando neótipos e topótipos através da abordagem cronológica de todos os nomes descritos na literatura.


**Figura 1.** Localidades de coleta de topótipos do gênero *Mazama* utilizados no estudo. Relação de espécimes coletados e análise realizada: Linha sólida – amostras de tecido para extração de DNA e análise filogenética; Linha pontilhada – amostra de tecido para cultivo celular e análise citogenética; Estrela azul – Espécime coletado para análise morfológica completa (craniometria e biometria). Linha amarela: Região do Chaco Central na América do sul (século XX).

## 2. REVISÃO DE LITERATURA

### 2.1 Família Cervidae

A família Cervidae caracteriza mamíferos ungulados dentro da ordem Artiodactyla distribuídas pelos continentes Europeu, Americano e Asiático e cujos machos possuem uma estrutura óssea maciça e renovável originada a partir do osso frontal (Zurano et al. 2019). Essa estrutura, também chamada de chifre, está ausente na espécie *Hydropotes inermis*, e se encontram presente em ambos os sexos na rena, *Rangifer tarandus* (Merino e Rossi, 2010). Cervidae é a segunda família mais diversa dentro da ordem Artiodactyla, com 55 espécies e 18 gêneros distribuídos, incluindo espécies de pequeno porte, como *Pudu mephistopheles* com peso aproximado de 6 kgs, até indivíduos de grande massa corporal, como a espécie *Alces alces*, que pode ultrapassar os 600 kgs (Merino e Rossi, 2010).

Acredita-se que no início do Mioceno os primeiros representantes da família viveram nas florestas tropicais da Eurásia (Eisenberg, 1987; Geist, 1998; Zurano et al., 2019). Durante a transição do Mioceno e Plioceno, os cervídeos chegaram a América do Norte através do Estreito de Bering (Webb, 2000). Posteriormente, expandiram-se para a América do Sul a partir da formação do istmo do Panamá, no grande intercâmbio americano que ocorreu provavelmente em dois momentos, um no início do Plioceno e outro no final da transição entre Plio-Pleistoceno (Gilbert et al. 2006; Duarte et al. 2008). Assim, o registro fóssil indica que o ancestral dos cervídeos sulamericanos apresentava tamanho grande e chifres desenvolvidos, características que evoluíram independentemente no gênero *Pudu* e nas espécies de *Mazama* como consequência a uma rápida radiação adaptativa originando o corpo pequeno e chifre simples destes táxons (Webb, 2000; Gilbert et al., 2006). Estas adaptações morfológicas possibilitariam a adaptação às florestas fechadas da América do sul, contrastando com as outras espécies Neotropicais dos gêneros *Blastocerus, Hippocamelus, Odocoileus* e *Ozotoceros*, que possuem grande porte e geralmente habitam áreas abertas (Merino and Rossi, 2010; Whitehead, 1994).

Há pouco, eram reconhecidas 17 espécies de cervídeos neotropicais, todos descritos entre os séculos XVIII e XX, com base em caracteres

morfológicos de indivíduos que ocupavam os diferentes biomas da América Latina (Allen, 1915; Duarte et al. 2008). Entretanto, o advento das técnicas genéticas das últimas décadas permitiu rediscutir a classificação da família, e apresentar novas abordagens para complementar os dados morfológicos e auxiliar no estudo das complexas relações entre os táxons (Duarte et al., 2008; Merino e Rossi, 2010; González e Duarte, 2020; Gutiérrez et al., 2017; Heckeberg, 2020; Bernegossi, et al. 2022).

Entre as diferentes abordagens, a citogenética tem se apresentado como uma importante ferramenta para auxiliar na identificação de espécies da família Cervidae (Yang et al., 1997ª; Mudd et al., 2020), especificamente no gênero *Mazama,* cujos indivíduos possuem grande diversidade cariotípica, que contrasta com a pouca diferenciação morfológica (González e Duarte, 2020; Cifuentes-Rincón et al., 2020). Assim, em conjunto com o estudo filogenético, é possível entender os processos de especiação, história evolutiva da família e resolver incertezas taxonômicas dentro de Cervidae (Duarte et al., 2008; Heckberg et al. 2020; Hassanin et al. 2012).

## 2.2 Taxonomia tradicional de *Mazama*

O gênero *Mazama* Rafinesque, 1817, agrupa cervídeos de pequeno a médio porte, com chifres simples e não ramificados nos machos (Merino e Rossi, 2010). De hábitos solitários, os *Mazama* se distribuem desde o sudeste do Mexico, até o norte da Argentina e Uruguai, ocupando habitats que incluem florestas tropicais fechadas, abertas, secas, montanhosas, savanas arbóreas, desde o nível do mar até 4000 m de altitude (Eisenberg, 1987; Allen, 1915).

Inicialmente foram descritos mais de 42 táxons caracterizados por possuir uma morfologia similar, destacando o porte, os membros posteriores maiores que os anteriores, pelagem cinzenta, parda ou avermelhada e chifres retos e simples (Merino e Rossi, 2010; Rossi, 2000; Figura 1). Devido a isso, várias descrições foram sinonimizadas ou consideradas subespécies de outras, no processo em que as diferentes revisões consideraram a existência de quatro a dezoito espécies (Cabrera, 1961; Allen, 1915).

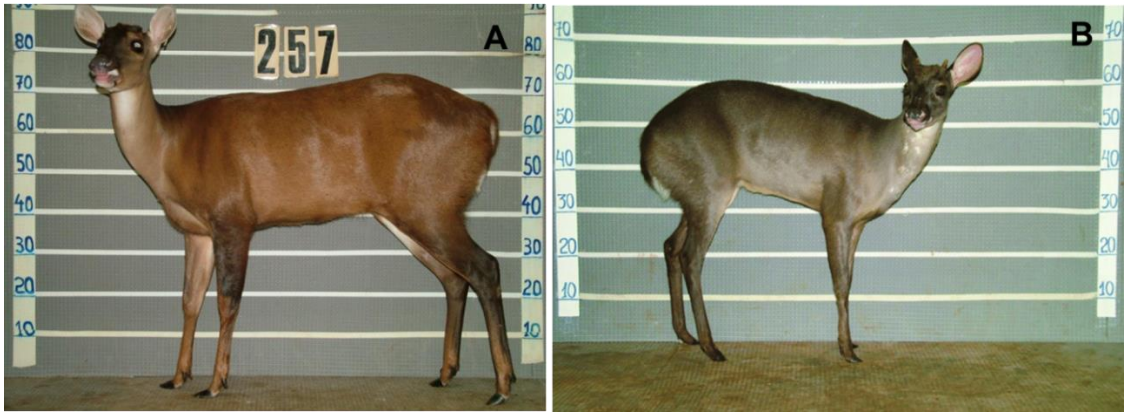


**Figura 2**. Coloração vermelha em *Mazama rufa* (A) e pardo ou cinza em *Mazama nemorivaga* (B). Padrão morfológico das espécies do gênero *Mazama*.

A morfologia externa e características do crânio foram os critérios utilizados para a descrição das espécies do gênero, entretanto, o advento de técnicas genéticas demonstrou que essa grande similaridade morfológica entre as espécies do gênero não reflete a alta variação cariotípica e origem polifilética de *Mazama* (Duarte et al. 2008). Contudo, as revisões taxonômicas modernas consideraram que os caracteres morfológicos não representam a grande diversidade do gênero, devido a um processo de homoplasia ou convergência em que o processo de seleção natural atuou para que houvesse diminuição do tamanho corporal e redução na complexidade do chifre para se adaptar as pressões do ambiente florestal (Duarte et al. 2008; Abril et al. 2010; Eisenberg, 1987, Webb, 2000). Também, foi sugerido que em algumas espécies houve a retenção de um padrão morfológico entre as linhagens e, portanto, a morfologia não é critério suficiente para avaliar questões taxonômicas do gênero (Peres et al. 2021).

Apesar de hoje em dia serem reconhecidas válidas as espécies *Mazama americana* (Erxleben, 1777), *Mazama temama* (Kerr, 1792), *Mazama nemorivaga* (Cuvier, 1817), *Mazama rufa (Illiger, 1815)*, *Mazama rufina* (Pucheran, 1852), *Mazama nana* (Hensel, 1872), *Mazama chunyi* Hershkovitz, 1959 e *Mazama jucunda* (Thomas, 1913) (Merino e Rossi, 2010; Gutiérrez et al., 2017), as incertezas taxonômicas ainda permanecem acerca da classificação das espécies do gênero (Duarte et al., 2008; Gutiérrez et al, 2017; Heckeberg, 2020). Assim, a complexa taxonomia de *Mazama* precisa ser revisada desde uma abordagem integrada utilizando técnicas moleculares, morfológicas e cariotípicas (Gutiérrez et al. 2017; Heckeberg, 2020; Bernegossi et al. 2022).

A citogenética, tem se mostrado como uma ferramenta promissora para auxiliar na taxonomia do grupo, contribuindo com o entendimento de delimitação de espécies, já que gera um forte mecanismo de isolamento reprodutivo entre populações com diferença numérica e estrutural dos seus cromossomos (Coyne e Orr, 1998). O conceito biológico de espécie define que se o descendente de um cruzamento for infértil, os pais devem ser considerados espécies distintas, pois haveria uma barreira reprodutiva eficiente entre elas (Coyne e Orr, 1998; Abril et al. 2010a; Cursino et al. 2012). Exemplo disso, a espécie *Mazama jucunda* (Duarte, 1996; Mantellato et al. 2022), descrita a partir de exemplares com cariótipos (2n = 32-34) diferenciados de *Mazama americana* (2n = 50-52) de quem era sinônimo e foram consideradas espécies distintas (Duarte e Jorge, 1996). O mesmo ocorreu com *Mazama temama*, que foi separada de *M. americana* através de análise citogenética de exemplares em cativeiro (Jorge e Benirschke 1977, Groves e Grubb, 1987). O isolamento reprodutivo entre variantes cariotípicas de *Mazama americana* foi demonstrado a partir da avaliação da fertilidade de animais híbridos entre diferentes citótipos. Eles apresentaram problemas reprodutivos importantes, que variaram de subfertilidade a infertilidade total (Cursino et al. 2014; Salviano 2017, Galindo et al. 2021).

Partindo deste princípio, a análise citogenética do neótipo de *M. americana* da Guiana Francesa mostrou que as formas brasileiras analisadas até o momento não pertencem a esse táxon (Cifuentes-Rincón et al. 2020). Figura 3

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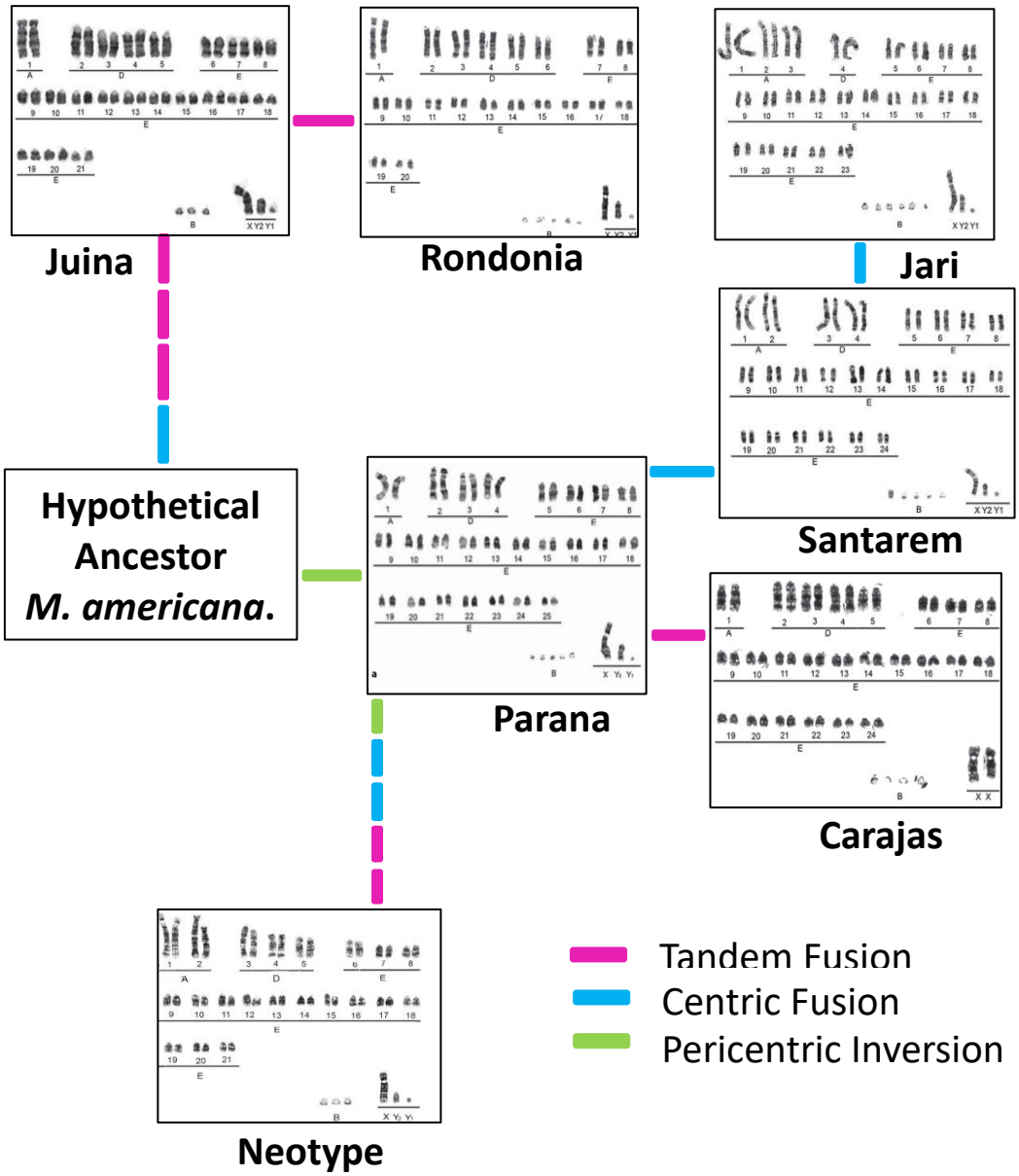


**Figura 3**. Cariótipo de indivíduos *Mazama americana* de diferentes localidades no Brasil (Juine: 2n = 44/45; Rondônia: 2n = 42/43; Santarém: 2n = 50/51; Jari: 2n = 48/49; Paraná: 2n = 52/53; Carajás: 2n = 50/51; Neotype: 2n) em comparação com o cariótipo do neótipo da Guiana Francesa (Neotype: 2n = 45). Retirado de Cifuentes-Rincón et al. 2020.

Também, a caracterização citogenética de espécime analisados do citótipo do Paraná mostrou um cariótipo diferente em comparação com a espécie de *M. americana sensu stricto*, permitindo a validação de *Mazama rufa* de Illiger (1815). Figura 3.

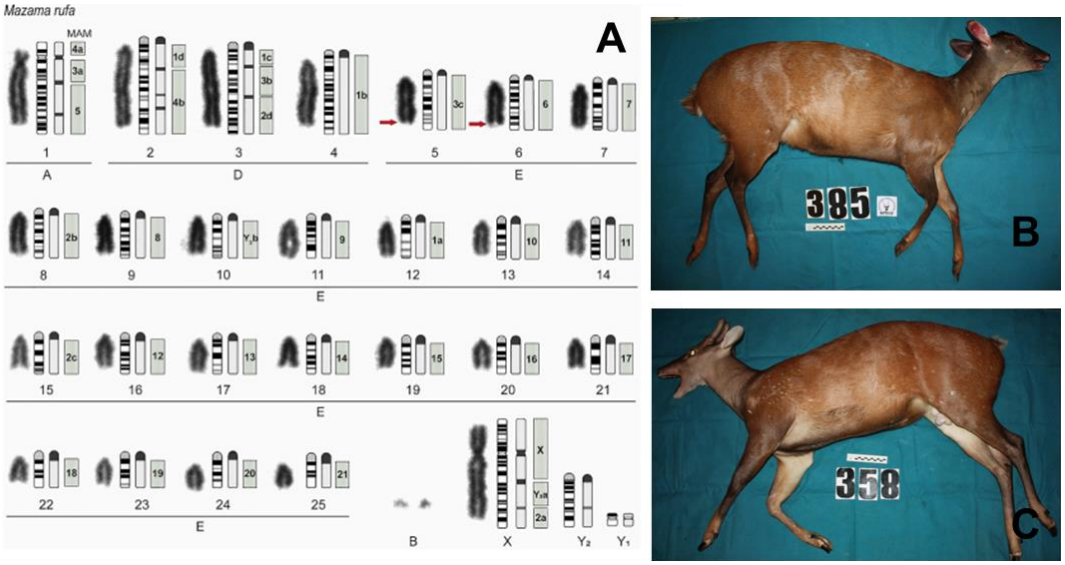


**Figura 4.** Neótipo de *Mazama rufa* (Illiger, 1815) e neótipo de *Mazama americana stricto sensu* (Erxleben, 1777). A) Divergências cromossômicas entre os espécimes, de esquerda à direita: cromossomos de *Mazama rufa* (2n = 53) em coloração de Giemsa, idiograma de Banda G, idiograma de Banda C, homeologia com *Mazama americana* (2n = 45). B) Foto lateral do corpo do neótipo de *M. rufa* C) Foto lateral do corpo do neótipo de *M. americana*. Adaptado de Peres et al. 2021.

A condição polifilética de *Mazama* tem sido extensamente relatada na literatura (Gilbert et al., 2006; Duarte et al., 2008; Hassanin et al., 2012; Gutiérrez et al., 2017; Heckeberg et al. 2020). Análises filogenéticas dos cervídeos da América do Sul separam as espécies de *Mazama* em duas subtribos, Blastocerina e Odocoileina, que divergiram há aproximadamente 5 milhões de anos (Duarte et al. 2008; Heckeberg et al. 2016; 2020). Dentro da subtribo Blastocerina estão alocadas as espécies *M. nemorivaga* e *Mazama chunyi*, e também antigamente nomeada *M. gouazoubira*, hoje reconhecida no gênero *Subulo*, por sua condição polifilética com as outras espécies do gênero (Bernegossi et al. 2022). Os estudos tem demonstrado que *M. chunyi* está intimamente relacionado com *S. gouazoubira*, assim, provavelmente sejam reconhecidas como espécies dentro do mesmo gênero (Gutiérrez et al. 2017; Bernegossi et al. 2022). Também, que *M. nemorivaga* não possui ancestral comum exclusivo em relação a *Subulo* e *Mazama* e por isso outro nome gênerico deve ser proposto (Gutiérrez et al. 2017). Para esse táxon, os autores consideram que o nome genérico *Passalites* Gloger, 1841, se encontre

disponível para alocar a espécie, que deveria ser nomeada *P. nemorivagus* (Cuvier, 1817) (Gutiérrez et al. 2017; Figura 4).

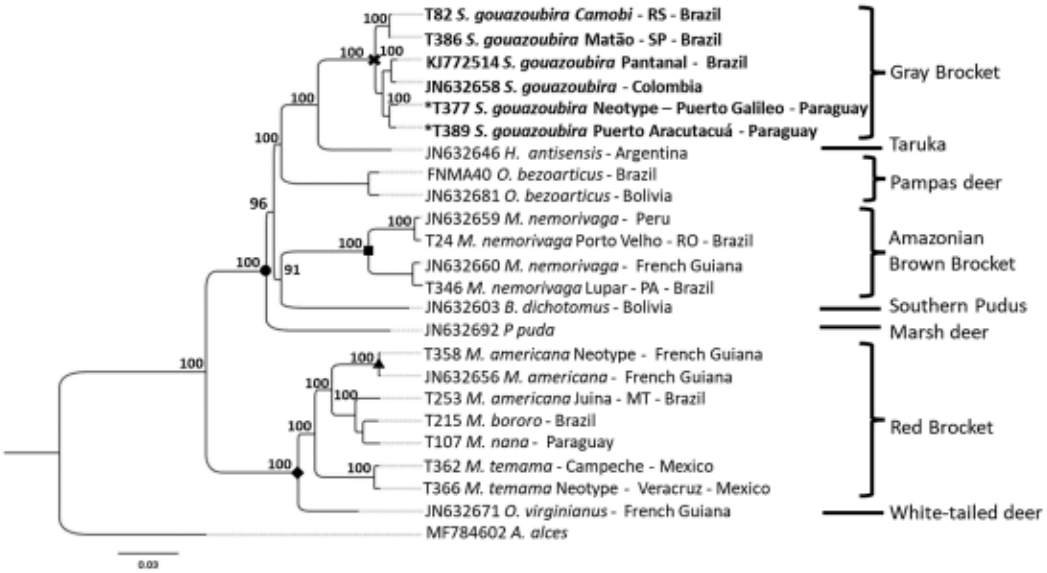


**Figura 5.** Árvore de Inferência Bayesiana do Mitogenoma dos cervídeos neotropicais. *Subulo gouazoubira* (cruz), neótipo de *S. gouazoubira* (asterisco), clado Blastocerina (círculo), *Mazama nemorivaga* (quadrado), clado Odocoileina (diamante), e *Mazama americana* sensu stricto (triângulo). Adaptado de Bernegossi et al. 2022.

A subtribo Odocoileina abrange as espécies *M. nana, M. jucunda, M. rufa, M. pandora*, duas linhagens de *M. americana* e o gênero *Odocoileus* (Duarte et al., 2008; Gutiérrez et al., 2017; Heckeberg, 2020; Bernegossi et al. 2022). Dentro da subtribo, a espécie *M. americana* tem se mostrado polifilética nos diversos estudos (Duarte et al. 2008; Peres et al. 2021). A relação não monofilética dos *M. americana* em conjunto com as divergências cariotípicas, foram argumentos para que vários autores considerassem a denominação *sensu stricto*, para os espécimes com origem na localidade tipo, Guiana Francesa, e *sensu lato*, para todos os espécimes com diferentes localidades no Brasil e da América latina (Bernegossi et al. 2022; Peres et al. 2021). Assim, se considera que a nomenclatura de *Mazama* seja exclusiva dos táxons relacionados com a espécie tipo do gênero *M. americana sensu stricto* (Gutiérrez et al., 2017; Cifuentes-Rincón et al., 2020). O estudo filogenético de Gutiérrez et al. (2017) evidenciou a espécie *M. pandora* como grupo irmão do gênero *Odocoileus*, e também que uma linhagem de *M. americana* que inclui indivíduos do Pará e Amazonas no Brasil, assim como a espécie *M. rufina,* não compartilham

ancestral comum com as outras espécies de *Mazama,* sugerindo que esses táxons sejam designados em gêneros separados de *Mazama* (Gutiérrez et al. 2017; Figura 5).

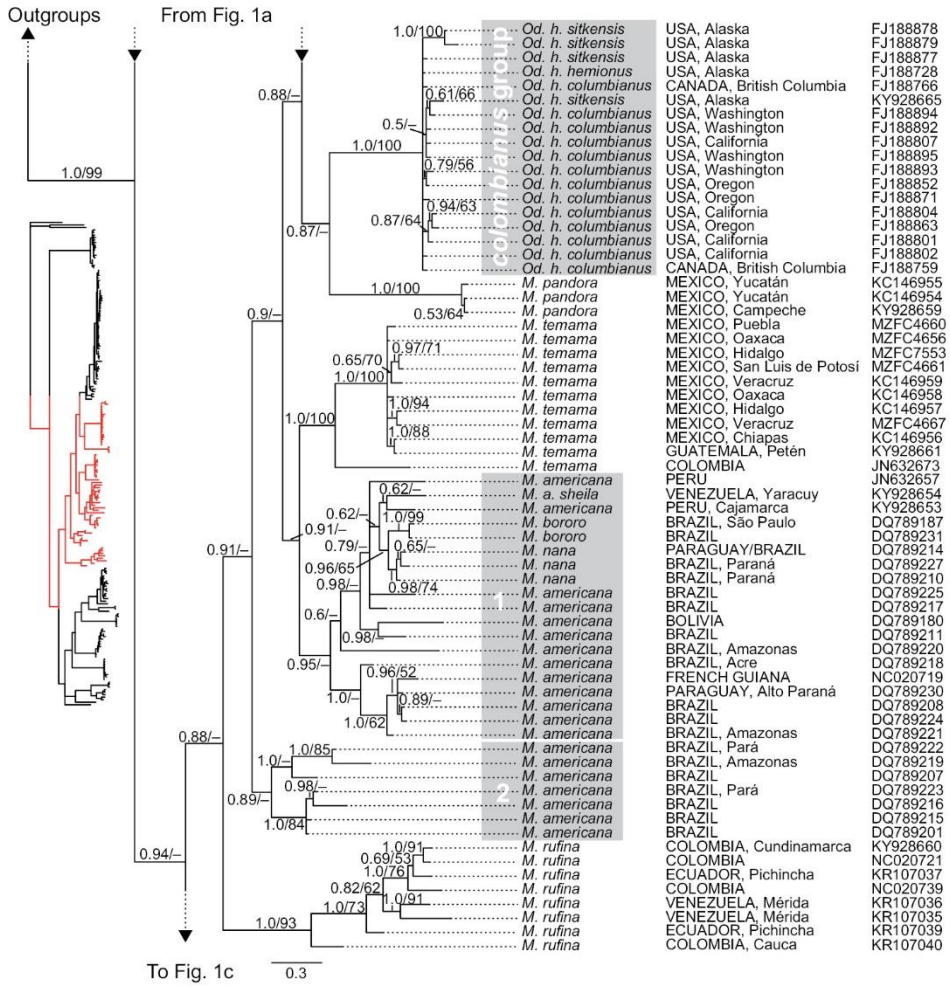


**Figura 6.** Árvore de Inferência bayesiana do gene Cytb. Subtribo Odocoileina sensu Heckeberg (2020). (1) Indivíduos *Mazama americana* recuperados com as espécies *Mazama nana* e *Mazama jucunda*. (2) Indivíduos *Mazama americana* recuperados em clado irmão das demais espécies da subtribo. Retirado de Gutiérrrez et al. 2017.

Os autores consideram que uma visão integrativa de *Mazama* poderia contribuir com o esclarecimento da complexa taxonomia do gênero (Gutiérrez et al. 2017; Heckeberg et al. 2020; Bernegossi et al. 2022). Como ponto de partida, é necessária a investigação de todos os táxons descritos a partir da amostragem de topotipos que possam ser caracterizados geneticamente.

## 3. Descrições de espécimes *Mazama* com origem no México, Venezuela e Argentina:

### 3.1 *Mazama pandora* Merriam, 1901

A partir de um holótipo macho coletado por E. W. Nelson e E. A. Goldman em Tunkas, Yucatan, México, no ano de 1901, Merriam (1901) descreveu a espécie *Mazama pandora*. Como caracteres relevantes, o autor citou a coloração geral do corpo cinzenta ou marrom pálida e os chifres retos pontiagudos presentes no espécime tipo com sulcos profundos longitudinalmente e 113mm de comprimento. A descrição também incluiu a presença de pelos no pescoço como no resto do corpo, pelos amarelados no queixo, lábios, região inguinal e face interna das coxas, cor branquicenta na região anal, orelhas marrons com mancha branca na base anterior. O autor compara o crânio do exemplar com a espécie *M. sartorii* (Saussure, 1860), um sinônimo de *M. temama* (Kerr, 1792), citando que o exemplar de Yucatán possui rosto mais largo, nasais mais longos, lacrimal maior e arredondado, basioccipital com uma constrição ou entalhe fortemente desenvolvido em cada lado imediatamente à frente dos côndilos, mastóides maiores, descendentes nos lados externos dos processos paroccipitais, comprimento total 1125 mm, cauda 140mm, metatarso 273mm, e altura 372mm. As medidas do crânio do holótipo e outro exemplar de Campeche foram citadas com um comprimento basal do crânio 163mm e 160mm respectivamente, assim como comprimento nasal com 59 e 57.5 mm respectivamente (Meriam, 1901).

O táxon descrito por Merriam (1901) foi considerada subespécie de *M. temama, M. americana* e *S. gouazoubira* (Goldman e Moore, 1945; Hershkovitz, 1951) até o estudo de Medellin et al (1998), que validou *Mazama pandora* como a espécie que ocupa as áreas mais abertas de Yucatán, México. Posteriormente, os estudos filogenéticos sugeriram a proximidade de *M. pandora* com as espécies da subtribo Odocoileina, como grupo irmão do gênero *Odocoileus* (Escobedo-Morales et al. 2016; Gutiérrez et al. 2017). As dúvidas sobre as características morfológicas da espécie em relação a outros *Mazama* junto a ausência de dados citogenéticos tornam necessárias análises genéticas mais

aprofundadas para conhecer o cariótipo de *M. pandora*, e também, estudar a relação com as outras espécies de *Mazama* da América Latina.

### 3.3 *Mazama americana citus Osgood, 1912*

Na beira do río Aurare, El Panorama, costa Oriental do Lago de Maracaibo na Venezuela, foi coletado um macho adulto no ano de 1911, por W. H. Osgood e S. G. Jewett, que posteriormente Osgood (1912) nomeou *Mazama americana citus*. Apesar do autor descrever a coloração pálida e cinzenta, o exemplar foi designado como subespécie de *M. americana,* uma vez que Osgood (1912) considerava *M. nemorivaga* um sinónimo junior da espécie de Erxleben, 1777. As características do tipo e de uma fêmea adulta foram descritas com comprimento total de 1090 e 1060 mm, macho e fêmea respectivamente, coloração geral da pele acinzentada, mandíbula grande, dimensão de crânio pequena (189 e 185mm, macho e fêmea respectivamente), corpo cinzento acanelado, região ventral pálida, cabeça e pescoço marrom pálido, mancha tarsal branca, mancha branca proeminente sobre cada olho.

Sucessivos autores renomearam a espécie de Osgood (1912) como *M. cita cita* (Allen, 1915), ou subespécie de *M. gouazoubira* (Cabrera, 1961; Bisbal, 1991; Pinder e Leewenberg, 1997). Bisbal (1991) confirmou a ocorrência da espécie de Osgood nos estados ao norte da Venezuela, com uma morfologia similar ao que considerava *M. gouazoubira nemorivaga* dos estados do sul da Venezuela. Posterior a este estudo, não houve nenhum outro estudo de taxonomia da espécie de *Mazama* cinza na Venezuela para esclarecer a identidade genética da espécie em relação aos outros *Mazama* da América Latina.

### 3.4 *M. rufa toba* Lönnberg, 1919

A partir de um macho coletado em 1896 por A. Ros no Chaco Central, entre o Rio Pilcomayo e Rio Bermejo na Argentina, foi descrito *M. rufa toba* (Lönnberg, 1919). O comprimento do crânio de 198mm, com todas as medidas menores que os descritos por Allen (1915) para *M. rufa rufa* (Illiger, 1815) foram citadas pelo autor (Lönnberg, 1919), que também destacou o pequeno tamanho

da região pré-orbital como a diferença mais importante com o *M. rufa* do Paraguai. A coloração geral avermelhada, com áreas de pelagem preta na cabeça e dorsal ao pescoço e áreas mais claras ventral ao pescoço e lateral ao rosto, ausência de pelos antevertidos, larga banda de pardo vermelho misturado com canela sobre os olhos, foram as principais características relatadas pelo autor (Lönnberg, 1919). A descrição foi considerada subespécie ou sinónimo de *M. americana* por Cabrera (1961) e Grubb (2005) respectivamente. Entretanto, não existe nenhuma revisão partindo de análises citogenéticas e moleculares de espécimes *Mazama* com coloração vermelha e origem na Argentina.

### 3.5 *M. simplicornis argentina* Lönnberg, 1919

A partir de um macho e uma fêmea adultos coletados no Chaco Central da Argentina pelo Capitão A. Ros, Lönnberg (1919) descreveu a subespécie *Mazama simplicicornis argentina.* O autor considerou a fêmea como espécime tipo devido à ausência de pele do macho para descrição, detalhando os caracteres de coloração geral cinza e marrom, com partes dorsais do pescoço mais cinza, pelos em direção não antevertida, cabeça pálida e marrom clara e macha branca labial abaixo da mufla (Lönnberg, 1919). As características das manchas faciais, menor dimensão de crânio (185mm no macho de *M. simplicicornis argentina*) e distância geográfica foram citadas como diferenças raciais entre o veado cinza do Chaco Argentino e o típico *M. simplicicornis* Illiger, 1815 do Paraguai.

Devido a essa descrição, vários autores consideraram ser *M. simplicicornis argentina* um sinônimo de *M. gouazoubira,* atualmente considerado *Subulo gouazoubira* (G. Fischer, 1814) (Cabrera, 1960; Czernay, 1987). Entretanto, a descrição de veado cinza do gênero *Mazama* na Argentina ainda não foram revisadas, partindo de análises citogenéticas e moleculares.

## 4. REFERENCIAS


Abril V.V., Carnelossi E.A., González S., Duarte J.M. 2010a. Elucidating the evolution of the red brocket deer *Mazama americana* complex (Artiodactyla; Cervidae). **Cytogenetic and Genome Research** 128:177–187.

Allen J.A. 1915. Notes on American deer of the genus *Mazama*. **Bulletin of the American Museum of Natural History** 34:521–553.

Bernegossi A. M., Borges C., Sandoval E.D.P., Cernohorska H., Svatava K., Vozdova M., Caparroz R., González S., Duarte JMB. 2022. Resurrection of the genus *Subulo* Smith, 1827 for the gray brocket deer, with designation of a neotype. **Journal of Mammalogy**.

Bisbal F. 1991. Distribución y taxonomíadelvenadomatacán (*Mazama* sp.) en Venezuela. **Acta Biológica Venezuélica** 13:89–104.

Cabrera A. 1961. Catálogo de los mamíferos de America der Sur. Revista del Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”. **Zoologia** 4(2):309–732.

Cifuentes-Rincón A., Morales-Donoso J.A., Sandoval E.D.P., Tomazella IM., Mantellatto AMB., de Thoisy B., Duarte JMB. 2020. Designation of a neotype for *Mazama americana* (Artiodactyla, Cervidae) reveals a cryptic new complex of brocket deer species. **ZooKeys** 958:143–164.

Coyne J. A., Orr A. H. 1998. The evolutionary genetics of speciation. Phil. Trans. R. Soc. Lond*. B*353287–305

Cursino M.S., Salviano M.B., Abril V.V., Zanetti E.d.S. Duarte J.M.B. 2014. The role of chromosome variation in the speciation of the red brocket deer complex: the study of reproductive isolation in females. **BMC Evolutionary Biology** 14:40.

Cuvier G.F. 1817. Dictionnaire des Sciences Naturales. Zoologie. Mammiferes. Paris, F. G. Levrault.

Duarte J.M.B. 1996. Guia de identificação dos Cervídeos Brasileiros. Jaboticabal: FUNEP. p.8. Duarte, JMB and Jorge, W. 1996. Chromosomal polymorphism in severalpopulationsofdeer (genus *Mazama*) fromBrazil. **Archivos de Zootecnia** 45 (170): 281–287.

Duarte J.M.B., González S., Maldonado JE. 2008. The Surprising Evolutionary History of South American Deer. **Molecular Phylogenetic and Evolution** 49:17–22.

Duarte J.M.B, Gonzalez S. 2010. Neotropical Cervidology. Biology and Medicine of Latin American Deer. Duarte JMB, Gonzalez S (Eds.) Jaboticabal: FUNEP e IUCN, 394p.

Eisenberg J.F. 1987. The evolutionary history of the Cervidae with special reference to the South American radiation. In.: Wemmer C (Ed.). **Biology and Management of the Cervidae**. Washington: Smithsonian Institution Press, p. 60-64

Erxleben J.C.P. 1777. Systema regni animalis per classes, ordines, genera, species, varietates cum synonymia et history animalium, Classis 1. Mammalia. Impensis Weygandianis, Lipsiae. p. 1-636.

Escobedo-Morales L.A., Mandujano, S., Eguiarte, L.E., Rodríguez-Rodríguez, M.A., and Maldonado, J.E. 2016. First phylogenetic analysis of Mesoamerican brocket deer *Mazama pandora* and *Mazama temama* (Cetartiodactyla:Cervidae) based on mitochondrial sequences: Implications on Neotropical deer evolution. **Mammalian Biology** 81: 303–313.

Fischer G. 1814. Zoognosia Tabulis Synopticis Illustrata. Typis Nicolai Sergeidis Vsevolozky. Moscow.

Galindo D.J., Martins G.S., Vozdova M., Cernohorska H., Kubickova S., Bernegossi A.M., Kadlcikova D., Rubes J., Duarte JMB. 2021. Chromosomal Polymorphism and Speciation: The Case of the Genus *Mazama* (Cetartiodactyla; Cervidae). **Genes** 12(2):165.

Geist V. 1998. Deer of the world. Their evolution, behaviour, and ecology. Pennsylvania: Stackpole Books, 421.p.

Gilbert C., Ropiquet A., Hassanin A. 2006. Mitocondrial and nuclear phylogenies of Cervidae (Mammalia; Ruminantia): Systematics, morfology, and biogeography. **Molecular Phylogenetics and Evolution** 40. P.101-117.

Gloger C. W. L. 1842. Gemeinnütziges hand-und Hilfsbuch der Naturgeschichte. Für gebildete Leser aller Stande, besonders für die reifere Jugend und ihre Lehrer. A. Dchulz, Breslau 1, 1–495.

Goldman e Moore. 1945. The biotic provinces of Mexico. **Journal Mammology** 26 (4). p.360

González S., Duarte J.M.B 2020. Speciation, evolutionary history and conservation trends of Neotropical deer. **Mastozoologia Neotropical** 27:37-47.

Groves C.P., Grubb, P. 1987. Relationships of living deer. In: Wemmer, C.M. Biology and management of the cervidae. Smithsonian Institution Press, Washington, pp. 21-59.

Groves G.C., Grubb P. 2011. Ungulate taxonomy. Johns Hopkins University Pres, Baltimore, Maryland 317 pp.

Grubb P. 2005. Order Artiodactyla. In: Wilson DE, Reeder DM, editors**. Mammal species of the world.** 3rded. Baltimore: Johns Hopkins University Press. P. 629–722.

Gutiérrez E.E., Maldonado J.E., Radosavljevic A., Molinari J., Patterson B.D., Martínez C.J.M., et al. 2015. The Taxonomic Status of *Mazama bricenii* and the Significance of the Táchira Depression for Mammalian Endemism in the Cordillera de Mérida, Venezuela. **PLoS ONE** 10(6): e0129113.

Gutiérrez E.E., Helgen K.M., McDonough M.M., Bauer F., Hawkins M.T.R., Escobedo-Morales L.A., Patterson B.D., Maldonado J.E. 2017. A gene-tree test of the traditional taxonomy of American deer: The importance of voucher specimens, geographic data, and dense sampling. **ZooKeys** 697:87–131.

Hassanin A., Delsuc F., Ropiquet A., Hammer C., van Vuuren BJ., Matthee C., Ruiz-Garcia M., Catzeflis F., Areskoug V., Nguyen TT., Couloux A. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. **Comptes Rendus Biologies** 335:32–50.

Hassanin A., Delsuc F., Ropiquet A., Hammer C., Van Vuuren B. J., Mathee C., Ruizgarcia M., Catzeflis F., Areskoug V., Nguyen T., Couloux A. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. **Comptes Rendus Biologies** 335:32-50.

Hershkovitz P. 1951. Mammals from British Honduras, Mexico, Jamaica and Haiti. **Fieldiana-Zoology Chicago Natural History Museum** 31:547–569.

Heckeberg N.S., Erpenbeck D., Wörheide G., Rössner GE. 2016. Systematic relationships of five newly sequenced cervid species. **PeerJ** 4:e2307.

Heckeberg N.S. 2020. The systematics of the Cervidae: A total evidence approach. **PeerJ** 8:e8114.

Hensel R. 1872. Beiträge zur Kenntniss der Säugethiere Süd-Brasiliens. Abhandl. König. **Sitzungsberichte der Bayerischen Akademie der Wissenschaften** 1-130.

Illiger J.K.W. 1815. Uebelblick der Säugethiere nach ihrer Vertheilung über die Welttheile. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlim 39–159.

Jorge W., Benirschke K. 1977. Centromeric heterochromatine and G-banding of the red brocket deer, *Mazama americana temama* (Cervoidea, Artyodactyla) with a probable non-robertsonian translocation. **Cytologia** 42(3- 4):711–21.

Kerr R. 1792. The animal kingdom; p. 303.

Lönnberg E. 1919. On some mammals from the Argentine Chaco. **Arkiv för Zoologi** 12:1-20.

Lydekker R. 1915. Catalogue of the ungulate mammals in the British Museum (Natural History). British Museum, London.

Mantellatto A.M.B., González S., Duarte, J. M. B. 2020. Molecular Identification of *Mazama* Species (Cervidae: Artiodactyla) from Natural History Collections. Genet. **Molecular Biology** 43, e20190008.

Medellín R.A., Gardner A.L., Aranda J.M. 1998. The taxonomic status of the yucatán brown brocket, *Mazama pandora* (Mammalia: Cervidae). **Proceedings of the Biological Society of Washington** 111(1):1–14.

Merino M.L., Rossi R.V. 2010. Origin, Systematics, and Morphological Radiation. In: J.M.B. Duarte and S. Gonzalez S. (Eds.) **Neotropical Cervidology, Biology and Medicine of Latin American Deer**. Funep/IUCN, Jaboticabal/Gland. pp. 2–11.

Merriam C.H. 1901. A new brocket from Yucatan**. Proceedings of the Biological Society of Washington** 14: 105-106.

Miranda-Ribeiro A. 1919. Os veados do Brasil segundo as coleções Rondon e de vários museus nacionais e estrangeiros. **Revista do Museu Paulista** 11: 209-308.

Mudd A.B, Bredeson J.V, Baum R, Hockemeyer D, Rokhsar D.S. 2020. Analysis of muntjac deer genome and chromatin architecture reveals rapid karyotype evolution. **Communications Biology** 3(1):480

Oliveira, M. L., Peres, P. H.F., Gatti, Andressa., Morales-Donoso, J. A., Mangini, P. R., Duarte, J. M. B. 2020. Faecal DNA and camera traps detect an evolutionarily significant unit of the Amazonian brocket deer in the Brazilian Atlantic Forest. **European Journal of Wildlife Research** 66: 28.

Osgood W. H. 1912. Field Museum Natural History, **Zoology** X:43.

Peres P.H.F., Luduvério D.J., Bernegossi A.M., Galindo D.J., Nascimento G.B., Oliveira M.L., Sandoval E.D.P., Vozdova M., Kubickova S., Cernohorska H., Duarte JMB. 2021. Revalidation of *Mazama rufa* (Illiger 1815) (Artiodactyla: Cervidae) as a Distinct Species out of the Complex *Mazama americana* (Erxleben 1777). **Frontiers in Genetics** 12: 1–18.

Pucheran J. 1851. Note sur une espèce nouvelle de Cerf (*Cervus rufinus* Bourc. et Puch). **Revue et Magasine de Zoologie** 2(III): 561–565.

Pinder L., Leeuwenberg F. 1997. Veado-catingueiro (*Mazama gouazoubira*, Fischer 1814). In: Duarte, J.M.B. (Ed). **Biologia e conservação de cervídeos sul-americanos: *Blastocerus, Ozotoceros* e *Mazama***. FUNEP, Jaboticabal, p.60-68.

Rossi, R. V. 2000. Taxonomia de *Mazama* Rafinesque, 1817 do Brasil (Artiodactyla, Cervidae). Dissertação de Mestrado – Universidade de São Paulo. São Paulo, Brasil.

Salviano M.B, Cursino M.S, Zanetti E.S, Abril V.V, Duarte J.M.B. 2017. Intraspecific chromosome polymorphisms can lead to reproductive isolation and speciation: an example in red brocket deer (*Mazama americana*). **Biology of Reproduction** 96(6):1279–1287.

Sarria-Perea J.A.S. 2012. **Taxonomia e filogenia de algumas espécies de *Mazama* (Mammalia; Cervidae) da Colômbia**. Dissertation, Universidade Estadual Paulista, São Paulo, Brazil.

Saussure H.L.F. 1860. Note sur quelques mammifères du Mexique. **Revue et Magasin de Zoologie**, Paris Series 2:122–152.

Thomas O. 1913. The smaller south American cervidae. **Annual Magazine of Natural History** (Series 8) 11: 587

Webb D.S. 2000. Evolutionary History of new world cervidae. In.: Vrba ES, Schaller GB (Eds.) **Antelopes, Deer, and Relatives: fossil record, behavioral ecology, systematic, and conservation.** New Haven: Yale University Press, p. 38-64.

Whitehead. 1994. The whitehead encyclopedia of deer: 493.

Yang F, Carter N.P., Shi L., Ferguson-Smith M.A. 1995. A comparative study of karyotypes of muntjacs by chromosome painting. **Chromosoma** 103:642- 652.

Zurano J.P, Magalhães F.M, Asato A.E, Silva G, Bidau C.J, Mesquita D.O, Costa G.C 2019. Cetartiodactyla: updating a time-calibrated molecular phylogeny. **Molecular Phylogenetics and Evolution** 133:256–262.

# CAPÍTULO 2 – Cytogenetic, Molecular and Morphological characterization of *Odocoileus pandora* Merriam, 1901 (ARTIODACTYLA, CERVIDAE)

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## ABSTRACT

The Yucatan brown brocket deer was initially described as *Mazama pandora* Merriam, 1901. Phylogenetic analysis of mitochondrial DNA recovered the species as sister group of the genus *Odocoileus* and subsequently the species was repositioned to this genus naming *Odocoileus pandora* (Merriam, 1901). However, there is still a lack of taxonomic consensus that could assists in nomenclature definition of the species. We aim to clarify the taxonomy of *O. pandora* through an integrative assessment using morphological, cytogenetic, and molecular data from recently collected specimens. Morphological characterizations identified *O. pandora* as a medium-sized brocket deer. The skull shows an inflated auditory bulla and a narrow zygomatic arch. Males present long, broad, spike-like antlers converging inward, marked with deep longitudinal grooves. *Odocoileus pandora* shows a karyotype with 2n = 60 FN = 74. Bacterial artificial chromosome clone hybridization showed that centric and tandem fusions, and inversions are involved in karyotypical divergences between *O. pandora*, *S. gouazoubira*, *M. americana* and *O. virginianus*. Our phylogeny based on Bayesian Inference of mitogenome recovered *O. pandora* as monophyletic within the subtribe Odocoileina, with *Odocoileus* as sister taxa. Morphological and genetic characteristics of *O. pandora* agrees in differencing the Yucatan brown brocket from *Mazama* and *Odocoileus* genera. Therefore, a new generic name should be indicated for this taxon.

Keywords: cervid, mitochondrial phylogeny, Yucatan brocket deer.

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## 1. INTRODUCTION

Native species of the family Cervidae distribute across Europa, America, and Asia (Zurano et al. 2019), and represents the second most diverse family of the order Artiodactyla (Taber et al. 2016). In the Neotropics, deer display great morphological diversity, with small species like the northern pudu (*Pudu mephistophiles* de Winton, 1896) with a body mass of less than 6 kg and larger species like the marsh deer (*Blastocerus dichotomus* Illiger, 1815) that weighs between 100-150 kg (Feldhamer and McShea 2012). Currently, experts recognize six genera: *Mazama* Rafinesque, 1817, *Odocoileus* Rafinesque, 1832, *Ozotoceros* Linnaeus, 1758, *Blastocerus* Illiger, 1815, *Pudu* Gray, 1850 and *Hippocamelus* Leuckart, 1816.

The genus *Mazama* comprises small to medium-sized species, with unbranched antlers in males (Merino and Rossi 2010; Gutiérrez et al. 2015), characterized by a morphological similarity with a wide karyotypic diversity, with diploid numbers (2n) ranging from 32 to 70 chromosomes (Abril and Duarte 2008; Abril et al. 2010). The genus represents a polyphyletic group, as revealed by mitochondrial DNA phylogenies (Gilbert et al. 2006; Duarte et al. 2008; Hassanin et al. 2012; Gutiérrez et al. 2017; Heckeberg 2020). Thus, the first phylogenetic analysis of mitochondrial cytochrome b gene (Cytb) recovered the *Mazama* species separated in two clades, red and gray brocket, in reference to the coat color pattern (Duarte et al. 2008).

Aiming to clarify the taxonomy of the genus *Mazama*, recent studies have assigned neotypes for *Mazama americana* (Erlxheben, 1777) (Cifuentes-Rincón et al. 2020), *Mazama temama* (Kerr, 1792) (Sandoval et al. 2022) and *Mazama rufa* (Illiger, 1815) (Peres et al. 2021) as a starting point for an integrated comparison with taxa. Also, the integrative analysis of *Mazama gouazoubira* (G. Fischer, 1814) detected the presence of distinctive traits in this species that suggest divergence from *Mazama*, placing it within the recently revalidated genus *Subulo* Smith, 1827 (Bernegossi et al. 2022). Thus, a taxonomic review of other *Mazama* species becomes necessary to resolve their supra-specific relationships, and also, to contrast the taxa that were positioned as *Mazama* due to morphological convergence, but actually belong to different genus. The Yucatán brown brocket (Temazate café in Spanish) is the common name of a

species first described as *Mazama pandora* by Merriam (1901). The species is a medium-sized deer with short and brown dorsal hair, large and long ears, often curved with separate bases deeply grooved and massive pedicels (Weber and Medellin 2010). This species occurs mainly in the tropical forest of the Yucatan Peninsula, Mexico, where uncontrolled hunting, habitat loss, and fragmentation are threatening the species. Therefore, it is considered vulnerable by the International Union for Conservation of Nature (IUCN) Red List (Weber et al. 2016).

Besides Merriam (1901) recognized *M. pandora* as a valid species, subsequent authors classified this taxon related to coat color pattern, as belonging to the gray or red brocket group, considering it as subspecies of the Central American red brocket deer *M. temama* (Goldman and Moore 1945; Hershkovitz 1966), and also as subspecies of the gray brocket deer *Subulo gouazoubira* (Allen 1915; Lydekker 1915; Hershkovitz 1959). After that, Medellín et al. (1998), regarding an extensive review of morphological traits of specimens of *M. americana*, *M. temama* and *S. gouazoubira,* raised its status again to a specific level, as previously suggested by Merriam (1901). The different classifications of the Yucatan brocket based on pelage coloration contrast with recent molecular phylogenies. Escobedo-Morales et al. (2016) recovered a monophyletic group that included this species, red brockets, and the white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780) based on mitochondrial genes ND2 and Cytb, and the control region. However, the authors warned that the taxonomic position of "*M. pandora"* remains unsolved, as topology varies depending on which mitochondrial dataset is used. Other phylogenetic studies corroborated this proximity, suggested the repositioning of the Yucatan brown brocket within the genus *Odocoileus*, naming *Odocoileus pandora*, and considered the denomination of subtribe Odocoileina (red clade) and subtribe Blastocerina (gray clade) more appropriate to these groups (Heckeberg et al. 2016; Gutiérrez et al. 2017; Heckeberg 2020). Concerns about the geographic intraspecific pattern of morphological variation o*f Odocoileus pandora*, together with the absence of cytogenetic data, accentuate the need of further analyses to understand the evolutionary patterns and processes in *O. pandora* and other Neotropical deer species.

The present study provides new information of diagnostic morphological, molecular, and cytogenetic characters for *O. pandora*. This specimen study represents a starting point to assist in comparative biology studies and taxonomic reviews of *Odocoileus pandora* and other Neotropical deer.

## 2. MATERIALS AND METHODS

### 2.1Sample collection

In partnership with Instituto de Ecología, A.C. of Mexico (collecting permit SGPA/DGVS n°06821), we collected an adult male in Zoh Laguna, Campeche, near the Calakmul Biosphere Reserve in Yucatán, Mexico (18°35'32.2" N, 89°24'55.3" W). The tissue samples were donated to the Deer Research and Conservation Center (NUPECCE), Brazil, where the biological material remain deposited. The specimen was tagged with field number NUPECCE (T365) and assigned catalog number (NPC093) at the NUPECCE Museum. Tissue samples (skin and hair) from four other *O. pandora* specimens were collected in the Yucatán Peninsula for molecular analysis, deposited at the Deer Research and Conservation Center (NUPECCE) and identified as Mx01, Mx02, Mx05 and Mx06.

### 2.2 Morphological characterization

We photographed and measured the specimen using a pendulum scale, a measuring tape and a digital caliper to the nearest 0.05 mm (Supplementary Table S1 and Fig. S1)[1]. The following traits were measured: width and length of the head, ear length, distance between eyes, mandible total length, neck circumference, thorax circumference, height of the body, metacarpal length, tail length, body mass, metatarsal length, abdominal circumference, and total body length (Supplementary Table S1[1])[1]. The skin was removed and treated with a

[1]Supplementary tables and figures are available with the article

tanning solution to dry the material. Aspects of general coat color, chromogenetic fields of the body (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 chromogenetic fields of the head were analyzed through the description of presence or absence of the criteria reported by Hershkovitz (1982) related with qualitative traits in Neotropical deer species. We took 36 cranial measurements following the criteria for the standard measurements for cervids (von den Driesch 1976). Different angles of the skulls were photographed to complement the documentation and description of the specimens.

### 2.3 Cytogenetic analysis

We collected and preserved in liquid nitrogen 5 x 2 cm skin samples from the inguinal region 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 (Howell and Black 1980). The chromosomes were classified as acrocentric, submetacentric, or metacentric according to their arm ratio (Levan et al. 1964) and separated into groups according to their relative lengths (Cifuentes-Rincón et al. 2020).

### 2.4 Fluorescence *in situ* hybridization (FISH)

FISH cytogenetic technique was performed using Bacterial artificial chromosome (BAC) clones derived from cattle on the karyotype of *Odocoileus pandora*. These BAC probes were selected from the CHORI-240 library based on the NCBI ARS-UCD1.2 Assembly data (BACPAC Genomics, Emeryville, CA, USA) and according the previous characterization on chromosomes of *S. gouazoubira* (2n = 70 and FN = 70), which retains the ancestral karyotype of the Cervidae family (Bernegossi et al. 2022). Using specific BAC probes we compared the karyotypes of *O. pandora* and *O. virginianus* (2n = 70 and FN =

74) to detect chromosomal divergences, and contrast our results with the previously characterized karyotype of *M. americana* (Peres et al. 2021). The list of BAC clones used in each individual is listed on Table 1.

**Table 1.** List of BAC clones from the CHORI-240 cattle (BTA) library and their correspondence in OPA (*Odocoileus pandora* (Merriam, 1901)) and SGO (*Subulo gouazoubira* (Fischer, 1814)) chromosomes (Bernegossi et al. 2022). The BAC clones marked with * were tested in OVI (*Odocoileus virginianus* (Zimmermann, 1780)).

| BTA chromosome | BAC clone (position Mb) | SGO homology | MPA homology |
|---|---|---|---|
| **1** | 106N15 (2.08-2.25)* | 16 | 19 |
| | 69G2 (57.29-57.48)*<br>109I18 (116.68-116.91)*<br>273F5 (154.36-154.55)* | 4 | 6<br>*OVI1 |
| **2** | 110M8 (44.41-44.65) | 3 | 8 |
| **2** | 124N14 (96.51-96.71)<br>437C7 (135.51-135.71)* | 30 | 2p<br>*OVI30 |
| **3** | 106P15 (98.75-98.97)<br>24H18 (4.38-4.62)* | 1 | 1q<br>*OVI2 |
| **4** | 259C9 (119.29-119.48) | 2 | 7 |
| **5** | 411D6 (5.81-5.99)<br>56D20 (55.50-55.75)* | 21 | 4q<br>*OVI21 |
| **5** | 100C21 (119.70-119.89)* | 23 | 3p<br>*OVI23 |
| **6** | 46O3 (61.12-61.34) | 20 | 21 |
| **6** | 200F18 (117.34-117.56) | 24 | 23 |
| **7** | 57O13 (1.36-1.56) | 7 | 11 |
| **8** | 71G4 (62.73-62.93) | 26 | 24 |
| **8** | 504A4 (112.10-112.29) | 33 | 28 |
| **9** | 78C10 (60.39-60.60) | 22 | 22 |
| **9** | 90A6 (103.38-103.59) | 34 | 29 |
| **10** | 214M18(103.02-103.24) | 6 | 10 |
| **11** | 98M8 (3.70-3.92) | 8 | 12 |
| **12** | 27O21 (36.43-36.65) | 9 | 13 |
| **13** | 174M6 (7.23-7.44) | 12 | 16 |
| **14** | 319C15 (0.87-1.06) | 10 | 14 |
| **15** | 121K12 (41.87-42.09) | 13 | 17 |
| **16** | 124O11 (38.84-39.04) | 11 | 15 |
| **17** | 63H8 (3.90-4.12)* | 19 | 1p<br>*OVI19 |
| **18** | 105L6 (65.58-65.76)* | 17 | 3q<br>*OVI17 |
| **19** | 50L8 (34.35-34.57)* | 15 | 2q<br>*OVI15 |
| **20** | 189H8 (5.16-6.89) | 18 | 20 |
| **21** | 95M7 (5.45-5.66) | 14 | 18 |
| **22** | 168H4 (38.93-39.12)<br>155B17 (59.06-59.29)* | 25 | 5p<br>*OVI25 |
| **23** | 77G24 (28.26-28.47) | 27 | 25 |
| **24** | 90M14 (32.92-33.16) | 28 | 26 |
| **25** | 124M6 (37.22-37.43) | 32 | 4p |

| | | | |
|---|---|---|---|
| | 89A17 (21.27-21.51)* | | *OVI32 |
| **26** | 47G11 (24.54-24.77) | 5 | 9 |
| **27** | 126M6 (41.90-42.14) | 31 | 27 |
| **28** | 108O21 (24.74-24.94) | 5 | 9 |
| **29** | 103L15 (28.99-29.16)* | 29 | 5q<br>*OVI29 |
| **Xp** | 159O16 (23.04-23.22)<br>67P21 (33.77-34.00) | Xq | Xp<br>*OVIXp |
| **Xq** | 311B9(47.76-47.97)<br>316D2 (68.49-68.68)<br>40H2 (74.95-75.12) | | Xq<br>*OVIXq<br>Xp<br>*OVIXp<br>Xq<br>*OVIXq |

We followed a modified protocol for DNA extraction from the Wizard® Plus SV Minipreps DNA Purification Systems method. BAC DNA was labeled using BioPrime® Array CGH Genomic Labeling (Invitrogen, Carlsbad, CA, USA) with Green-DdUTP (Abbott, IL, USA), biotin 16-dUTP or digoxigenin-11-dUTP (Roche, Mannheim, Germany) and FISH was performed according Vozdova et al. (2019). All chromosome preparations were photographed with a Zeiss AxioCam MRm camera attached to a Olympus BX60 microscope (100x objective), equipped with appropriate fluorescence filters.

### 2.5 DNA extraction, amplification and sequencing

Genomic DNA extraction from tissue samples (muscle and skin) of *Odocoileus pandora* T365, Mx02 and Mx06 was performed using the Qiagen DNeasy® Blood Qiamp Tissue kit following the manufacturer's instructions. Approximately 1 µg of DNA from each specimen was sent to NGS Soluções Genômicas (https://ngsgenomica.com.br) for massively parallel DNA sequencing. First, the DNA samples were fragmented by Covaris ultra-sonication to an average size of 300 base pairs. Then, these fragmented DNA samples were used as input for genomic library preparation using the TruSeq Nano DNA sample preparation kit (Illumina, USA) following the manufacturer's instructions. All genomic libraries were pooled and sequenced on the NextSeq 2000 Platform (Illumina, San Diego, CA, USA), 100 bp paired-end sequencing, generating a mean of 13 million reads per sample. Data conversion and adapter removal was done automatically on BaseSpace (Illumina´s cloud platform) by Illumina´s BCL Convert software. Quality control of the reads was performed using FastOC within BaseSpace, using a K-mer size of 5 and contamination filtering.

Unmerged reads were aligned to the *O. virginianus* mitogenome (GenBank accession number JN632671) as a reference using Bowtie2 v2.4.1 (Langmead and Salzberg 2012) on the Galaxy online server (Afgan et al. 2018), resulting in a minimum coverage depth of 30x per sample. Annotation was performed using the MITOS (Bernt et al. 2013) web-based platform (http://mitos.bioinf.uni-leipzig.de/index.py) and manually adjusted based on comparisons to the reference mitogenome using Geneious Prime 2021.2.2 (Biomatters; http://www.geneious.com/).

We also extracted genomic DNA from tissue samples of the *Odocoileus pandora* specimens Mx01 and Mx05 from Yucatan using a modified protocol described by Sambrook et al. (1989). We amplified mitochondrial cytochrome b gene (Cytb, 1,140 bp) using primers to obtain a fragment of length 480 bp (L14124/H15149) designed by Kocher et al. (1989) and a second primers pair for a fragment of length 660 bp (FARH/FARL) designed by Duarte et al. (2008). The PCR was performed in a conventional thermocycler “Biometra T1 Thermocycler” to amplify the DNA fragments using 3,6 mM dNTPs, 1.5 mM MgCl, 1x Buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 1U Taq Polymerase, 0.5 pM of forward primer, 0.5 pM of reverse primer and 50 ng/ul of DNA, in a final volume of 30 µL. After one initial cycle of 2 min at 94° for DNA denaturation, the amplification program consisted in 35 cycles of 1 min at 94°, 30 sec at 55° (hybridization temperature), 30 sec at 72°, with final extension of 10 min at 72°. The PCR product was analyzed by electrophoresis in 2% agarose and purified with the Wizard SV gel and PCR Clean-Up System kit (Promega TM), followed by sequencing using the ABI BigDye Terminator kit (Applied Biosystems) in an ABI 3130xl automatic sequencer (Applied Biosystems).

### 2.6 Alignment and Bayesian phylogenetic inference

The sequences were deposited in GenBank, mitogenome sequences with accession numbers OQ731410, OQ731408 and OQ731409 and Cytb sequences with accession numbers OM401530, OM543536, OM401527 and OM543537 (Table 2). We downloaded available sequences in GenBank from Neotropical deer species *M. americana*, *M. rufa*, *M. nemorivaga* (Cuvier, 1817), *Mazama nana* (Hensel, 1872), *O. pandora*, *Mazama rufina* (Pucheran, 1851)*, M. temama*,

*Subulo gouazoubira*, *O. virginianus*, *Odocoileus hemionus* (Rafinesque, 1817), *Blastocerus dichotomus*, *Ozotoceros bezoarticus* (Linnaeus, 1758) *and Mazama jucunda* (Thomas, 1913). For the latter, we retrieved sequences labeled as *M. bororo,* a junior synonym of *M. jucunda (Mantellato et al. 2022)*. *Alces alces* (Linnaeus, 1758) was used as outgroup. Both datasets, complete mitogenome and Cytb gene, were aligned separated using the Clustal X program (Thompson et al. 1997). For the mitochondrion genome, we excluded the control region from the alignment because of the tandem repeat located inside them and the high incidence of potential homoplasy. The optimal partitioning scheme was selected by the partitionFinder 2 (Robert et al. 2017) under the Bayesian information criterion. The partition included 13 protein-coding genes (ND1, ND2, COI, COII, ATP8, ATP6, COIII, ND3, ND4L, ND4, ND5, ND6 and Cytb, 22 transfer RNAs (tRNA)and two ribosomal subunits RNAs (srRNA and lrRNA).

For the Cytb data set we selected the best molecular evolution model using the jModelTest v. 0.1.1 (Posada and Crandall 1998), following the corrected Akaike information criterion, AICc (Akaike 1973).

The Bayesian inference (BI) analysis was performed in two independent runs (mitogenome and Cytb gene) using multiple four chain Metropolis-coupled analysis (with default heating) using MrBayes v3.2.7 (Huelsenbeck and Ronquist 2001) with 10,000,000 generations, and a tree sampling frequency of 1,000. We checked for convergence of the runs with Tracer v1.7.1 (Rambaut et al. 2018), then we discarded the first 25% of the trees to exclude suboptimal topologies. We also recovered the 50% majority rule consensus tree and displayed it using FigTree v1.4.0 (Rambaut 2012). Additionally, we used MEGA X software (Kumar et al. 2018) to estimate genetic distances among groups using a K2P model.

**Table 2.** List of mitochondrial sequences used in the phylogenetic analysis of *Odocoileus pandora* Merriam, 1901, and other neotropical cervids.

| Species | ID | Origin | Mitogenome | CYTB |
|---|---|---|---|---|
| | | | Access number | Access number |
| ***Mazama pandora*** | T365 | Yucatan, Mexico | OQ731410 | OQ731410 |
| ***Mazama pandora*** | Mx01 | Yucatan, Mexico | | OM401530/ OM543536 |
| ***Mazama pandora*** | Mx02 | Yucatan, Mexico | OQ731408 | OQ731408 |
| ***Mazama pandora*** | Mx05 | Yucatan, Mexico | | OM401527/ OM543537 |
| ***Mazama pandora*** | Mx06 | Yucatan, Mexico | OQ731409 | OQ731409 |
| ***Mazama temama*** | T366 | Veracruz, Mexico | MW047275 | MW047253 |

| | | | | |
|---|---|---|---|---|
| ***Mazama temama*** | T362 | Campeche, Mexico | MW047273 | MW047254 |
| ***Mazama americana*** | T358 | Cayenne, French Guiana | MW047272 | MN726911 |
| ***Mazama americana*** | JN632656 | French Guiana | JN632656 | |
| ***Mazama americana*** | T018 | Pará,Brazil | | DQ789211 |
| ***Mazama americana*** | T297 | Carajas, Brazil | OQ731411 | |
| ***Mazama americana*** | T253 | Mato Grosso, Brazil | MZ350856 | MZ350856 |
| ***Mazama rufa*** | T385 | Parana, Brazil | OQ198444 | DQ789215 |
| ***Mazama americana*** | T310 | Amazonas, Brazil | | MW047256 |
| ***Mazama nana*** | T107 | Paraguay | MZ350863 | |
| ***Mazama jucunda**** | T215 | Brazil | MZ350859 | MZ350859 |
| ***Mazama jucunda**** | T071 | Parana, Brazil | | DQ789231 |
| ***Mazama rufina*** | JN632661 | Colombia | JN632661 | JN632661 |
| ***Mazama nemorivaga*** | T024 | Porto Velho, RO, Brazil | MZ350861 | MZ350861 |
| ***Mazama nemorivaga*** | T346 | Pará, Brazil | | MZ350867 |
| ***Subulo gouazoubira*** | T377 | Asunción, Paraguay | MZ350858 | MZ350858 |
| ***Blastocerus dichotomus*** | CV21 | Brazil | OM543540 | OM543539 |
| ***Ozotoceros bezoarticus*** | FNMA40 | Brazil | MZ350859 | MZ350859 |
| ***Subulo gouazoubira*** | T314-KJ772514 | Pantanal, Brazil | KJ772514 | T314- KJ772514 |
| ***Odocoileus virginianus*** | HQ332445 | USA | HQ332445 | HQ332445 |
| ***Odocoileus virginianus*** | JN632671 | French Guiana | JN632671 | JN632671 |
| ***Odocoileus hemionus*** | FJ188884; | Sonora, Mexico | | FJ188884 |
| ***Odocoileus virginianus*** | KX171718; | Florida, USA | | KX171718 |
| ***Odocoileus hemionus*** | FJ188882 | Sonora, Mexico | | FJ188882 |
| ***Odocoileus hemionus*** | JN632670 | Arizona, USA | JN632670 | |
| ***Odocoileus hemionus*** | CM014077 | Canadá | CM014077 | CM014077 |
| ***Alces alces*** | MF784602 | Poland | MF784602 | MF784602 |
| ***Odocoileus hemionus*** | FJ188879 | Alaska, USA | | FJ188879 |
| ***Odocoileus. virginianus*** | KM612278 | Mexico | KM612278 | KM612278 |
| ***Odocoileus. virginianus*** | KM612271 | Mexico | KM612271 | KM612271 |
| ***Odocoileus. virginianus*** | KM612279 | Mexico | KM612279 | KM612279 |
| ***Odocoileus hemionus*** | KY928664 | Arizona, USA. | | KY928664 |
| ***Mazama pandora*** | KC146954 | Yucatán, México | | KC146954 |
| ***Mazama pandora*** | KC146955 | Yucatán, México | | KC146955 |
| ***Mazama pandora*** | KY928659 | Campeche, México | | KY928659 |
| ***Mazama temama*** | KY928661 | Guatemala | | KY928661 |

| | | | | |
|---|---|---|---|---|
| ***Mazama temama*** | KC146956 | Chiapas, Mexico | | KC146956 |
| ***Mazama temama*** | KP954717 | Hidalgo, Mexico | | KP954717 |
| ***Mazama temama*** | KC146958 | Chiapas, Mexico | | KC146958 |
| ***Subulo gouazoubira*** | T386 | Brazil | MZ350865 | MZ350865 |
| ***Hippocamelus antisensis*** | JN632646 | Argentina | JN632646 | JN632646 |
| ***Blastoceros dichotomus*** | JN632603 | Bolivia | | JN632683 |
| ***Ozotoceros bezoarticus*** | JN632681 | Bolivia | | JN632680 |
| ***Mazama nemorivaga*** | JN632659 | Peru | JN632659 | JN632659 |
| ***Subulo gouazoubira*** | JN632658 | Colombia | JN632658 | JN632658 |

*Sequences identified as *M. bororo* Duarte, 1996, a junior synonym of *M. jucunda* (Thomas, 1913) (Mantellato et al. 2020).

## 3. RESULTS

### 3.1 Morphological characterization of *Odocoileus pandora* Merriam, 1901 (MAMMALIA, CERVIDAE):

The specimen has a general light brown coat color, grayish in the dorsal region and whitish in the inguinal region. Gray brown head with white in ventral neck region. Whitish spot below the orbit. Presence of white gular spot. Dark brown rostral band. White opening of preorbital pouch. Pointed ears with white inner auricular surface and brown outer auricular surface. Hair absence in the white anterior and posterior auricular border. Light brown color in the posterior and anterior craniolateral limbs region. Humped posterior half of nasals in lateral profile. Whitish ventral-caudal region. Brown dorsal tail and white ventral tail. Relatively long and thick antlers, inclined postero-dorsally, longitudinally grooved and curved. Broad and massive pedicels curved at 45 to 60 degrees, originated from the posterior region of the orbit, converging at the tips. Skull with large and long supraorbital groove. Extended vomerine septum typical of Capreolinae. Inflated auditory bullae. Two lacrimal foramina externally at the orbital border. Flat lacrimal fossa. Large preorbital cavity with angular and inverted triangular shape. (Figure 1). Dorsal margin of squamosal root of zygoma narrowly arched above glenoid fossa. Presence of wide premaxillary and inflated auditory bulla.

**Record location:** Zoh Laguna, Campeche, Yucatan Peninsula Mexico.

**Specimen deposited in:** Deer and Conservation Center (Nupecce), Sao Paulo State University (UNESP) – Campus de Jaboticabal.

**Deposit Number.:** NPC093 (full skeleton and skull, taxidermized skin) – NUPECCE Museum.

**Tissue sample deposited in:** NUPECCE Tissue and Cell banking. TESE 365. Complete mitochondrion DNA sequence Deposit Number: OQ731410.

**Karyotype:** 2N=60 /NF=74, XY sexual system

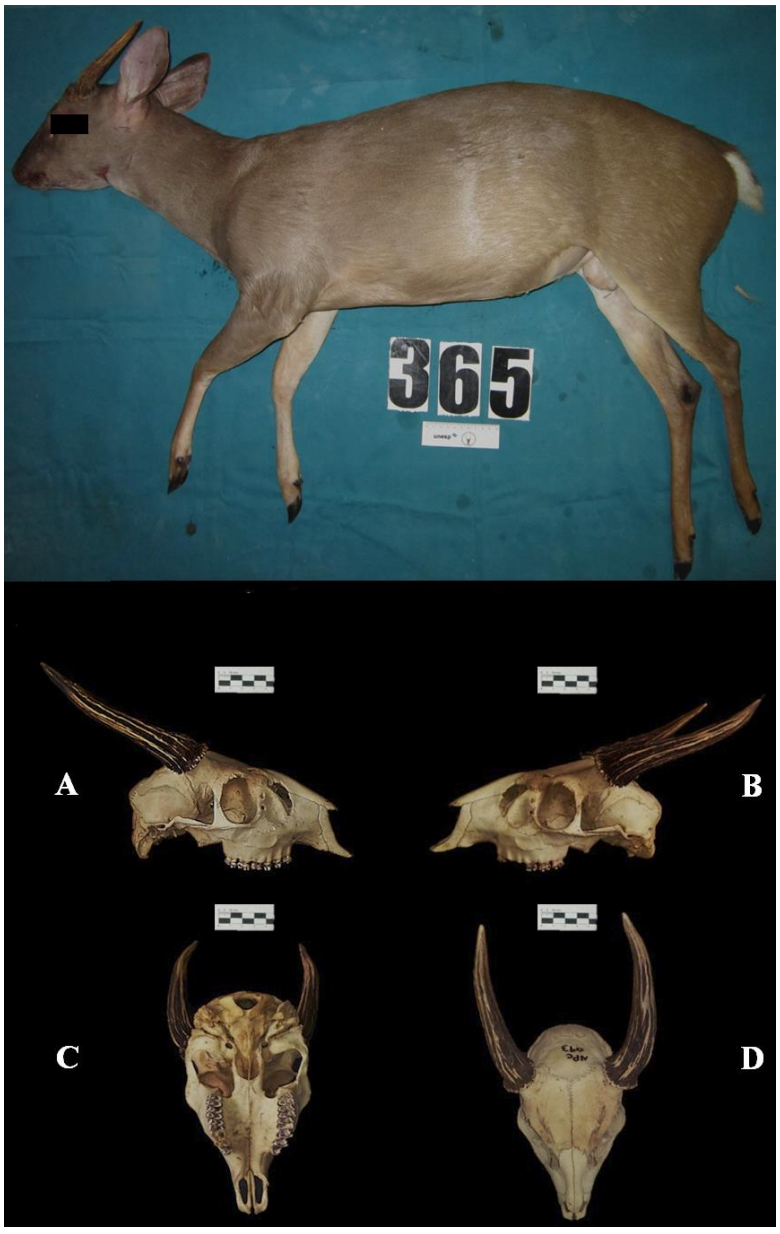


**Figure 1.** Adult male of *Odocoileus pandora* (Merriam, 1901), collected in the Yucatan Peninsula, Mexico. ID: T365. I: Lateral view of body. II: Macerated skull of *Odocoileus pandora* (Merriam, 1901). (A) Right lateral view (B) Left lateral view (C) Ventral view (D) Dorsal view. Material deposited at the NUPECCE museum identified under voucher NPC093.

### 3.2 Cytogenetic analysis

The *O. pandora* displays a karyotype with 2n = 60 and FN = 74, 29 pairs of autosomal chromosomes and a simple sexual system XY (Figure 2). The X chromosome appeared as a submetacentric chromosome and the Y as a small metacentric. Chromosomal pairs 1–6 were classified as belonging to group C (small autosomal of two arms) and all acrocentric chromosomes belonging to group E (small chromosomes of one arm). The presence of supernumerary chromosomes (Bs) was not observed (Figure 2A).

C-banding showed constitutive heterochromatin blocks in the pericentromeric region of all autosomal and X chromosomes (Figure 2B). The Ag-NOR staining showed four nucleolus organizing regions in the telomeric area of the long arms of the two chromosomes of pair 1, and in the telomeric region of the long arms of the two chromosomes of pair 10. Occasionally, metaphases were also found with marking in the telomeric region of the long arm in one chromosome in pair 14 (Figure 2C).

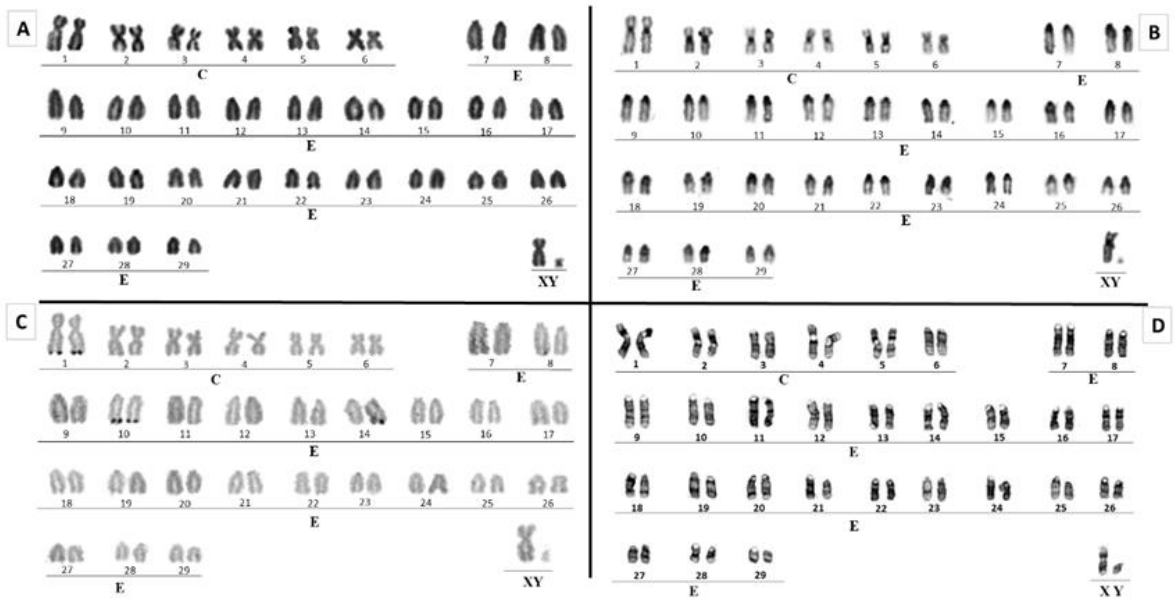


**Figure 2.** Karyotype of a *Odocoileus pandora* male (Merriam, 1901), collected in the Yucatan Peninsula, Mexico. ID: T365. 2n = 60 and NF = 74. (A) Conventional Giemsa staining (B) C-Banding (C) Ag-RON staining with five nucleolar organizer regions (D) G-Banding.

G-Banding (Figure 2D) associated with FISH demonstrated that *Odocoileus pandora* underwent chromosomal rearrangements compared to the

hypothetical ancestral deer karyotype retained by *Subulo gouazoubira* (2n = 70 and FN = 70). We observed that *O. pandora* presented centric fusions involving the pairs 19 and 1, 29 and25, 30 and 15, 23 and 17 and 32 and 21 from the basal state presented in *S. gouazoubira*, that formed the large chromosomes in group C of *O. pandora* (Table 1 and Figure 3). It also showed a centromeric shift of the chromosome 4 from *S. gouazoubira* that resulted in the submetacentric morphology of *O. pandora* chromosome 6 (Figure 4). This rearrangement was also evidenced in *Odocoileus virginianus* karyotype, resulting in the only bi-armed autosome of this species.

The X chromosome of the *O. pandora* individual showed a submetacentric morphology with a change in the centromere position when compared to the acrocentric X of *S. gouazoubira*. In addition, the proximal region of the X chromosome of *O. pandora* showed a change in the hybridization pattern of BAC clones. From the putative basal stare, the proximal region of X chromosome appears inverted in the short arm of the *O. pandora*, while the long arm preserved the same hybridization pattern as those shown for the distal region of the X chromosome in *S. gouazoubira* (Table 1 and Figure 4). FISH analysis in *O. virginianus* revealed the submetacentric morphology of X chromosome, in which each BAC clone position was similar to *O. pandora* (Figure 4). The chromosomes that appeared fused in *O. pandora* were confirmed as acrocentric in *O. virginianus* (Figure 3 and 4). Inferring that all other acrocentric autosomes of *O. virginianus* are homologous to one *S. gouazoubira* chromosome, we noted that divergences between *O. virginianus* and *O. pandora* karyotypes involves at least five chromosomal fusions. For the Figure 3, we classified *O. pandora* and *O. virginianus* chromosomes according to *S. gouazoubira* idiogram.

In addition, the comparative analysis between the *M. americana* and *O. pandora* (Figure 3 and 4) showed that the chromosomal rearrangements found in both karyotypes are not homologous. Example of this, the long arm of chromosomes 2 and 3, and the chromosomes 11 and 20 of *O. pandora* appeared fused in *M. americana* pair 1, and the short arm of chromosome 3, and chromosomes 6, 9, 10 and 12 of *O. pandora* are fused in *M. americana* pair 2. The composition of sex chromosomes also differed between the species. *M. americana* has a multiple sex chromosome system $XY_1Y_2$ formed by X-autosomal fusions that involves the homologous to pairs 15 and 17 of *O. pandora*.

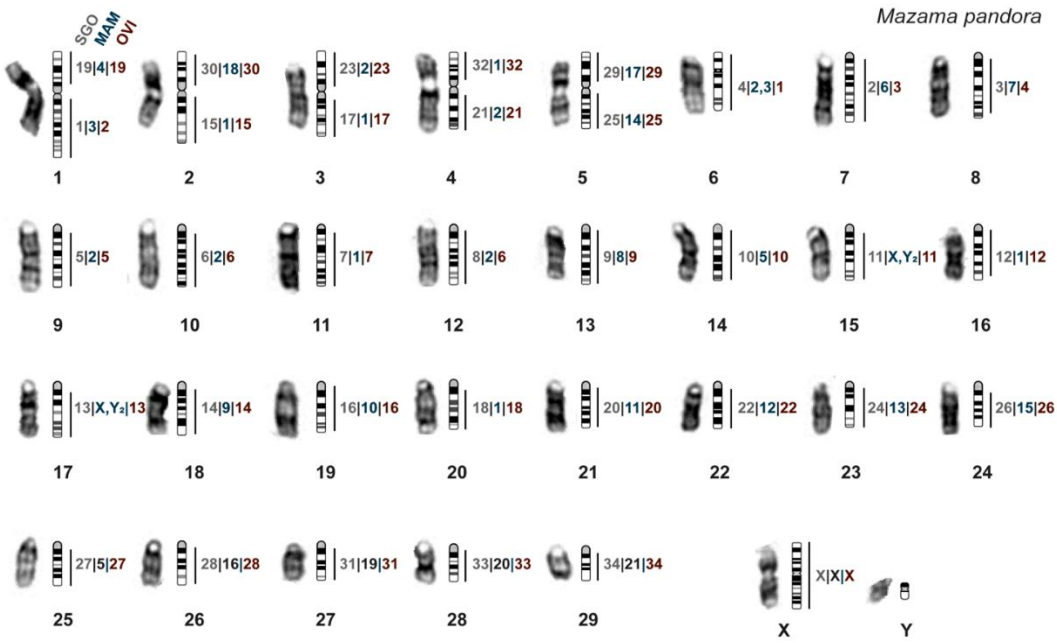


**Figure 3.** FISH results in *Odocoileus pandora* male (Merriam, 1901) (OPA) karyotype: G-banded chromosomes, idiogram of *O. pandora* readapted from the karyotype of *Subulo gouazoubira* (Fischer, 1814) (Bernegossi et al. 2022), homology with *Subulo gouazoubira* (SGO), *Mazama americana* (Erxleben, 1777) (MAM) and *Odocoileus virginianus* (Zimmermann, 1780) (OVI).

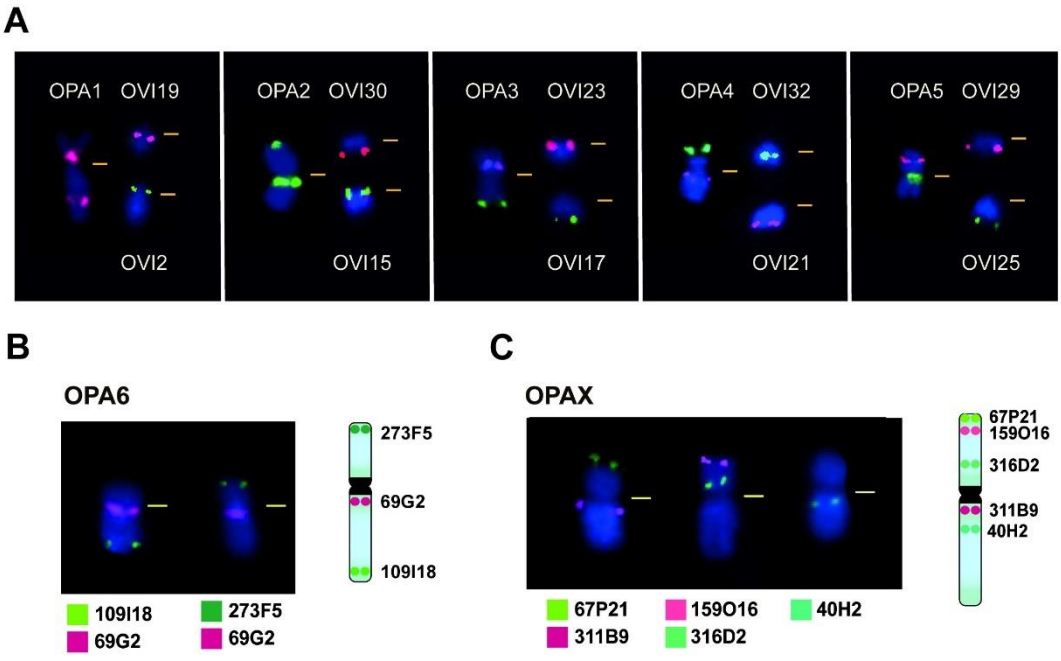


**Figure 4.** BTA homologies in *Odocoileus pandora* (Merriam, 1901) (OPA) chromosomes. (A) OPA1 to 5 homologies with OVI (*Odocoileus virginianus* Zimmermann, 1780). (B) BTA1 homologies in OPA6. (b) BTAX homologies in OPAX.

### 3.3 Mitochondrial phylogenetic analysis

The evolutionary model with the highest AIC for the Cytb dataset was GTR+I+G. The partial mitogenome (excluding control region) alignment comprised 15,410 bp in length and the best-ft partitioning scheme selected a separate substitution model for each gene and each codon position for protein-coding genes. The mitochondrion resulting topology recovers two strongly supported clades, Blastocerina and Odocoileina sensu Heckeberg (2020), and also confirmed the polyphyly of the genus *Mazama*, with the gray brocket species within the Blastocerina This subtribe forms a clade that include *O. bezoarticus*, *B. dichotomus, Hippocamelus antisensis* (d'Orbigny, 1834) and the gray brockets *M. nemorivaga* and *S. gouazoubira* (Figure 5).

Odocoileina is formed by three clades, the first represent the early divergence of *M. rufina*, a second clade with the red brocket species and the third clade formed by the other species *Odocoileus* as sister taxa of *O. pandora*. The analysis evidenced the monophyly of *O. pandora* with 100% posterior probability, diverging separately from *Odocoileus virginianus and Odocoileus hemionus* with 97% posterior probability. The species *O. hemionus* was paraphyletic and *O. virginianus* polyphyletic (Figure 5). In the red brocket clade, *M. temama* proved to be monophyletic, *M. americana* was recovered as polyphyletic, with the specimens from French Guiana recovered in a different clade than the Brazilian individual from Carajás nested with one individual of the species *M. rufa,* and individuals from Juína, Brazil, grouped within a clade with *M. jucunda* and *M. nana.* (Figure 5). The BI of partial Cytb gene evidenced a similar result, the monophyletic *O. pandora* was recovered within Odocoileina subtribe with the other *Odocoileus* species clade as sister taxa. It is noteworthy that *O. pandora*–*Odocoileus* is nested within other red brocket species (Fig. S2)[1].

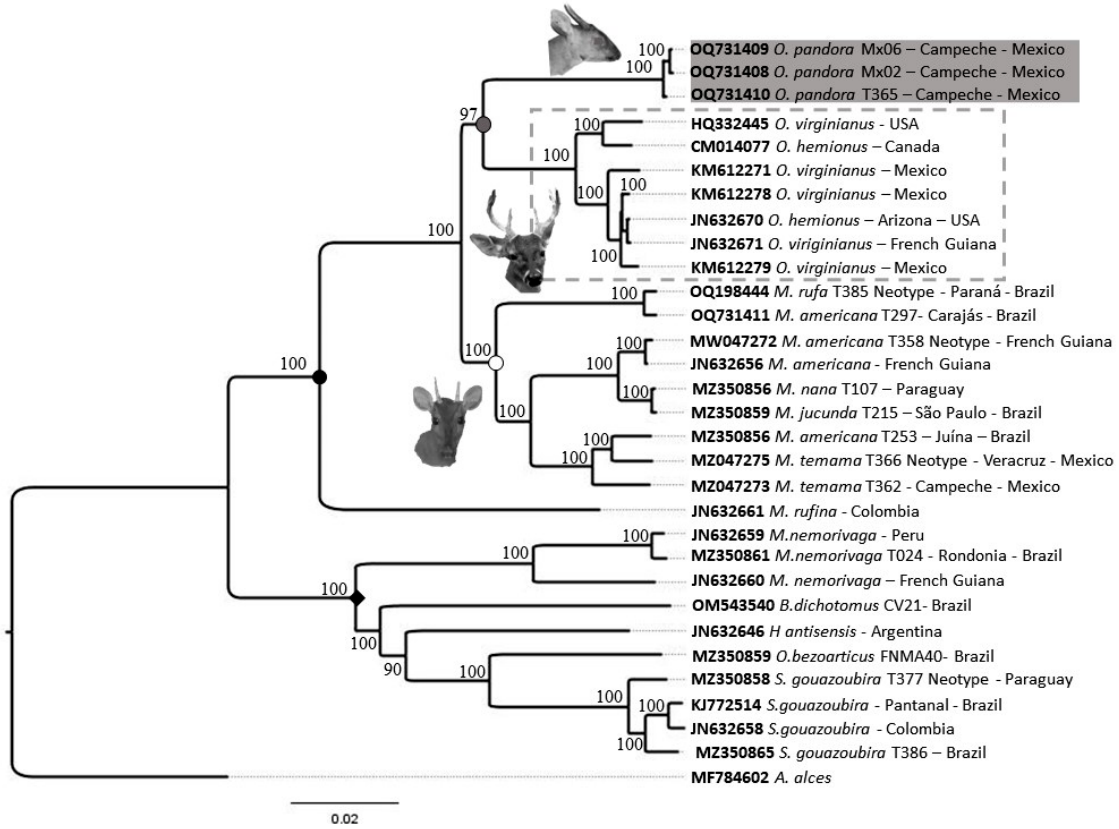


**Figure 5.** Bayesian inference tree of the mitogenomes from several species of Neotropical cervids. The value above the clades represents the posterior probability of the analysis. Mx06, Mx02 and T365: specimens collected in Campeche, Mexico. Clade markers: *Odocoileus pandora* (Merriam, 1901) species (gray square), *Odocoileus* clade Rafinesque, 1832 (gray circle), *Odocoileus virginianus* (Zimmermann, 1780) – *Odocoileus hemionus* Rafinesque, 1817, clade (dashed line square) Odocoileina subtribe black circle), *Mazama* Rafinesque, 1817 clade (white circle) and Blastocerina subtribe (black diamond). Outgroup: *Alces alces* Linnaeus, 1758.

We assessed a genetic distance of 0.07 and 0.08 between *O. pandora* regarding genera *Odocoileus* and *Mazama*, respectively (Table S2)[1], based on the complete mitochondrion distance matrix.

## 4. DISCUSSION

### 4.1 Morphological characterization

The morphological characterization of *O. pandora* has been discussed by many authors with contrasting hypotheses, using skin color as a trait that determines the inclusion of the species within the red or gray brocket (Hershkovitz

1951; 1966). The specimen analyzed here showed characteristics similar to the type specimen of Merriam (1901), and to the morphotypes described by Medellin et al. (1998), with a homogeneous light brown coat with whitish areas in the inguinal and submandibular region, inner part of the hind and fore limbs, as well as ear base. The cranial and external body measurements of the examined individual also agreed with the pattern described for the species (Merriam 1901; Medellin et al. 1998; Weber and Medellín 2010).

The specimen described here presents narrow zygomatic arch, curved nose, wide premaxillary and inflated auditory bulla. These characters differentiate *O. pandora* from *M. americana* and *M. temama*, both species with a broad zygomatic arch, straight nasal, and flat auditory bulla (Medellin et al. 1998).

The skull presents an antler-shape in accordance with previous descriptions to a long, broad, massive, striated, longitudinally grooved and curved antlers, converging towards each other (Medellin et al. 1998; Duarte and Gonzalez 2008). The antler size and complexity may vary depending on animal´s age, reproductive status and worn condition (Heckeberg et al. 2023). Medellín et al. (1998) reported that *Odocoileus pandora* males have fluted antlers in the unworn condition, separated by ridges deeply plicated and that this furrowed appearance is not always evident in heavily worn antlers. The individual variation related to age and antler cycle is similar to other deer in which body growing and reproductive status are influencing the antler size and velvety or unvelvety condition (Versiani et al. 2009; Heckeberg et al. 2023). In all cases, the massive, grooved and curved shape of the antlers in *O. pandora* contrasts with those observed in *Mazama* and *Subulo* species, which simple and straight antlers, and with the branching pattern of the antlers in *Odocoileus*, *Blastocerus* and *Ozotoceros* (Merino and Rossi 2010). Therefore, the distinctive shape of the antlers could be useful to recognize *O. pandora* from other deer species. However, it is important to note the difficulty of using antler traits to estimate the relationship among deer species as reported in other studies that described convergent development and subsequent homoplasy of these characters (Pitra et al. 2004; Heckeberg 2020)

### 4.2 Cytogenetic description

The complex taxonomy of Neotropical deer has been characterized by morphological similarities with a wide karyotypic diversification among the species (Duarte et al. 2008). This group shows a karyotypic variation from 2n = 32 to 70, with chromosomal polymorphisms described in closely related species (Abril et al. 2010; Peres et al. 2021). These authors had reported chromosomal rearrangements as an efficient post-zygotic reproductive barrier that leds to sub-fertility influenced by a decrease on gamete production (Cursino et al. 2014; Salviano et al. 2017) and it is considered one of the main drivers of speciation in deer (Galindo et al. 2021). The cytogenetic characterization of *O. pandora* may be considered as a diagnostic character, useful in the identification of the species respect to genera *Mazama* and *Odocoileus*.

This study contributes with the first karyotypic description of *Odocoileus pandora*. This karyotype displays significant differences when compared with *M. americana* (2n = 45 FN = 51; Cifuentes-Rincon et al. 2020), *M. temama* (2n = 44 FN = 70; Sandoval et al. 2022), *Odocoileus virginianus* (2n = 70, FN = 72; Aguilera et al. 2008) and *S. gouazoubira* (2n = 70 NF = 70; Bernegossi et al. 2022).

The absence of B chromosomes was evidenced in the different metaphases of *O. pandora*, similar to *O. virginianus, O. hemionus*, *B. dichotomus* and *O. bezoarticus*, contrasting with the presence of supernumerary chromosomes in the karyotypes of *Mazama* (Abril et al. 2010; Valeri et al. 2018). Likewise, the analysis of the classical banding allowed us to observed 6 pairs of two-armed chromosomes in *O. pandora*, a differentiated karyotype from *Blastocerus*, *Odocoileus* and *Ozotoceros* with three, two and one, pair of two-armed chromosomes, respectively (Duarte and Giannoni 1995*a*; Duarte and Giannoni 1995*b*; Aguilera et al. 2008).

The graphical representation of the G-banding associated to the FISH technique may help in the comparative study of brockets (Duarte and Jorge 2003; Abril and Duarte 2008; Abril et al. 2010; Vargas-Munar et al. 2010; Valeri et al. 2018) reducing possible misinterpretations with classical banding studies due to the high rate of karyotypic evolution and chromosomal polymorphisms reported in Neotropical deer (Frohlich et al. 2016; 2017). In this study, the G-banding allowed an accurate comparison of the idiogram for *O. pandora* with that

described for *S. gouazoubira* (Bernegossi et al. 2022). Here, we detected that *O. pandora* differed from the ancestral *S. gouazoubira* by five centric fusions and a centromere shift in the autosome chromosomes, and also by the submetacentric morphology of X chromosome in comparison with the acrocentric X of *S. gouazoubira*.

Specifically, the X chromosome in *O. pandora* followed the description of *Capreolus capreolus* (Frohlich et al. 2017) with the same order and position of each X probe used in the proximal region and in the qproximal and qdistal regions. This pattern was also observed in the X chromosome of *O. virginianus*. The difference with *M. americana* and *M. rufa* resides in the X-autosome fusion, as reported in Peres et al. (2021).

The BAC clones also demonstrated that *O. pandora* diverged in at least five and 20 chromosomal rearrangements compared with *O. virginianus* and *M. americana* (*sensu stricto*), respectively, characterized by distinct processes of mainly centric and tandem fusions during their karyotypic evolutions. Therefore, we confirm through cytogenetic data that the Yucatan brocket deer is an independent evolutionary lineage, separated from *Odocoileus* and *Mazama* genera, and considered as a valid species as Merriam (1901) and Medellin et al. (1998) did previously. Further studies with all the species of the group may be performed to detect chromosomal rearrangements involved in the inter- and intra-specific divergences that could assist in the understanding of chromosomal evolution associated with the speciation process within Neotropical deer.

### 4.3 Phylogenetic characterization

The phylogenetic tree of the complete mitochondrion DNA confirmed the polyphyly of the genus *Mazama* and the paraphyly of the “red *Mazama* clade” (Gilbert et al. 2006; Duarte et al. 2008; Hassanin et al. 2012; Gutiérrez et al. 2017; Heckeberg 2020).

*Odocoileus pandora* was recovered within subtribe Odocoileina, with *M. americana* sensu lato, *M. rufa*, *M. rufina*, *M. nana*, *M. temama*, *M. jucunda*, *O. virginianus* and *O. hemionus*. This corroborates the results of Escobedo-Morales et al. (2016) who performed the first phylogenetic analysis of *O. pandora* using mtDNA, placing the species in the red clade. These authors found two different

topologies depending on the mitochondrial dataset used, one recovered from the Cytb-only matrix in which *O. pandora* was recovered as a monophyletic haplogroup sister of *O. virginianus*, and a second one derived from a Cytb-ND2-Control region with an early divergence respect to *Odocoileus* (Escobedo-Morales et al. 2016).

Other studies have also described *O. pandora* within a clade composed of sequences from *Odocoileus sp.* (Heckeberg et al. 2016), while a broader approach performed by Gutiérrez et al. (2017) associated the species as a sister taxon of *O. h. columbianus*, *O. h. sitkensis* and *O. h. hemionus*. Recently, Heckeberg (2020) proposed a close relationship between *O. pandora* and the species of the genus *Odocoileus* through skull qualitative characters and concatenated mtDNA and nuclear genes analyses. Our Cytb phylogenetic analysis agreed with the previous results with *O. pandora* recovered in a monophyletic clade within the genus *Odocoileus* clade, however, the branch value was low and we cannot confirm the ancestry relationship between these taxa. Previous studies differ in the topologies derived from analyses of different mitochondrial datasets. This issue suggests the need for a multi-locus approach and comprehensive species sampling (Gutiérrez et al. 2017; Escobedo-Morales et al. 2016; Peres et al. 2021). Based on the complete mitochondrion, the recovered phylogeny showed higher node support than those using only one mtDNA gene (Hassanin et al. 2012; Heckeberg 2020; Bernegossi et al. 2022). Our mitogenome phylogeny evidenced that *O. pandora* is not nested within the genus *Odocoileus* clade, but in a separated monophyletic clade from the *Odocoileus* species.

In addition, the complete mitochondrion DNA matrix showed that the genetic distance between the Yucatan brocket deer and the genus *Odocoileus*, and between Yucatan brocket deer and the genus *Mazama* is similar than the genetic distance between *Subulo* and *Hippocamelus*, different well-established genera within Blastocerina subtribe. The latter genera were recovered as sister groups in mitochondrial DNA phylogeny, in which the main diagnostic characters are based on antler morphology, body size and external coloration. Similar to this, *O. pandora* and the others species *Odocoileus* are sister taxa that contrast morphologically and also cytogenetically. Future research should be performed

to discuss this issue in a broader scale analyzing the speciation rates in Odocoileina and Blastocerina subtribe as a best parameter to generic definition.

### 4.4 Taxonomic implications

The Yucatan brown brocket was described in 1901 by Merriam, from a male holotype (USNM 108273), morphologically characterized as belonging to the genus *Mazama,* currently recognized as *Odocoileus pandora* (Gutiérrez et al. 2017) Morphological reviews performed by Medellin et al. (1998) with a larger sample size and our analysis allowed us to describe differentiated qualitative characters that are present in the skull of *O. pandora* specimens as narrow zygomatic arch, nasal curved, wide premaxillary and inflated auditory bulla. The antlers of the species are simple, unbranching, thick, massive and long, classified as Morphotype 1 sensu Heckeberg (2020), different from the branching antler present in other *Odocoileus* species. The Yucatan brown brocket deer presents an inclined postero-dorsally, longitudinally grooved and convergent antlers, compared to the simple, straight (not convergent curved) antlers of the other species of the genus *Mazama*.

Cytogenetically, *Odocoileus pandora* presents an exclusive karyotype for the species, with 2n = 60 FN = 74, completely different from the 2n <52 >70 described in species of the genus *Mazama*, *Odocoileus*, *Ozotoceros* and *Blastocerus*. Currently, the only Neotropical deer species with a karyotype described with 2n = 60 is *M. rufina*, similar to *O. pandora*, however, it is characterized by having all the acrocentric autosome chromosomes, contrasting with the six pairs of two-armed autosomes found in *O. pandora*.

Mitogenome phylogeny revealed the *O. pandora* specimens in a separated monophyletic clade from the other *Odocoileus* species, as sister taxa. Although they share an immediate common ancestral, the Phylogenetic tree showed *O. pandora* is not nested within *Odocoileus*. This result, allows us to consider two options. The first, is confirming the transfer of the Yucatan Brown brocket deer to the genus *Odocoileus*. The use of *O. pandora* as a synonym of *M. pandora* is increasing in the scientific literature and electronic databases. However, it is necessary to update the diagnostic description of *Odocoileus* to include a species with simple antlers and differentiated external morphology features, as size, mass, body coloration and others. In addition, this action may affect the

taxonomic clarification of the Yucatan brown brocket transferred from *Mazama*, a genus with complicated taxonomic issues, to *Odocoileus*, another genus that need clarification related to the polyphyletic and paraphyletic relationship of the species currently allocated within the genus. The second option and our current consideration is to unlink the Yucatan brown brocket from both *Mazama* and *Odocoileus* genera, suggesting a new generic name for this taxon through the monophyletic evidence of the mitogenome tree obtained here together with the external morphological difference of antler shape, auditory bullae, size, body coloration and mass.

A more complete review of the species should be performed through a comprehensive molecular (including nuclear markers and a population approach) and cytogenetic characterization of more specimens *O. pandora* (preferably including the holotype of the species) from Yucatan Pensinsula to clarify the generic position of this taxon.

## 5. CONCLUSION

A male of known origin enabled an amended description for *Odocoileus pandora*. The complete mitogenome tree showed the monophyly of *Odocoileus pandora* as a separate clade from the genus *Mazama* with the other species *Odocoileus* as sister taxa This is the first karyotypic study of *Odocoileus pandora*. The morphological, cytogenetic and molecular characterization of the Yucatán brown brocket suggest that a new generic name should be assigned for the species.

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## 6. REFERENCES


Abril, V.V., and Duarte, J.M.B. 2008. Chromosome polymorphism in the Brazilian dwarf brocket deer, *Mazama nana* (Mammalia, Cervidae). Genetics and Molecular Biology. **31**: 53–57. doi:10.1159/000298819

Abril, V.V., Carnelossi, E.A.G., Gonzalez, S., and Duarte, J.M.B., 2010. Elucidating the evolution of the red brocket deer *Mazama americana* complex (Artiodactyla; Cervidae). Cytogenetic and Genome Research. **128**: 177–187. doi:10.1159/ 000298819

Aguilera, M.M., Exposito, A., and La Rocca, O. 2008. Cytogenetics of two subspecies of White-tailed deer (*Odocoileus*) from Venezuela. Caryologia. **61**:19–25. doi:10.1080/00087114.2008.10589606

Allen, J.A. 1915. Notes on American deer of the genus *Mazama.* Bulletin of the American Museum of Natural History. **34**:521–553.

Bernegossi, A.M., Borges, C.H.S., Sandoval, E.D.P., Cartes, J.L., Cernohorska, H., Kubickova, S., Vozdova., Caparroz, R., Gonzalez, S., and Duarte, J. M.B. 2022. Resurrection of the genus *Subulo* Smith, 1827 for the gray brocket deer, with designation of a neotype. Journal of Mammalogy. doi:10/1093/jmammal/gyac068

Cifuentes-Rincón, A., Morales-Donoso, J.A., Sandoval, E.D.P., Tomazella, I.M., Mantellatto, A.M.B., de Thoisy, B., and Duarte J.M.B. 2020. Designation of a neotype for *Mazama americana* (Artiodactyla, Cervidae) reveals a cryptic new complex of brocket deer species. ZooKeys. **958**: 143–164. doi: 10.3897/zookeys.958.50300

Cursino, M.S., Salviano, M.B., Abril, V.V., Zanetti, E.S., and Duarte, J.M.B. 2014. The role of chromosome variation in the speciation of the red brocket deer complex: the study of reproductive isolation in females. BMC Evolutionary Biology **14**: 40. doi:10.1186/1471-2148-14-40

Cuvier G.F. 1817. Dictionnaire des Sciences Naturelles. Zoologie. Mammiferes. F.G. Levrault, Paris

d'Orbigny, A. 1834. Rapport sur les résultats scientifiques du voyage de M. Alcide d'Orbigny dans L'Amérique du Sud, pendant les années 1826, 1827, 1828,

1829, 1830, 1831, 1832 et 1833. Partie Zoologique, commissaires MM. Isidore Geoffroy Saint-Hilaire et de Blainville. Nouvelles Annales du Muséum d'Histoire Naturelle Paris **3**:84−99.

de Winton, J. 1896. On some mammals from Ecuador. Proceedings of the Zoological Society of London **64**(2) 507–513.

Duarte, J.M.B., and Giannoni, M.L. 1995a. Cytogenetics analysis of the marsh deer, *Blastocerus dichotomus* (Mammalia, Cervidae). Revista Brasileira de Genética. **18**: 245–248.

Duarte, J.M.B., and Giannoni, M.L. 1995b. Cytogenetic analysis of the Pampas deer *Ozotoceros bezoarticus* (Mammalia, Cervidae). Brazilian Journal of Genetics. **18**: 485–488.

Duarte, J.M.B., and Jorge, W. 1996. Chromosomal polymorphism in several populations of deer (genus *Mazama*) from Brazil. Archivos de Zootecnia. **45**: 281–287.

Duarte, J.M.B., and Jorge, W. 2003. Morphologic and cytogenetic description of the small red brocket (*Mazama bororo* Duarte, 1996) in Brazil. Mammalia. **67**: 403–410. doi:10.1515/mamm.2003.67.3.403

Duarte, J.M.B., Gonzalez, S., and Maldonado, J.E. 2008. The surprising evolutionary history of South American deer. Molecular Phylogenetics and Evolution. **49**: 17–22. doi:10.1016/j.ympev.2008.07.009

Duarte, J.M.B., Boer, J.A., Sandoval, E.D.P., Bernegossi, A.M., and Tomazella, I.M. 2021. Skin Freezing Technique for Living Cell Bank. GenProtocols. Available from https://genprotocols.genengnews.com/protocols/skin-freezing-technique-for-living-cell-bank/1041. Accessed in December 2021.

Erxleben, J.C.P. 1777. Systema regni animalis per classes, ordines, genera, species, varietates cum synonymia et history animalium, Classis 1. Mammalia. Impensis Weygandianis **1**:1–636.

Escobedo-Morales, L.A., Mandujano, S., Eguiarte, L.E., Rodríguez-Rodríguez, M.A., and Maldonado, J.E. 2016. First phylogenetic analysis of Mesoamerican brocket deer *Mazama pandora* and *Mazama temama* (Cetartiodactyla:Cervidae) based on mitochondrial sequences:

Implications on Neotropical deer evolution. Mammalian Biology. **81**: 303–313. doi:10.1016/j.mambio.2016.02.003

Feldhamer, G.A., and McShea, W.J. 2012. Deer: the animal answer guide. The Johns Hopkins University Press.

Fischer, G. 1814. Zoognosia Tabulis Synopticis Illustrata. Typis Nicolai Sergeidis Vsevolozky. Mosquae. **3**:17–32.

Fitzinger, L.J.F.J. 1879. Untersuchungen über die Arten der natürlichen Familie der Hirsche (Cervi). Hirsche (Cervi). Sitzungsbericht Konning Akademie Wissenschaften Wien. **79**: 332–362.

Frohlich, J., Kubickova, S., Cernohorska, H., Musilová, P., Muskova, H., and Rubes, J. 2016. A comparative study of pygmy hippopotamus (*Cheropsis liberiensis*) karyotype by cross-species chromosome painting. Journal of Mammalian Evolution. **24**: 465–474. doi:10.1007/s10914-016-9358-5

Frohlich, J., Kubickova, S., Musilova, P., Cernohorska, H., Muskova, H., Vodicka, R., and Rubes, J. 2017. Karyotype relationships among selected deer species and cattle revealed by bovine FISH probes. PLoS ONE e0187559. doi: 10.1371/journal.pone.0187559

Galindo D.J., Martins G.S., Vozdova M., Cernohorska H., Kubickova S., Bernegossi A.M., Kadlcikova D., Rubes J., and Duarte J.M.B. 2021a. Chromosomal polymorphism and speciation: the case of the genus *Mazama* (Cetartiodactyla; Cervidae). Genes **12**(2):165. doi: 10.3390/genes12020165

Gilbert C., Ropiquet A., and Hassanin A. 2006. Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): systematics, morphology, and biogeography. Molecular Phylogenetics and Evolution **40**:101–117.

Gloger, C.W.L. 1841. Gemeinnütziges hand-und Hilfsbuch der Naturgeschichte. Für gebildete Leser aller Stände, besonders für die reifere Jugend und ihre Lehrer. A. Dchulz, Breslau 1:1–495.Goldman, E., and Moore, R.T. The biotic provinces of Mexico. Journal Mammalogy. **26**: 347–360.

Gray, J.E. 1850. Synopsis of the species of deer (Cervina), with the description of a new species in the Gardens of the Society. Proceedings of the Zoological Society of London. **18**:222–242.

Gutiérrez, E.E., Maldonado, J.E., Radosavljevic, A., Molinari, J., Patterson, B.D., Martínez, C.J.M., Rutter, A. R., Hawkins, M. T. R., Garcia, F. J., and Helgen, K. M. 2015. The Taxonomic Status of *Mazama bricenii* and the Significance of the Táchira Depression for Mammalian Endemism in the Cordillera de Mérida, Venezuela. PLoS ONE **10**(6): e0129113.

Gutiérrez, E.E., Helgen, K.M., McDonough, M.M., Bauer, F., Hawkins, M.T.R., Escobedo-Morales, L. A., Patterson, B.D., and Maldonado, J.E. 2017. A gene-tree test of the traditional taxonomy of american deer: The importance of voucher specimens, geographic data, and dense sampling. ZooKeys. **697**: 87–131. doi:10.3897/ zookeys.697.15124

Hassanin, A., Delsuc, F., Ropiquet, A., Hammer, C., van Vuuren, B.J., Matthee, C., Ruiz-Garcia, M., Catzeflis, F., Areskoug, V., Nguyen, T.T., and Couloux, A. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes Rendus – Biologies. **335**: 32–50. doi: 10.1016/j.crvi.2011.11.002

Heckeberg, N.S., Erpenbeck, D., Wörheide, G., and Rössner, G.E. 2016. Systematic relationships of five newly sequenced cervid species PeerJ 4:e2307. doi:10.7717/PEERJ.2307

Heckeberg, N.S. 2020. The systematics of the Cervidae: A total evidence approach. PeerJ. doi:10.7717/peerj.8114

Heckeberg, N.S., Zachos, F. R., and Kierdorf, U. 2023. Antler tine homologies and cervid systematic: A review of past and present controversies with special emphasis on *Elaphurus davidianus. The Anatomical Record*. **306**(1): 5– 28. doi:10.1002/ar.24956.

Hensel, R. 1872. Beiträg zur Kenntniss der Säugethiere Süd-Brasiliens. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlim.

Hershkovitz, P. 1951. Mammals from British Honduras, Mexico, Jamaica and Haiti. Fieldiana-Zoology Chicago Natural History Museum. **31**:547–569.

Hershkovitz, P. 1959. A new species of South American brocket, genus *Mazama* (Cervidae). Proceedings of the Biological Society Washington **72**: 45–54.

Hershkovitz, P. 1966. Field Mus. Nat. Hist., Chicago, XII+861: 567.

Hershkovitz, P. 1982. Neotropical deer (Cervidae). Part I. Pudus, genus *Pudu* Gray. Fieldiana-Zoology news series. **11**: 1–86. doi:10.5962/bhl.title.5080

Howell, W., and Black, D. A. 1980. Controlled silver-staining of nucleous organizer regions with a protective coloidal developer. Experientia. **36**: 1014–1015. doi:10.1007/BF01953855

Huelsenbeck, J., and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics. **17**: 754–755. 5. doi:10.1093/bioinformatics/17.8.754

Illiger, A. 1815. Uberblick der Saughiere nach inhrer Vertheilung uber die welttheile. Akademie der Wissenchaften. 1804–1811.

Kerr, R. 1792. The animal kingdom. *Cervus temama*. . 303.

Kocher, T.D., and White, T.J. 1989. Evolutionary analysis via PCR. *In*: PCR technology. Principles and applications for DNA amplification *Edited* by H. A. Erlich. Stockton Press, New York. pp 137–147.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution. **35**:1547–1549. doi: 10.1093/molbev/msy096.

Levan, A., Fredga, K., and Sandberg, A.A. 1964. Nomenclature for centromeric position of chromosomes. Hereditas. **52**: 201–219. 9. doi:10.1111/j.1601-5223.1964.tb01953.x

Leuckart, F. S. 1816. Dissertatiuncula inauguralis de Equo bisulco Molinre, Facultatis Medicae Gottingensis con sensu pro obtinendis doctoris medicinae honoribus scripsit. Georg-August-Universitat Gottingen, Gottingen, Germany

Linnaeus, C. 1758. Systema natura per regna tria naturae secundum: classes, ordines, genera, species, cum characteribus, differentiis, synonymis locis **1**: 1–824.

Lydekker, R. 1915. Catalogue of the ungulate mammals in the British Museum (Natural History). British Museum, London.

Medellín, R.A., Gardner, A.L., and Aranda, J.M. 1998. The taxonomic status of the yucatán brown brocket, *Mazama pandora* (Mammalia: Cervidae). Proceedings of the Biological Society of Washington. **111**(1): 1–14.

Merino, M.L., and Rossi, R.V. 2010. Origin, Systematics, and Morphological Radiation. *In:* Neotropical Cervidology, Biology and Medicine of Latin American Deer. *Edited by* J.M.B. Duarte and S. Gonzalez S. Funep/IUCN, Jaboticabal/Gland. pp. 2–11.

Merriam, C. H. 1901. Seven new mammals from Mexico, including a new genus of rodents. Proceedings of the Washington Academy of Sciences. **3**: 559–563.

Mantellato, A.M.B., Gonzalez S., and Duarte, J.M.B. 2022. Cytochrome b sequence of the *Mazama americana jucunda* Thomas, 1913 holotype reveals *Mazama bororo* Duarte, 1996 as its junior synonym. Genetics and Molecular Biology **45**(1):e20210093. doi:10.1590/1678-4685- gmb-2019-0008

Peres, P.H. F., Luduvério, D. J., Bernegossi, A.M., Galindo, D.J., Nascimento, G.B., Oliveira, M.L., Sandoval, E.D.P., Vozdova, M., Kubickova, S., Cernohorska, H., and Duarte, J.M.B. 2021. Revalidation of *Mazama rufa* (Illiger 1815) (Artiodactyla: Cervidae) as a Distinct Species out of the Complex *Mazama americana* (Erxleben 1777). Frontiers in Genetics. **12**: 1–18. doi: 10.3389/fgene.2021.742870

Pitra, C., J. Fickel, E. Meijaard and C. P. Groves. 2004. Evolution of old world deer. Molecular Phylogenetic and Evolution. **33**: 880-895.

Posada, D., and Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics. **14**: 817–818. doi:10.3389/fgene.2017.00010

Pucheran, J. 1851. Note sur une espèce nouvelle de Cerf (Cervus rufnus Bourc. et Punch). Revue et Magasin de Zoologie Pure et Appliqueé **2**(3):561–-565.

Rafinesque, C. S. 1817. Descriptions of seven new genera of North American quadrupeds. American Monthly Magazine. **1**: 363–364.

Rafinesque, C. S. 1832. Atlantic Journal and Friend of Knowledge. **1**: 109.

Rambaut, A. 2012. FigTree v1.3.1. Computer program and documentation. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Drummond, A., Dong, X., Baele, G., and Suchard, M. A. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7, *Systematic Biology*. **67**(5): 901–904. doi: 10.1093/sysbio/syy032

Robert, L., Frandsen, P. B., Wright, A. M., Senfeld, T., and Calcott, B. 2017. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses, Molecular Biology and Evolution. **34**(3): 772–773. doi:10.1093/molbev/msw260).

Sambrook, J., Fritsch E., and Maniatis T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Press, 16.

Salviano, M.B., Cursino, M.S., Zanetti, E.D.S., Abril, V.V., and Duarte, J.M.B. 2017. Intraspecific chromosome polymorphisms can lead to reproductive isolation and speciation: an example in red brocket deer (*Mazama americana*). Biology of Reproduction. **96**:1279–1287. doi:10.1093/biolre/iox041

Sandoval, E.D.P., Rola, L.D., Morales-Donoso, J.A., Gallina, S., Reyna-Hurtado, R. and Duarte, J.M.B. 2021. Integrative analysis of *Mazama temama* (Artiodactyla; Cervidae) and designation of a neotype for the species. Journal of Mammalogy. **103**:447–458. doi: 10.1093/jmammal/gyab169

Seabright, M. 1971. A rapid banding technique for human chromosomes. Lancet. **11**: 971-972. doi:10.1016/S0140-6736(71)90287-X

Smith, H.C. 1827. The seventh order of the Mammalia. The Ruminantia. In: Griffith E., Smith C.H., Pidgeon E., (Eds.). The animal kingdom arranged in conformity with its organization, by the Baron Cuvier, with additional descriptions of all the species hitherto named, and many not before noticed, by Edward Griffith and others. Geo B. Whitaker, London. 5:298–376.

Sumner, A.T. 1972. A simple technique of demonstrating centromeric heterochromatin. Experimental Cell Research. **75**: 304–306. doi:10.1016/0014-4827(72) 90558-7

Taber, A., Beck, H., Gonzalez, S., Altrichter, M., Duarte, J.M.B., and Reyna-Hurtado R. 2016. Why neotropical forest ungulates matter, the case for deer, peccary and tapir conservation. *In:* Tropical conservation: perspectives on local and global priorities *Edited by* Aguirre A., Sukumar, R. Oxford University Press. pp. 238–254.

Thomas, O. 1913. The smaller south American cervidae. Ann Mag Nat Hist (Series 8) **11**:587

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research. **25**:4876–4882. doi:10.1093/nar/25.24.4876

Valeri, M.P., Tomazella, I.M., and Duarte, J.M.B. 2018. Intrapopulation chromosomal polymorphism in *Mazama gouazoubira* (Cetartiodactyla; Cervidae): The emergence of a new species? Cytogenetic and Genome Research. **154**: 147–152. 2. doi: 10.1159/000488377

Vargas-Munar, D.S.F., Sarria-Perea, J.A., and Duarte, J.M.B. 2010. Different responses to doxorubicin induced chromosome aberrations in Brazilian deer species. Genetics and Molecular Research. **9**:1545–1549. doi:10.4238/vol9-3gmr822

Verma, R.S., and Babu, A. 1995. Human Chromosomes: Principles and Techniques. New York: McGraw-Hill Inc.

Versiani, N., Pereira, R., and Duarte, J. M. B. 2009. Annual variations in fecal androgen metabolites and antler cycle of captive red brocket bucks (*Mazama americana*) in Southeast Brazil. European Journal of Wildlife Research. **55**(5):535–538

von den Driesch, A. 1976. A guide to the measurement of animal bones from archaeological sites. Harvard University, Peabody Museum of Archaeology and Ethnology, Peabody Museum Bulletin 1.

Vozdova, M., Kubickova, S., Cernohorska, H., Fröhlich, J., Vodicka, R., and Rubes, J. 2019. Comparative study of the bush dog (*Speothos venaticus*) karyotype and analysis of satellite DNA sequences and their chromosome distribution in six species of Canidae. Cytogenetic and Genome Research. **159**: 88–96. doi:10.1159/000503082

Weber, M., and Medellin, R. 2010. Yucatan Brown Brocket Deer *Mazama pandora* (Merriam 1901). *In:* Neotropical Cervidology, Biology and Medicine of Latin American Deer. *Edited by* J.M.B. Duarte and S. Gonzalez S. Funep/IUCN, Jaboticabal/Gland. pp. 166–170.

Weber, M., de Grammont, P.C., and Cuarón, A.D. 2016. *Mazama pandora*. The IUCN Red List of Threatened Species. doi:10.2305/IUCN.UK.2016-2.RLTS.T29622A22154219

Zimmermann, E.A.W. 1780. Geographishe Geischichte des Menschen, und der allgemein verbreiteten vierfüßigen Thiere. Zweiter Band. Enthält ein vollständiges Verzeichniß aller bekannten Quadrupeden. 1–432.

Zurano J.P, Magalhães F.M, Asato A.E, Silva G, Bidau C.J, Mesquita D.O, and Costa G.C 2019. Cetartiodactyla: updating a time-calibrated molecular phylogeny. Molecular Phylogenetics and Evolution **133**:256–262.

## SUPPLEMENTARY DATA

**Table S1.** External body measurements (in mm) of a male *Odocoileus pandora* (Merriam, 1901).

| CHARACTER | T365 | CHARACTER | T365 |
|---|---|---|---|
| **Head length** | 230 | **Right testicle length** | 44 |
| **Head width** | 79 | **Left testicle length** | 22 |
| **Ear length** | 110 | **Right testicle diameter** | 45,5 |
| **Distance between eyes** | 56 | **Left testicle diameter** | 27 |
| **Mandible total length** | 78 | **Metatarsal length** | 220 |
| **Neck circumference** | 340 | **Tail length** | 110 |
| **Thorax circumference** | 600 | **Mass** | 23,5 |
| **Height** | 585 | **Right antlers diameter** | 23 |
| **Metacarpal length** | 140 | **Left antlers diameter** | 22 |
| **Body length** | 820 | **Right antlers length** | 105 |
| **Abdominal circumference** | 740 | **Left antlers length** | 110 |

**Table S2.** Genetic distance of *Odocoileus pandora* (Merriam, 1901) compared to Neotropical genera: *Subulo* (Smith, 1827), *Hippocamelus* Leuckart, 1816, *Ozotoceros* Linnaeus, 1752, *Mazama* Rafinesque, 1817, Blastoceros *Illiger, 1815*, *Odocoileus* Rafinesque, 1832 and *Mazama nemorivaga* species (Cuvier, 1817).

| | ***Subulo*** | ***Hippocamelus*** | ***Ozotoceros*** | ***Nemorivaga*** | ***Blastoceros*** | ***Mazama*** | ***Odocoileus*** |
|---|---|---|---|---|---|---|---|
| ***Subulo*** | | | | | | | |
| ***Hippocamelus*** | 0,07 | | | | | | |
| ***Ozotoceros*** | 0,09 | 0,09 | | | | | |
| ***Nemorivaga*** | 0,10 | 0,11 | 0,11 | | | | |
| ***Blastoceros*** | 0,10 | 0,10 | 0,10 | 0,12 | | | |
| ***Mazama*** | 0,13 | 0,14 | 0,13 | 0,14 | 0,14 | | |
| ***Odocoileus*** | 0,13 | 0,13 | 0,13 | 0,14 | 0,14 | 0,07 | |
| ***Odocoileus pandora*** | 0,13 | 0,14 | 0,14 | 0,14 | 0,14 | 0,08 | 0,07 |

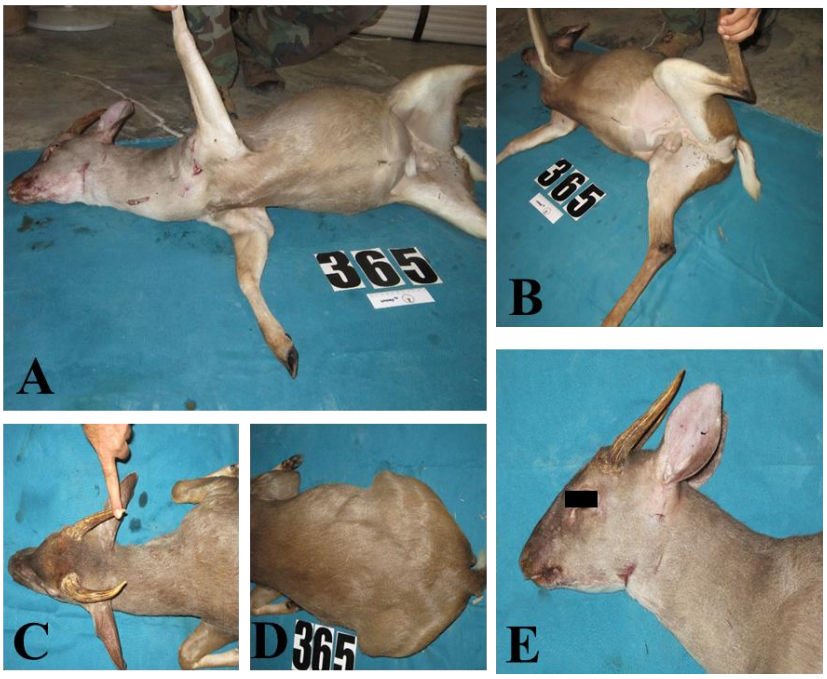



**Fig. S1.** *Odocoileus pandora* (Merriam, 1901) male collected in Yucatan, Mexico. (a) Ventral view of the body. (b) Ventral caudal view of the body. (c) Dorsal cranial view of the body. (d) Caudal dorsal view of the body. (e) Lateral close-up of the head.

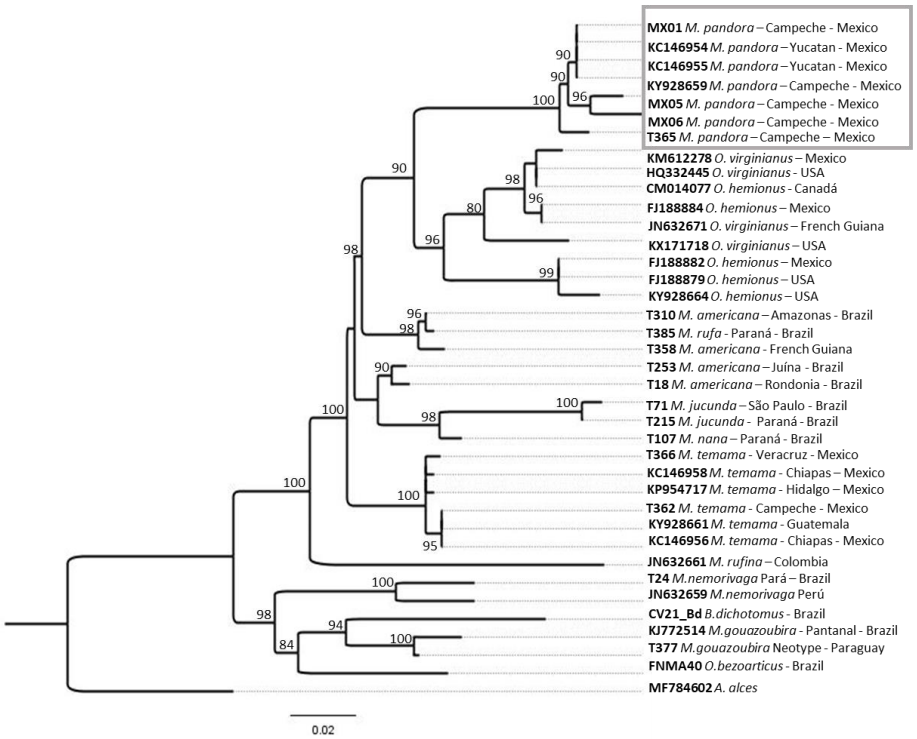



**Fig. S2.** Phylogenetic tree of the Cytb gene. Bayesian Inference Analysis. Gray square: *Odocoileus pandora* (Merriam, 1901) clade. The value above the clades represents the posterior probability of the analysis. Mx01, Mx05, Mx06 and T365: specimens collected in Campeche, Mexico. Outgroup: *Alces alces* Linnaeus, 1758.

## CAPÍTULO 3 – Species-level validation, and a new genus-name to reallocate the gray brocket deer from northwestern Venezuela and neighboring Colombia, *Mazama americana citus* Osgood, 1912 (Artiodactyla: Cervidae)

**Abstract**

The genus *Mazama* is characterized by homoplasic morphological characters, and a high karyotypic diversity. It has been shown to be polyphyletic by several authors, who have allocated the species of the genus to two multigeneric lineages, the subtribes Odocoileina and Blastocerina, of the tribe Odocoileini (New World deer) of the family Cervidae. Within the Blastocerina, gray brocket deer include two nonsister species, viz. *Subulo gouazoubira*, found to the south of the Amazon region, and *M. nemorivaga*, from the Guianas and Amazon region. In this study, we clarify its taxonomic status and phylogenetic position of *Mazama americana citus* Osgood 1912, referred to as either *S. gouazoubira* or *M. nemorivaga* by subsequent authors. We collected a topotype of *M. a. citus* in the eastern shore of Lake Maracaibo (NW Venezuela), describe its morphology and morphometry, characterize it cytogenetically (conventional staining, Ag-NOR, G and C- banding, fluorescence *in situ* hybridization), and include it in a phylogenetic analysis (based on whole mitogenomes and Cytb) along with two additional specimens of *M. americana citus* from northwestern Venezuela. Our analyses reveal the topotype to represent a large gray brocket, with a pale brownish gray coloration, and with a cinnamon band above the eyes. Bayesian inference based on the complete mitochondrion and Cytb recovered *M. a. citus* as the earliest diverging clade of the Blastocerina (100% branch value), thus separated phylogenetically from all other gray brockets. The karyotype of the topotype has a 2n = 61, and a FN = 70. Using an entire set of cattle whole chromosome painting probes, and a BAC X probe, we determined this karyotype to differ in at least 10 rearrangements from the ancestral karyotype with *S. gouazoubira*. Based on these findings, we recognize the Osgood´s gray brocket as a valid species, and discussed that a new generic name should be created to allocate this taxon.

**KEY-WORDS:** Animal cytogenetic, mitochondrial phylogeny, subtribe Blastocerina, taxonomy

## 1. INTRODUCTION

Neotropical brockets are small to medium-sized (13 to 40 kg) deer, with short and unbranched antlers in males, inhabiting forests from southeastern Mexico to northern Argentina (Merino and Rossi, 2010). Traditional taxonomic reviews of the Neotropical deer possessing this morphotype have allocated them to the genus *Mazama* Rafinesque, 1817, thought to include between 4 to 17 species and subspecies (Allen, 1915; Cabrera, 1961; Czernay, 1987).

With the advance of genetic techniques, several authors have shown the genus *Mazama* to be polyphyletic, and its species to be karyotypically diverse. These findings indicate that, owing to high levels of homoplasy, external and cranial morphologies alone are not sufficient to distinguish species and to establish phylogenetic relationships in these deer (Gutiérrez et al. 2017; Heckeberg et al. 2020; Peres et al. 2021; Cifuentes-Rincón et al. 2020).

Phylogenies based on mitochondrial genes have shown species traditionally allocated to *Mazama* to represent at least three genera distributed in the two subtribes, Odocoileina and Blastocerina, of the tribe Odocoileini (New World deer) of the family Cervidae (Gutiérrez et al. 2017; Duarte et al. 2008).

The taxonomy of deer traditionally assigned to the genus *Mazama* is in state of rapid flux. Within the subtribe Odocoileina, red brockets have been deemed to represent the true *Mazama*, with *M. americana* (Erxleben, 1777) as the type species (Gutiérrez et al. 2017; Bernegossi et al. 2022). Also, within this subtribe, *M. bricenii* Thomas, 1908, was synonymized with *M. rufina* Pucheran, 1851 (Gutiérrez et al. 2015); the Yucatan brown brocket deer, formerly *M. pandora* Merriam, 1901, was transferred to the genus *Odocoileus* Rafinesque, 1832 (Gutiérrez et al. 2017); *Mazama rufa* (Illiger, 1815) was validated (Peres et al. 2021); and *M. bororo* Duarte, 1996, was synonymized with *M. jucunda* (Thomas, 1913) (Mantellatto et al. 2022). Within the subtribe Blastocerina, the monophyletic Azara's gray brocket, *M. gouazoubira* (G. Fischer, 1814), has been proposed to belong to the genus *Subul*o Smith, 1823, thus its updated name would be *Subulo gouazoubira* (G. Fischer, 1814); and the Amazonian gray brocket, *Mazama nemorivaga* (Cuvier, 1817) has been proposed to represent another genus within the Blastocerina given that it does not share an exclusive

common ancestor with *S. gouazoubira* (Gutiérrez et al. 2017; Heckeberg et al. 2020). Much is yet to be learnt on the alpha and beta taxonomy of the genus *Mazama*, thus the analysis of taxa described from type localities across the Neotropics needs to be continued.

One subspecies needing clarification of its phylogenetic relationships is *Mazama americana citus* Osgood,1912, whose type locality is “El Panorama, Rio Aurare, eastern shore of Lake Maracaibo, Venezuela. The specific allocation of this putative subspecies was based on the assumption that *Cervus nemorivagus* Cuvier, 1817, is synonym of *Cervus americanus* Erxleben, 1777 (Osgood, 1912; Thomas, 1913; Cabrera, 1961). The description related the specimens as paler and more grayish than those of typical “*M. americana*”, with upper parts grizzled cinnamon gradually paler on the sides and under parts, slightly larger with especially large cheek teeth and minor cranial features, white spot at tarsal gland, on each side of the rhinarium and above each eye (Osgood, 1912).

Contrary to Osgood (1912), Allen (1915) deemed *M. nemorivaga* (Cuvier, 1817) a valid species distinct from *M. americana*. Moreover, he deemed *M. americana citus* a valid species on its own, for which he used the name *Mazama cita*. He divided this species into two subspecies, *M. cita cita*, the form described by Osgood (1912), and *M. cita sanctaemartae*, which he described. Allen (1915) referred to *M. nemorivaga* and *M. americana citus* as the *M. nemorivaga* group, which he differentiated from other congeners based on possession of a short (about 50% of condylobasal length) preorbital portion of the skull. According to Allen (1915), compared to *M. nemorivaga* from the Guianas, *M. americana citus* differs in being slightly larger, in possessing a heavier dentition, and in showing a “very considerably” distinct coloration. Osgood (1912) did not provide etymologies. However, in the same paper he described nine additional species and subspecies of mammals, for which he proposed epithets clearly based on Latin. If *citus* is also based on Latin, it represents the perfect passive participle (verbal adjective) of the verb cieō, and means “put in motion,” or “swift.” In Latin this tense must be masculine, feminine, or neuter. This explains why most subsequent authors have used *cita* instead of *citus* to match gender with the feminine name *Mazama*.

Tate (1939) grouped *Mazama* into two “Divisions,” the first of which (“A”) he distinguished from the second (“B”) based on the possession of elongate and

narrow (as opposed to short and thick) antlers, and large (as opposed to small) teeth; and on geographic distribution, with members of Division “A” (*americana* group, large brockets) occurring in South America and eastern Panama, and members of Division “B” (*M. simplicicornis* (Illiger, 1815) group, small brockets) occurring in South and Central America, and Mexico. Tate (1939) included *M. americana citus* in his Division “A.” Previously, Lydekker (1915) had deemed *M. americana citus* a subspecies of Azara’s gray brocket, i.e., *M. simplicicornis citus*; whereas subsequently, others (Cabrera, 1961; Whitehead, 1994; Pinder and Leewenberg, 1997) deemed it a subspecies of the southern gray brocket, i.e., *M. gouazoubira cita*. In his review of the genus *Mazama* in Venezuela, Bisbal (1991, 1994) applied the name *Mazama gouazoubira* (G. Fischer, 1814) to all the gray or brown brockets from Venezuela, and recognized two subspecies: *M. g. nemorivaga*, occurring south of the Orinoco (Amazonas and Bolívar states); and *M. g. cita*, occurring in northern Venezuela (Zulia and Falcón states, central coastal region).

Based on novel morphological, classical cytogenetic, molecular cytogenetic, and mitogenomic data, the present study aims to resolve the species-level, and generic status within the subtribe Odocoileina, of Osgood’s gray brocket, *Mazama americana citus.*

## 2. MATERIALS AND METHODS

### 2.1 Specimens and samples

We collected an adult male topotype in El Consejo de Ciruma, ca. 35 km ESE from the type locality of *M. americana citus*, in partnership with Instituto Venezolano de Investigaciones Científicas of Venezuela (permission license for collection SISTRA n°20356). The topotype received an identification number NUPECCE (T410) and catalog number CVULA-9109 (CVULA = Colección de Vertebrados de la Universidad de Los Andes, at Mérida, Venezuela). In addition, muscle tissue samples (VEN04 and VEN05) of two gray brocket specimens were collected in the state of Falcon, Venezuela, for molecular analysis.

## 2.2 Morphological characterization

The freshly collected topotype was photographed, and its external body measurements (mm) and body mass (kg) were determined using a pendulum scale, measuring tape and digital caliper 0.05 mm. The following were measured: width and length of the head, ear length, between eyes, mandible, neck circumference, thorax circumference, height at shoulder, metacarpus length, tail length, mass in kilograms, metatarsus length, abdomen circumference, and total body length (Table S1). The skin was removed and treated with a tanning solution to dry the material. Aspects of general coat color, chromogenetic fields of the body (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 tuft in the tarsal region were examined. Additionally, the chromogenetic fields of the head were analyzed (Hershkovitz 1982). A total of 38 standard cranial measurements for cervids (von den Driesch, 1976, see Table S2) were taken. Available skull was photographed at different angles to complement the documentation and description of the specimens.

## 2.3 Statistical analyses

The body and cranial measurements of the collected topotype were compared to those of adult specimens of the gray brockets *M. nemorivaga* and *S. gouazoubira* in the NUPECCE (Museum of Deer and Conservation Center) database (Fig. S1). For each separated matrix, we used the “Paleontological Statistics” PAST program (Hammer et al. 2001) to perform cluster analysis by the Unweighted Pair Group Method with Arithmetic mean method (UPGMA) to measure the similarity between individuals, as well as to discriminate species and populations (Sneath and Sokal, 1973).

## 2.4 Cytogenetic analysis

Skin fragments (5 x 2 cm) from the inguinal region of the topotype 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 (Howell and Black, 1980). The chromosomes were classified as acrocentric, submetacentric, or metacentric according to their arm ratio (Levan et al. 1964) and separated into groups according to their relative lengths (Cifuentes-Rincón et al. 2020). The chromosomes were numbered according to the *S. gouazoubira* (SGO) karyotype published in Bernegossi et al. 2022.

**2.5 FISH**

Fluorescence *in situ* hybridization was performed using cattle whole chromosome painting (WCP) probes on the karyotype of the *Mazama americana citus* topotype and characterized the homologies with *S. gouazoubira* (2n = 70 and NF = 70), which retained the ancestral karyotype of the Cervidae family (Bernegossi et al. 2022). For probe preparation, whole cattle chromosomes were isolated by microdissection in the PALM Microlases system (Carl Zeiss MicroImaging GmbH, Munich, Germany) or by flow cytometry using MoFlo XDP Cell Sorter (Beckman Coulter, USA) as described in Frohlich et al. (2017). Five BAC clones were selected from the CHORI-240 library based on the NCBI ARS-UCD1.2 Assembly data and obtained from BACPAC Genomics, Emeryville, CA, USA (Table S3). For DNA extraction, we use a protocol adapted from the method included in Wizard® 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® Array CGH Genomic Labeling (Invitrogen, Carlsbad, CA, USA).

The selected BAC clones were selected to be distributed along p and q arms of the submetacentric sex chromosome X of cattle. For the pseudoautosomal region (PAR) probe, two BAC clones were selected and used together as a single probe. The amplification was performed using degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) (Telenius et al. 1992) and probe labeling, which was done during the second PCR with Green-dUTP or Orange-dUTP (Abbott Park, IL, USA) (Kubickova et al. 2002). 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.

### 2.6 DNA extraction and sequencing

Genomic DNA extraction from tissue samples of *Mazama cita* topotype was performed using the Qiagen DNeasy® Blood Qiamp Tissue kit following the manufacturer's instructions. Approximately 1 µg of DNA from each specimen was sent to NGS Soluções Genômicas (https://ngsgenomica.com.br) for massive parallel DNA sequencing. First, the DNA samples were fragmented by Covaris sonication to an average size of 300 base pairs. Then, these fragmented DNA samples were used as input for genomic library preparation using the TruSeq Nano DNA sample preparation kit (Illumina, USA) following the manufacturer's instructions. All genomic libraries were pooled and sequenced on the Illumina HiSeq 2500 Platform (Illumina, San Diego, CA, USA), 100 bp paired-end sequencing. Unmerged reads were aligned to the *S. gouazoubira* mitogenome (GenBank accession number KJ772514) as a reference using Bowtie2 v2.4.1 (Langmead and Salzberg, 2012) on the Galaxy online server (Afgan et al. 2018). Annotation was performed using the MITOS (Bernt et al. 2013) web-based platform (http://mitos.bioinf.uni-leipzig.de/index.py) and manually adjusted based on comparisons to the reference mitogenome using GENEIOUS (Biomatters; http://www.geneious.com/). We obtained mitogenome sequences of *S. gouazoubira*, *M. nemorivaga*, *M. americana*, *M. nana*, *M. jucunda*, *M. temama*, *Odocoileus virginianus*, *Pudu puda*, *Blastocerus dichotomus*, *M. rufa*, *O. bezoarticus*, and *Hippocamelus antisensis* from GenBank to perform the phylogenetic analyses, using sequence of *Alces alces* as the outgroup (see Table 1, for details).

We also extracted genomic DNA from hair and muscle samples of five gray brocket deer from northwestern Venezuela identified as *M. americana citus*, using a modified protocol based on the methodology described by Sambrook et al. (1989). The partial cytochrome b (Cytb 1040bp) fragment was amplified, the PCR products were purified with the Wizard SV gel and PCR Clean-Up System

kit (Promega TM), followed by sequencing using the ABI BigDye Terminator kit (Applied Biosystems) in an ABI 3130xl automatic sequencer (Applied Biosystems). Thereafter, we aligned with others sequences available in the GenBank for neotropical deer (Table 1).

### 2.7 Alignment and Bayesian phylogenetic inference

Both datasets, complete mitogenome and Cytb gene, were aligned separated using the Clustal X program (Thompson et al. 1997). For the mitochondrion genome, we excluded the control region from the alignment because of the tandem repeat located inside them and the high incidence of potential homoplasy. The optimal partitioning scheme was selected by the Partitionfinder 2.0 tool (Cognato and Vogler, 2001) at CIPRES Science Gateway (Miller et al. 2010) under the Bayesian information criterion. For the Cytb data set we selected the best molecular evolution model using the jModelTest v. 0.1.1 (Posada and Crandall, 1998), following the corrected Akaike information criterion, AICc (Akaike, 1973).

Bayesian inference (BI) analysis was performed using multiple four chain Metropolis-coupled analysis (with default heating) using MRBAYES 3 (Huelsenbeck and Ronquist, 2001) with 10,000,000 generations, sampling every 1,000 generations, until a variance of <0.01 was obtained. The first 25% of the sampled trees were discarded as burn-in and the remaining trees were used to construct a 50% majorityrule consensus tree. The consensus tree was edited using FigTree v.1.4.0 (Rambaut, 2012).

**Table 1**. Mitochondrial sequences used in the phylogenetic analysis of *Mazama americana citus* Osgood, 1912, topotype and other neotropical cervids.

| SPECIES | MITOGENOME | CYTB | ORIGIN |
|---|---|---|---|
| *Mazama americana citus T410 topotype* | OQ198443 | OQ198443 | El Consejo de Ciruma, Zulia, Venezuela |
| *Mazama americana citus VEN04 (JMA4031)* | | OQ658775 | Paraguana, Falcon, Venezuela |
| *Mazama americana citus VEN05 (JMA4035)* | | OQ658776 | Paraguana, Falcon, Venezuela |
| *Subulo gouazoubira T377* | MZ350858 | MZ350858 | Puerto Galileo, Paraguay |
| *Subulo gouazoubira T082* | MZ350862 | MZ350862 | Camobi-RS, Brazil |
| *Subulo gouazoubira* | KJ772514 | KJ772514 | Pantanal, Brazil |
| *Subulo gouazoubira MRGsp2* | JN632658 | JN632658 | Colombia |
| *Mazama nemorivaga T24* | MZ350861 | MZ350861 | Porto Velho-RO, Brazil |
| *Mazama nemorivaga* | JN632659 | JN632659 | Peru |
| *Mazama nemorivaga* | JN632660 | JN632660 | French Guiana |
| *Mazama nemorivaga* | | KY928656 | Guyana |
| *Mazama nemorivaga* | | KY928858 | Bolívar, Venezuela |
| *Mazama americana MAZ9472* | JN632656 | JN632656 | French Guiana |
| *Mazama americana T358* | MZ350857 | MZ350857 | Cayenne, French Guiana |
| *Mazama americana T253* | MZ350856 | MZ350856 | Juina, Brazil |
| *Mazama americana* | | KY928654 | Yaraguay, Venezuela |
| *Mazama rufa T385* | OQ198444 | | Foz de Iguaçú-PR, Brazil |
| *Mazama nana T107* | MZ350863 | MZ350863 | Paraguay |
| *Mazama jucunda T071* | | DQ789231 | Paraná, Brazil |
| *Mazama jucunda T215* | MZ350859 | MZ350859 | P. E. Intervales-SP, Brazil |
| *Mazama temama T366* | MZ350864 | MZ350864 | Veracruz, México |
| *Mazama temama T362* | MZ362858 | MZ362858 | Campeche, México |
| *Odocoileus virginianus* | KM612278 | | USA |
| *Odocoileus virginianus* | | HQ332445 | USA |
| *Ozotoceros bezoarticus* | JN632681 | | Brazil |
| *Ozotoceros bezoarticus FNMA40* | MZ350860 | MZ350860 | Brazil |
| *Blastocerus dichotomus CV21* | This study | | Brazil |
| *Blastocerus dichotomus* | | JN632603 | Brazil |
| *Hippocamelus antisensis* | JN632646 | | Argentina |
| *Alces alces* | MF784602 | MF784602 | Poland |
| *Pudu puda* | | JN632692 | Unknown M92144-JP_MNHN |

# 3. RESULTS

## 3. 1 Morphological characterization

The topotype of *Mazama americana citus* (Osgood, 1912), has a general grayish brown color, light brown laterally and whitish ventrally. Dorsal of the body, head and neck dark brown, gradually paler in the submandibular region with yellowish hairs. White yellowish nasal patch, presence of a dark lateral rostral band, inferior orbital band with yellow hairs and superior orbital band with yellow pale color projected to rostral band. Whitish color in the base of the ears and in the anterior auricular border, dark brown in the posterior auricular border. Presence of long and white hairs in the inner auricular surface. Brown cinnamon in the outer auricular surface. Mental patch pale brown, grayish mandibular band with yellowish buccal, and gular patch. Presence of tuft with dark brown and yellowish hairs. Opening of preorbital pouch pale brown. Forelegs grayish brown in the proximal lateral region, paler brown in the distal lateral region and whitish in the ventral region. Proximal hindlegs grayish brown in the lateral region and whitish in the ventral region, distal hindlegs dark brown in lateral and ventral region. Absences of white spot at tarsal gland, tail white below, pale cinnamon dorsally. Whitish inguinal region. Short, fine hairs all over the body. Small flattened auditory bulla and two separate lacrimal holes in the orbit. Short and deep supraorbital foramen, deep lacrimal fossa. Preorbital fossa with triangular and elongated shape (Figure 1 and 2).

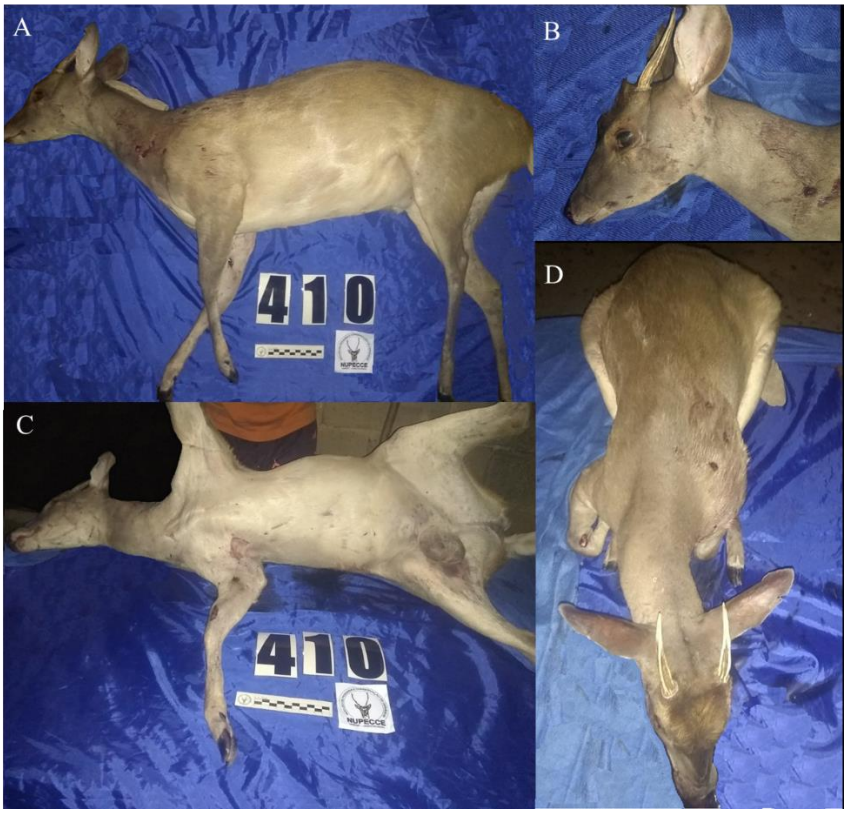



**Figure 1.** Male topotype of *Mazama americana citus* (Osgood, 1912), collected in El Consejo de Ciruma, Zulia, Venezuela. (A) Lateral view of the body (B) Close up of the head. (C) Ventral view of the body. (D) Dorsal view of the body

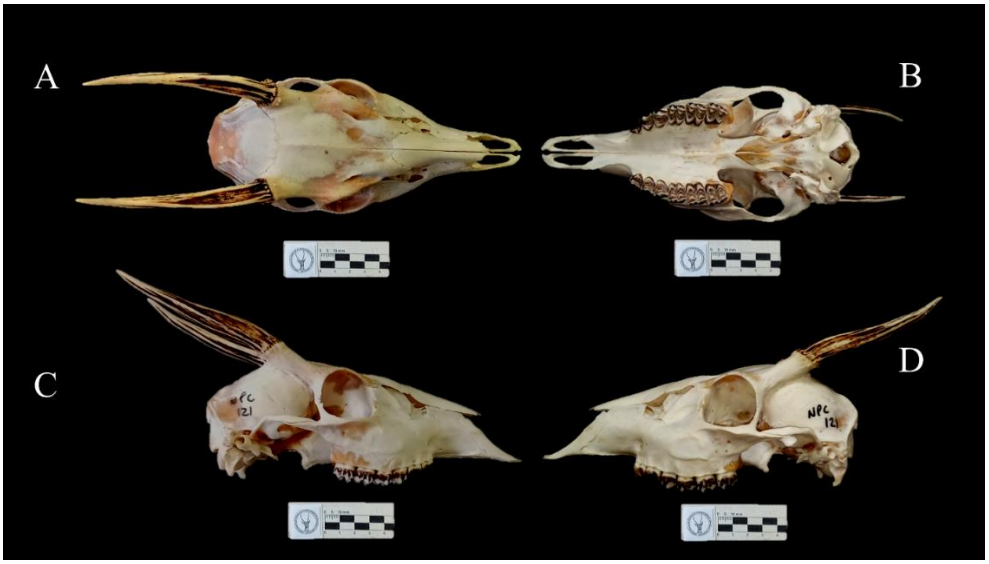



**Figure 2.** Skull of the male topotype of *Mazama americana citus* (Osgood, 1912), collected in El Consejo de Ciruma, Zulia, Venezuela (A) Dorsal view (B) ventral view (C) Right lateral view (D) Left lateral view.

**Record location:** El Consejo de Ciruma, eastern shore of Lake Maracaibo, Zulia state, Venezuela.

**Coordinates:** 10°29’33,3’’N x 71°08’46,6’’ W.

**Specimen deposited in:** Los Andes University Vertebrates Collection (CVULA), Mérida, Venezuela. **Deposit Number.:** CVULA-9109.

**Tissue sample deposited in:** NUPECCE Tissue and Cell banking. TESE 410.

DNA sequence Deposit Number: OQ198443 (complete mitochondrion).

**Karyotype:** 2N=61 /FN= 70.

The topotype of *M. americana citus* has greater body and skull sizes than other gray brockets included in the analysis (Table S2). The body length of the topotype was 850 mm, and the skull length was 189.39 mm, whereas the condylobasal length was 185.60 mm, the basal length 166.29mm, the zygomatic breadth 81.27 mm, and the cheektooth row length 58.16 mm. The UPGMA distance trees (Figure 3) showed the *M. americana citus* topotype separated from specimens of *S. gouazoubira* and *M. nemorivaga*

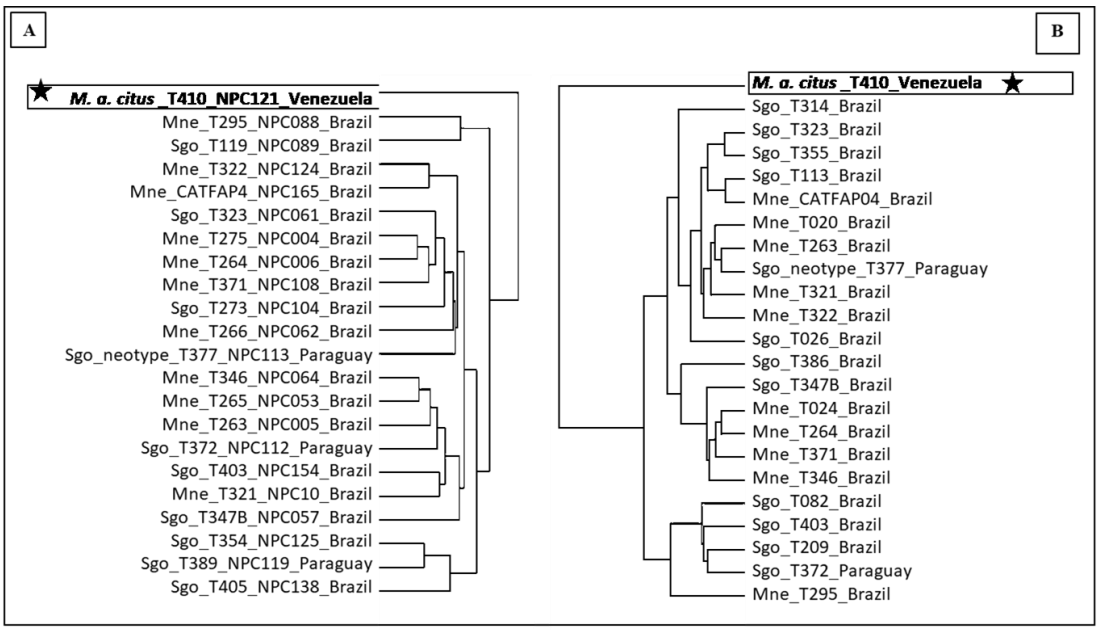



**Figure 3.** UPGMA distance tree of the collected male topotype (star) of *Mazama americana citus* (Osgood, 1912), compared with other brocket deer (A) skull measurement. NPC= catalog number in Nupecce´s Museum (B) body measurement T= voucher number; Mne= *M. nemorivaga*, *Sgo= S. gouazoubira*

### 3.2 Cytogenetic description

The *M. americana citus* (MCI) topotype showed a karyotype with 2n = 61 and FN=70, 29 pairs of autosomal chromosomes and a simple sexual system XY (Figure 4). The X chromosome is a submetacentric chromosome and the Y appeared as a small metacentric. Chromosomal pair MCI1 (large biarmed autosome) was classified in the group A, chromosomal pairs MCI2 to 4 were classified as belonging to group C (small biarmed autosomes) and all acrocentric chromosomes were included into group E. The presence of supernumerary chromosomes (Bs) was observed as variable from 0 to 5 in different metaphases.

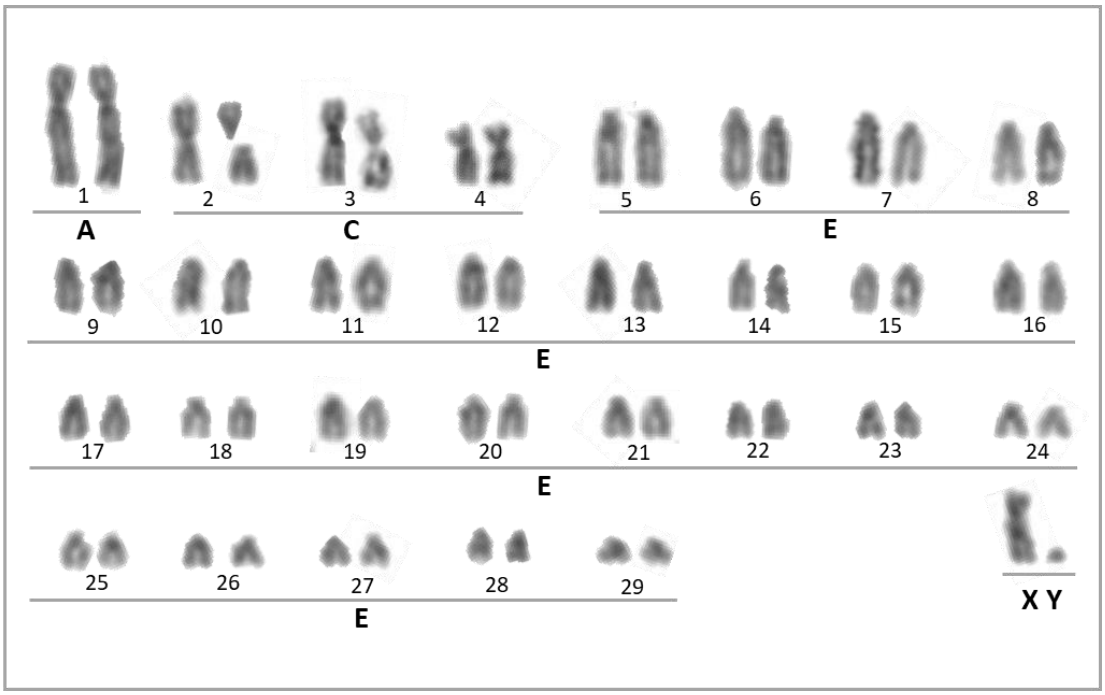



**Figure 4**. Karyotype of a male topotype of *Mazama americana citus* (Osgood, 1912), under conventional Giemsa staining.

The Ag-NOR staining showed nucleolus organizing regions in the telomeric area of the long arms of the two chromosomes of pair 1, and in the telomeric region of the long arms of the two chromosomes of pair 7. C-banding showed constitutive heterochromatin blocks in the pericentromeric region of all autosomes and X (Figure 5).

The bovine WCP chromosomal painting demonstrated that *M. americana citus* (MCI) (2n = 61 and FN=70) underwent chromosomal rearrangements in comparison with the hypothetical ancestral deer karyotype retained by *Subulo gouazoubira* (2n =70 and FN=70). The q-arm of the large submetacentric MCI1

is the result of tandem fusion of the chromosomes corresponding to SGO21 and SGO1 (BTA5 proximal region and BTA3). The p-arm of the MCI1 corresponding to SGO19 (BTA 17) was fused by centric fusion with the q-arm. The MCI2 was heterozygotic for a centric fusion involving SGO15 (BTA19) and SGO7 (BTA7) (Figure 5). We also observed an homozygotic centric fusions in MCI3 corresponded to SGO 14/11 (BTA21/16) and homozygotic centric fusion in MCI4 corresponded to SGO 32/12 (BTA25/13), that formed the largest chromosomes in group C (Figure 5). The X chromosome of the *M. americana citus* topotype showed a submetacentric morphology with a centromeric shift when compared with the acrocentric X of *S. gouazoubira*, in which the proximal region of SGO X was inverted in the p arm of the MCI X as showed by the hybridization pattern of BAC clones. The q arm preserved the same hybridization pattern as those shown for the distal region of the X chromosome in SGO. Figure 5.

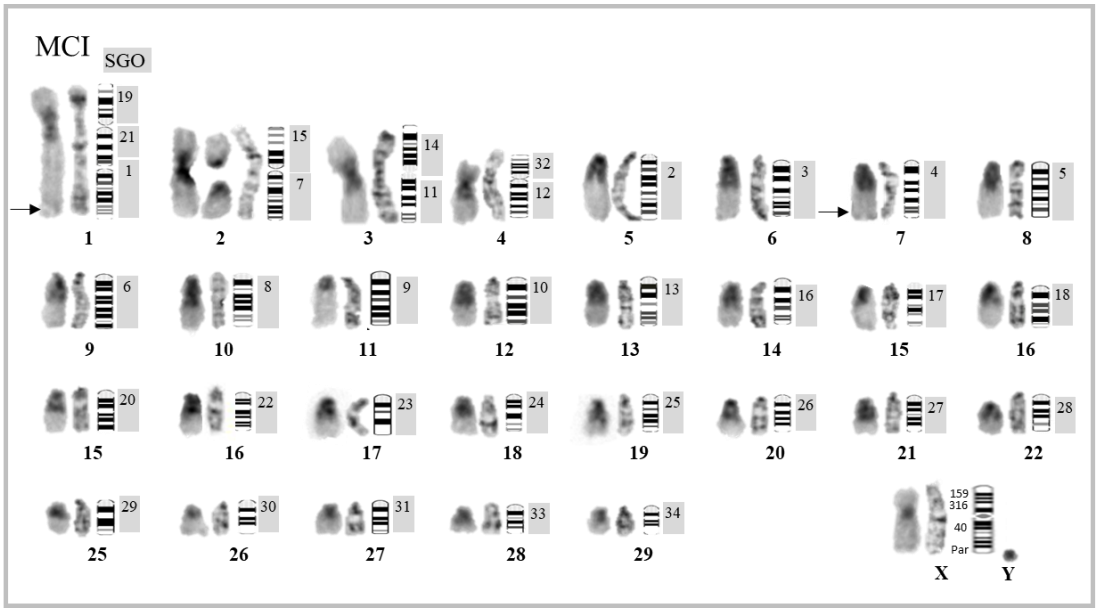



**Figure 5.** Karyotype of a male topotype of *Mazama americana citus* (Osgood, 1912) (MCI), and homologies to *Subulo gouazoubira* (SGO). Left to right: C-band, G-band, schematic representation of G-band of MCI and homology with *Subulo gouazoubira* SGO (Bernegossi et al. 2022 adapted). Localization of Ag-NOR staining is indicated by arrows. Positions of bovine BAC probes on the MCI X chromosome are shown.

### 3.3 Phylogenetic analysis

The BI tree of the mitogenomes (Figure 6B) showed the species *M. americana*, *M. rufa*, *M. nana*, *M. jucunda*, *M. temama*, and *O. virginianus,* composed the great clade of Odocoileina subtribe with 100% posterior probability. The topotype of *M. americana citus* was recovered within the Blastocerina subtribe with 100% of posterior probability as sister taxon of the other species of the subtribe, followed by *P. puda*. Also, in the Blastocerina clade, *M. nemorivaga* was recovered as a monophyletic species with *B. dichotomus* as its sister taxon, separated from the clade with a monophyletic *O. bezoarticus*, and from the clade of *S. gouazoubira* and *H. antisensis* as sister taxa. A similar result (two main clades) was obtained with the partial Cytb BI tree (Figure 6A). The first corresponds to the Odocoileina subtribe (100% pp) and includes *M. americana* sensu lato, *M. rufa*, *O. virginianus*, *M. nana*, *M. jucunda* and *M. temama*. The other corresponds to the Blastocerina subtribe, *M. nemorivaga*, *S. gouazoubira*, *O. bezoarticus*, *B. dichotomus* and *M. americana citus*. This clade was divided in tree subclades, the monophyletic *M. nemorivaga* subclade (100% pp), separated from the monophyletic *S. gouazoubira* subclade (97% pp) with *B. dichotomus* as sister taxa, and the *M. americana citus* subclade (100% pp).

The topotype of *M. americana citus* and other specimens from the northwestern Venezuelan states of Zulia and Falcon were the first branch that diverged within the Blastocerina subtribe in a separated monophyletic clade with 100% posterior probability. Within *M. americana citus* clade, the individuals from Paraguana Pensinsula (Falcón state) are more closely related to each other than to the individuals from Zulia state. The *M. nemorivaga* clade split into two clades with a strong support, one formed by the gray brocket individuals from Bolivar state in Venezuelan south of the Orinoco, French Guiana and Guyana (98%pp), separated from the other clade, that recovered the specimens from Peru and Brazil with 100% pp.

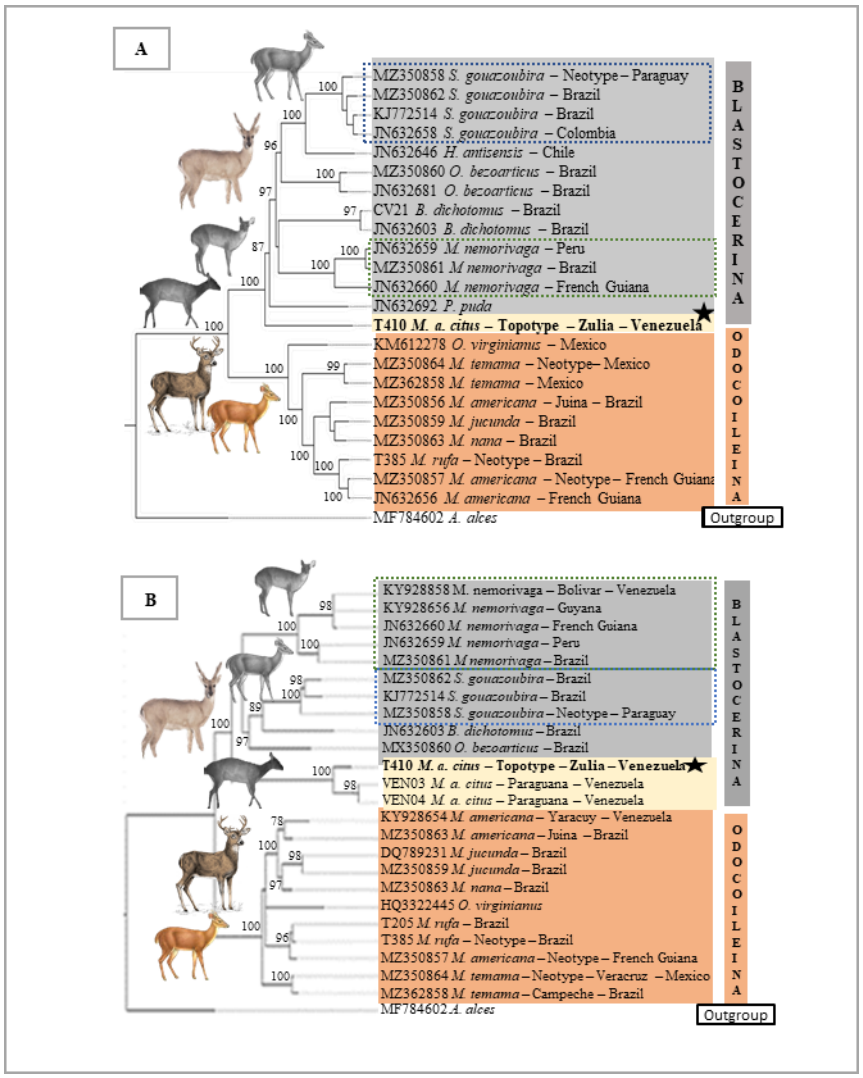



**Figure 6.** Bayesian Inference (BI) of the mtDNA from several species of Neotropical deer (A) Complete mitochondrion; (B) partial Cytb 1040bp. The values above the clades represents the posterior probability of the analysis. Clade markers: *Mazama americana citus* (gray square) and collected topotype (star). Outgroup: *A. alces*

## 4. DISCUSSION

### 4.1 Morphological characterization

The description of the gray brocket deer from Venezuela has been based on morphological features noticed as unique for the type series of *M. americana citus* (Osgood, 1912) when compared with other gray brockets. The pale brownish and yellowish gray coloration of the topotype matches previous descriptions of *M. americana citus*, with the only exception of absence of a white

spot on the tarsal gland, described by Osgood for the holotype (1912). However, this character has been reported to be a variable in other brocket species, such as *S. gouazoubira* (Duarte and Jorge 1998; Rossi 2000; Bernegossi et al. 2022). The general coloration of *M. americana citus*, together with the yellowish spot over its eyes and the white color around the muzzle have been proposed to be useful characters to distinguish *M. americana citus* from other gray brockets (Allen, 1915). However, other authors have considered these characters to be variable, arguing that body and skull size are more important than coloration to differentiate the species (Lydekker 1915; Bisbal 1991). The body and skull measurements of our topotype are close to those published for the gray brockets from northern Venezuela (Osgood, 1912; Allen, 1915; Bisbal, 1991), specifically, the greatest length of skull, greatest nasal length, maxillary toothrow, zygomatic broad, width of head, height at shoulder, and chest circumference.

Our morphological analyses show that our topotype of *M. americana citus* does not group with small gray brocket species. Most of measurements are greater in the topotype than in other gray brockets. Owing to the availability for morphometric analyses of a single specimen of *M. americana citus*, we could not test statistically the difference. However, a similar result was presented by Bisbal (1991), who despite deeming "*M. gouazoubira nemorivaga*" and "*M. gouazoubira cita*" morphometrically indistinguishable, provided measurement ranges indicating that cranially the latter is greater than the former. Our measurements, considered together with Osgood's (1912) and Bisbal's (1991) measurements, suggest that *M. americana citus* is indeed larger than *M. nemorivaga* and *S. gouazoubira*, both in body and in skull size. Tate (1939) had already assumed that this is the case by assigning *M. americana citus* to his large-brocket group. A descriptive study of specimens from localities across northern Venezuela is needed to provide a more definitive morphometric diagnosis of *M. americana citus*.

### 4.2 Cytogenetic description

The cytogenetic characteristic of the gray brocket species *S. gouazoubira* and *M. nemorivaga* had been reported by many authors (Valeri et al. 2018; Fiorillo et al. 2013; Bernegossi et al. 2022). Neitzel (1979) described the first karyotype

of *S. gouazoubira* with 2n and FN equal to 70, that was recently corroborated from current topotypes with a difference of 2n = 69 and FN = 70 due to the presence of a centric fusion in a heterozygous state (Bernegossi et al. 2022). Karyotypical studies with *M. nemorivaga* from Brazil showed a 2n range from 67 to 69 and NF = 70, with a submetacentric X sexual chromosome unlike the acrocentric X chromosome of *S. gouazoubira* (Fiorillo et al. 2013). In contrast, our cytogenetical result of the gray brocket topotype collected in Venezuela presented 2n = 61 and NF = 70, with two submetacentric pairs, one metacentric pair, one heterozygotic fusion and 25 acrocentric pairs. We observed a simple sexual system, with submetacentric X and metacentric Y chromosomes.

Comparing to the current karyotype of *S. gouazoubira*, species that retains the hypothetical ancestral karyotype of cervids (Neitzel et al. 1979), the fluorescence *in situ* hybridization FISH showed that *M. americana citus* topotype shared the fusion between autosomes 7 and 15 in heterozygosis that was reported in the *S. gouazoubira* specimens (Bernegossi et al. 2022). Otherwise, the topotype´s karyotype differed from *S. gouazoubira* by tree centric fusions and one tandem fusion in the autosome chromosomes. The X chromosome also differed by the submetacentric morphology in comparison with the acrocentric X of *S. gouazoubira*. In contrast, the X chromosome in *M. americana citus* had similarity to the X chromosome of *Capreolus capreolus*, with the same order and position of each X probe used in the proximal region and in the qproximal and qdistal regions (Frohlich et al. 2017). These karyotypic differences are efficient reproductive barriers that could potentially isolate sympatric populations (Cursino et al. 2014; Salviano et al. 2017; Galindo et al. 2022). Therefore, we confirm through cytogenetic, that *M. americana citus* has a distinctive karyotype from *S. gouazoubira* (Bernegossi et al. 2022). Moreover, we observed that the differentiated diploid number is unique for the taxon, when compared with cytogenetics studies in other brockets as *M. nemorivaga*, *M. temama, M rufa* and *M. americana* (Fiorillo et al. 2013; Cifuentes-Rincón et al. 2020; Peres et al. 2021; Sandoval et al. 2022).

### 4.3 Phylogenetic analysis

Mitochondrial DNA has been widely used to reconstruct the phylogenetic relationships among Neotropical deer (Hassanin et al. 2012; Caparroz et al. 2015; Bernegossi et al. 2022). Studies with separated gene cytochrome b and complete mitogenome had proven the efficiency in recovery this relationship and enhance the understanding of the evolutionary history of this group (Gutiérrez et al. 2017; Heckeberg et al. 2020). Our BI analyses of mtDNA confirmed previous studies about the polyphyly of the genus *Mazama* and reinforced the need of designating new generic names for the *Mazama* species recovered within Blastocerina subtribe (Bernegossi et al. 2022; Duarte et al. 2008; Gutiérrez et al. 2017).

In both trees of Cytb and complete mitogenome, the Osgood´s gray brocket topotype was recovered as monophyletic within Blastocerina. Despite the limited sample size of mitogenome sequences available for Neotropical deer, *M. americana citus* was recovered as a lineage that diverged early within Blastocerina subtribe. Even Cytb tree with more *M. americana citus* sequences from different localities confirmed this relationship in which the gray brocket specimens from northwestern Venezuela formed a monophyletic clade separated of both the *M. nemorivaga* and the genus *Subulo*. We found *M. americana citus* to be a separate species that does not share exclusive common ancestors with the other gray brockets, *M. nemorivaga* (type locality Cayenne, French Guiana) and *Subulo gouazoubira* (type locality Asunción, Paraguay) (Gutiérrez et al. 2017; Bernegossi et al. 2022). Furthermore, it confirms that *M. americana citus* is a valid taxonomic unit that should be recognize as formal species with a separated genus name within Blastocerina subtribe.

In the Cytb analysis, the two *M. americana citus* from Paraguana Peninsula were close related relative to the topotype. In this context, the possibility of an isolated gray brocket population in the Paraguana peninsula was reported by Bisbal (1991), who considered morphological particularities of one individual from this locality when compared with specimens *M. americana citus* and *M. nemorivaga*. The author mentioned the low sample quantity accessed as a limiting factor to discuss the hypothesis of variability and speciation process of this population (Bisbal, 1991). Therefore, as noticed in other neotropical deer studies (Bernegossi et al. 2022, Peres et al. 2021), only an integrative review

including a multi-loci analysis with more samples from this locality and other areas of occurrence in Venezuela and northern South America, may confirm the hypothesis of species´ distribution limits.

Finally, the phylogenetic results for our single Cytb sequence from Venezuela south of the Orinoco (Bolívar state) is consistent with the hypothesis that Venezuelan, Guyanese, and French Guianan *M. nemorivaga* form a monophyletic clade separated from Brazilian and Peruvian *M. nemorivaga* (see Gutiérrez et al. 2017). This confirms the presence of at least of two gray brocket species, *M. americana citus* and *M. nemorivaga*, in Venezuela.

### 4.4 Species-level validation of Osgood's gray brocket:

Taxonomic reviews based on integrative approaches have been advocated in the literature as an effective way of taxa designation (Fujita et al. 2012). The *Mazama americana citus* topotype was accessed following the description of Osgood (1912) from specimens collected in the eastern shore of Lake Maracaibo in Venezuela, at 40km near the Aurare river. The morphology of the topotype was in concordance with the description of Osgood (1912) and Allen (1915) referred to a gray brocket, paler brown and grayish than other gray brockets described previously by author as *M. nemorivaga* (Cuvier, 1817), and *S. gouazoubira* (G. Fischer, 1814). The morphometry analysis revealed the *M. cita* topotype as a large brocket separated of the small gray brockets group formed by *M. nemorivaga* and *S. gouazoubira*. Cytogenetic assessment of *M. americana citus* revealed an unique karyotype with 2n = 61 and NF = 70, simple sexual system with submetacentric X and exclusive chromosomal rearrangement to form the pairs 1 to 4 when compared with the hypothetical ancestral karyotype of *S. gouazoubira*. Mitochondrial phylogenomic allocated the topotype within Blastocerina separated from the *M. nemorivaga* and *S. gouazoubira* clade, also separated from other Neotropical deer of the genus *Hippocamelus*, *Pudu*, *Ozotoceros* and *Blastocerus*. Performing partial Cytb phylogenetic tree with more *M. americana citus* samples from Venezuela, revealed a monophyletic clade with high posterior probability confirming the separation from other gray deer species. Thus, the morphological, cytogenetic and phylogenetic results complement the

description of the Osgood´s gray brocket and support the recognition as a valid species within Blastocerina subtribe.

### 4.5 A new genus of Neotropical deer:

Mitochondrial phylogenies indicate that the genus *Mazama*, as traditionally defined, is polyphyletic since it includes at least three unrelated clades (Duarte et al. 2008; Heckeberg et al. 2020; Gutiérrez et al. 2017). This has led to a revised taxonomy, according to which the application of the genus name is restricted to the brocket deer of the clade that includes the type species, *M. americana*, in the subtribe Odocoileina (Gutierrez et al. 2017; Bernegossi et al. 2022). As a consequence of this new taxonomic paradigm, it has become necessary to reassign members of the subtribe Blastocerina to other genera, as has been amply discussed by several authors (Duarte et al. 2008; Gutiérrez et al. 2017; Bernegossi et al. 2022). In this context, with the support of further genetic analyses, *M. gouazoubira* has been transferred to the resurrected genus *Subulo* Smith, 1827 (Bernegossi et al. 2022). Because the Amazonian brown brocket, *Mazama nemorivaga*, does not share a common ancestor with *Subulo*, it also needs a different generic name (Gutiérrez et al. 2017; Heckeberg et al. 2020; Bernegossi et al. 2022). For the latter species, *Passalites* Gloger, 1941 has been proposed for designation (Gutiérrez et al. 2017); indeed Jasper et al. (2022) list *Cervus nemorivagus* Cuvier, 1817 as the type species of *Passalites* by monotypy.

The subtribe Blastocerina also includes the genera *Hippocamelus*, *Blastocerus*, *Ozotoceros*, and *Pudu*. The recovery in our phylogeny of *M. americana citus* as the first lineage to have diverged within the Blastocerina clade indicates that this species cannot be placed in the brocket deer genus *Subulo*, or in any of the other of the genera mentioned above. Four of the existing generic names available for *Mazama* (*Homelaphus* Gray, 1872; *Doryceros* Fitzinger, 1873; *Nanelaphus* Fitzinger, 1873; *Coassus* Gray, 1843) could not have been applied to the unique lineage represented by the gray brockets from northern Venezuela because they were proposed long before any material of *M. americana citus* was known to science. The genus name *Mazama* and *Homelaphus* are associated with *M. americana* (Jasper et al. 2022). As we have noted, *M. americana citus* has been deemed a synonym or subspecies of *M.*

*nemorivaga* or *S. gouazoubira* (Cabrera, 1961; Whitehead, 1994; Hummelink, 1940). The genus names *Doryceros* (= *Doratoceros*), *Nanelaphus*, and *Coassus* are associated with the latter two species *(*Bernegossi et al. 2022), none of which share an exclusive common ancestor with *M. americana citus* in our phylogeny. Therefore, *M. americana citus* needs to be reallocated to a new monotypic genus.

## 5. CONCLUSION

In the present study, we provide a morphometric, cytogenetic and phylogenetic evidences that support *Mazama americana citus* Osgood, 1912, topotype as a monophyletic lineage within Blastocerina subtribe separated from other Neotropical deer, specially from other brocket deer species. Thus, we recognized the validity of this species and discussed the need of a new genus-name proposal for this taxon. Our description should be followed by a more complete review of the species applying the criteria of Peres et al. (2021). In this context, it is necessary to carry out a comprehensive molecular (including nuclear markers and a population approach) and cytogenetic characterization of more specimens of *Mazama americana citus* Osgood, 1912 (preferably including the male holotype and the female paratype) from throughout the range of the species in Venezuela (including the Paraguaná Peninsula) and Colombia.

## 6. REFERENCES

Allen J.A. (1915). Notes on American deer of the genus *Mazama.* Bulletin of the **American Museum of Natural History** 34:521–553.

Afgan E., Baker D., Batut B., van den Beek M., Bouvier D., Cech M., Chilton J., Clements D., Coraor N., Grüning B.A., et al. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: update. **Nucleic Acids Research** 46:537–544.

Akaike H. (1973). Information theory and an extension of the maximum likelihood principle. In: Petrov B.N, Csaki F., editors. **Second international symposium on information theory.** Akadémiai Kiadó, Budapest; 26715281

Bernegossi A, M., Borges C., Sandoval E. D. P., Cernohorska H., Svatava K., Vozdova, M., Caparroz R., González S., Duarte, J. M. B. (2022). Resurrection of the genus *Subulo* Smith, 1827 for the gray brocket deer, with designation of a neotype. **Journal of Mammalogy.** doi: 10.1093/jmammal/gyac068

Bernt M., Donath A., Jühling F., Externbrink F., Florentz C., Fritzsch G., Pütz J., Middendorf M., Stadler P.F. (2013). MITOS: Improved de novo Metazoan Mitochondrial Genome Annotation. **Molecular Phylogenetics and Evolution** 69(2):313–319.

Bisbal F. (1991). Distribución y taxonomía del venado matacán (*Mazama* sp.) en Venezuela. **Acta Biológica Venezuélica** 13:89–104.

Bisbal F. (1994). Biología poblacional del venado matacán (*Mazama* spp.) (Artiodactyla: Cervidae) en Venezuela. **Revista Biológica Tropical** 42(112):305–313.

Cabrera A. (1961). Catálogo de los mamíferos de America der Sur. Revista del Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”. **Zoologia** 4(2):309–732.

Cifuentes-Rincón A., Morales-Donoso J.A., Sandoval E.D.P., Tomazella I.M., Mantellatto A.M.B., de Thoisy B., Duarte J.M.B. (2020). Designation of a neotype for *Mazama americana* (Artiodactyla, Cervidae) reveals a cryptic new complex of brocket deer species. **ZooKeys** 958:143–164.

Caparroz R., Mantellatto A M.B., Bertioli D.J., Figueiredo M. G., Duarte J.M.B. (2015). Characterization of the complete mitochondrial genome and a set of polymorphic microsatellite markers through next-generation sequencing for the brown brocket deer *Mazama gouazoubira*. **Genetics and Molecular Biology** 38(3):338–345.

Cognato A.I., Vogler A.P. (2001). Exploring data interaction and nucleotide alignment in a multiple gene analysis of Ips (Coleoptera: Scolytinae). **Systematic Biology** 50(6):758–80.

Cuvier G.F. (1817). Dictionnaire des Sciences Naturelles. Zoologie. Mammiferes. F.G. Levrault., Paris.

Czernay S. (1987). Die Spiesshirsche und Pudus: die Gattungen *Mazama* und *Pudu* A. Ziemsen, Wittenberg Lutherstadt. Die NeueBrehm-BuchereiHeft. V. 581, 84p.

Cursino M., Salviano M., Abril V., Zanetti E. D., Duarte, J. M. (2014). The Role of Chromosome Variation in the Speciation of the Red Brocket Deer Complex: The Study of Reproductive Isolation in Females. **BMC Evolotionary Biology.** 14: 40.

Duarte J.M.B., Jorge W. (1998). Análise citotaxonômica dos *Mazama* cinzas do Brasil (*Mazama gouazoubira* e *Mazama rondoni*). In: Duarte, J. M. B., (Eds.). **Análise citogenética e taxonômica do gênero *Mazama* (Cervidae; Artiodactyla) no Brasil**. Ph.D. Dissertation, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil

Duarte J.M.B., González S., Maldonado J.E. (2008). The surprising evolutionary history of South American deer. **Molecular Phylogenetics and Evolution** 49:17–22.

Duarte J.M.B., Boer J.A., Sandoval E.D.P., Bernegossi A.M., Tomazella I.M. (2021). Skin Freezing Technique for Living Cell Bank. GenProtocols. Available from https://genprotocols.genengnews.com/protocols/skin-freezing-technique-for-living-cell-bank/1041

Erxleben J.C.P. (1777). Systema regni animalis per classes, ordines, genera, species, varietates com synonymia et history animalium, Classis 1. Mammalia. Imprensis Weygandianis.

Fiorillo B.F., Sarria-Perea J.A., Abril V.V., Duarte J.M.B. (2013). Cytogenetic description of the Amazonian brown brocket *Mazama nemorivaga* (Artiodactyla, Cervidae). **Comparative cytogenetics** 7(1):25–31.

Fischer G. (1814). Zoognosia Tabulis Synopticis Illustrata. Typis Nicolai Sergeidis Vsevolozky. Moscow.

Fitzinger L.J.F.J. 1873. Die Gattungen der Familie der Hirsche (Cervi) nach ihrer Natürlichen Verwandtschaft. Sitzungsbericht Konning Akademie Wissenschaften Wien 68:332–362.

Frohlich J., Kubickova S., Musilova P., Cernohorska H., Muskova H., Vodicka R., Rubes J. (2017). Karyotype relationships among selected deer species and cattle revealed by bovine FISH probes. **PLOSone** 12(11): e0187559.

Fujita M. K., Leaché A. D., Burbrink F. T., McGuire J. A., Moritz C. (2012). Coalescent-based Species Delimitation in an Integrative Taxonomy. **Trends in Ecology and Evolutio**n 27: 480–488.

Galindo D.J., Martins G.S., Vozdova M., Cernohorska H., Kubickova S., Bernegossi A.M., Kadlcikova D., Rubes J., Duarte J.M.B. (2021). Chromosomal Polymorphism and Speciation: The Case of the Genus *Mazama* (Cetartiodactyla; Cervidae). **Genes** 12(2):165.

Gray J.E. 1843. List of the specimens of Mammalia in the collection of the British Museum. British Museum, London, United Kingdom.

Grubb, P. (2005). Order Artiodactyla. In: Wilson D.E, Reeder, D. M. (Eds.) **Mammal species of the world: a taxonomic and geographic reference**. Johns Hopkins University Press. Baltimore. pp: 637–722

Gutiérrez E.E., Maldonado J.E., Radosavljevic A., Molinari J., Patterson B.D., Martínez C. J.M., et al. (2015). The Taxonomic Status of *Mazama bricenii* and the Significance of the Táchira Depression for Mammalian Endemism in the Cordillera de Mérida, Venezuela. **PloS ONE** 10(6): e0129113.

Gutiérrez E.E., Helgen K.M., McDonough M.M., Bauer F., Hawkins M.T.R., Escobedo-Morales L.A., Patterson B.D., Maldonado J.E. (2017). A gene-tree test of the traditional taxonomy of American deer: The importance of voucher specimens, geographic data, and dense sampling. **ZooKeys** 697:87–131.

Hammer O., Harper D.A.T., Ryan P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. **Palaeontologia electronica** 4(1):9.

Hassanin A., Pasquet E., Vignes J.D. (1998). Molecular systematics of the subfamily Caprinae (Artiodactyla, Bovidae) as determined from cytochrome b sequences. **Journal of Mammalian Evolution** 5:217–236.

Hassanin A., Delsuc F., Ropiquet A., Hammer C., van Vuuren B.J., Matthee C., Ruiz-Garcia M., Catzeflis F., Areskoug V., Nguyen T.T., Couloux A. (2012). Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes Rendus. **Biologies** 335:32–50.

Heckeberg N.S. (2020). The systematics of the Cervidae: A total evidence approach. **PeerJ** 8:e8114.

Hershkovitz P. (1982). Neotropical deer (Cervidae). Part 1. Pudus, genus *Pudu* Gray. **Fieldiana Zoology, New Series** 11:1–86.

Howell W., Black D. A. (1980). Controlled silver-staining of nucleous organizer regions with a protective 79ervidae79 developer. **Experientia** 36:1014–1015.

Huelsenbeck J., Ronquist F. (2001). MrBayes: Bayesian inference of phylogenetic trees. **Bioinformatics** 17:754–755

Illiger J.K.W. (1815). Uebelblick der Säugethiere nach ihrer Vertheilung über die Welttheile. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlim 39–159.

Jasper J.G., Lee Jr. T.E., Zabel C.J., Twohy CL, Lane K.K., Robertson C.S. (2022). *Mazama rufina* (Artiodactyla: Cervidae). **Mammalian Species**, 54(1016), 212–219.

Kubickova S., Cernohorska H., Musilova P., Rubes J. (2002). The use of laser microdissection for the preparation of chromosome-specific painting probes in farm animals. **Chromosome Research** 46:143–147.

Langmead B., Salzberg S. (2012). Fast gapped-read alignment with Bowtie2. **Nature Methods** 9:357–359.

Levan A., Fredga K., Sandberg A. (1964). Nomenclature for centromeric position of chromosomes. **Hereditas** 52:201–219.

Lydekker R. (1915). Catalogue of the ungulate mammals in the British Museum (Natural History). British Museum, London.

Merino M.L., Rossi R.V. (2010). Origin, Systematics, and Morphological Radiation. *In:* J.M.B. Duarte and S. Gonzalez S. (Eds.) **Neotropical Cervidology, Biology and Medicine of Latin American Deer.** Funep/IUCN, Jaboticabal/Gland. pp. 2–11.

Miller M.A., Pfeiffer W., Schwartz T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway **Computing Environments Workshop** (GCE) 1–8.

Merriam C. H. (1901). Seven new mammals from Mexico, including a new genus of rodents. **Proceedings of the Washington Academy of Sciences** 3: 559–563.

Neitzel H. (1987). Chromosome evolution of Cervidae: karyotypic and molecular aspects. In: Obe G., Basler A., editors. **Cytogenetics, Basic and applied aspects.** Springer Verlag. Berlin; p. 90–112.

Osgood W. H. (1912). **Field Mus. Nat. Hist., Zool.,** X, p. 43, Jan. 10.

Peres P.H. F., Luduvério D. J., Bernegossi A.M., Galindo D.J., Nascimento G.B., Oliveira M.L., Sandoval E.D.P., Vozdova M., Kubickova S., Cernohorska H., Duarte J.M.B. (2021). Revalidation of *Mazama rufa* (Illiger 1815) (Artiodactyla: Cervidae) as a Distinct Species out of the Complex *Mazama americana* (Erxleben 1777). **Frontiers in Genetics** 12: 1–18. Doi: 10.3389/fgene.2021.742870

Pinder L., Leeuwenberg F. (1997). Veado-catingueiro (*Mazama gouazoubira*, Fisher 1814). In: Duarte J.M.B., editor. Biologia e conservação de cervídeos sul-americanos: *Blastocerus*, *Ozotoceros* e *Mazama.* Funep, Jaboticabal; p. 60–68.

Pucheran J. (1851). Note sur une espèce nouvelle de Cerf (Cervus rufinus Bourc. Et Puch). **Revue et Magasine de Zoologie 2**(III): 561–565.

Posada D., Crandall K.A. (1998). Modeltest: testing the modelo f DNA substitution. **Bioinformatics** 14:817–818.

Rafinesque G.S. (1817). No title. **American Monthly Magazine** 1:363–364.

Rambaut A. (2012). FigTree version 1.4.0. Computer program and documentation. University of Oxford. United Kingdom. http://tree.bio.ed.ac.uk/software/figtree/

Rossi R.V. (2000). **Taxonomia de *Mazama* Rafinesque, 1817 do Brasil (Artiodactyla, Cervidae)**. Dissertação de Mestrado – Universidade de São Paulo. São Paulo, São Paulo, Brasil.

Salviano M. B., Cursino M. S., Zanetti E. D. S. S., Abril V. V., Duarte J. M. B. (2017). Intraspecific Chromosome Polymorphisms Can lead to Reproductive Isolation and Speciation: An Example in Red Brocket Deer (Mazama A). **Biology of Reproduction** 96: 1279–1287.

Sandoval E.D.P., Rola L.D., Morales-Donoso J.A., Gallina S., Reyna-Hurtado R., Duarte J.M.B. (2021). Integrative analysis of *Mazama temama* (Artiodactyla; Cervidae) and designation of a neotype for the species. **Journal of Mammalogy 103(2)447–458.**

Seabright M. (1971). A rapid banding technique for human chromosomes. **Lancet** 11:971–972

Sneath P.H.A., Sokal R.R. (1973). Numerical taxonomy: the principles and practice of numerical classification. San Francisco, W. H. Freeman and Co.

Smith H.C. (1827). The seventh order of the Mammalia. The Ruminantia. In: Griffith E., Smith C.H., Pidgeon E. (Eds.) **The animal kingdom arranged in conformity with its organization, by the Baron Cuvier, with additional descriptions of all the species hitherto named, and many not before noticed, by Edward Griffith and others**. Vol. 5. Geo B. Whitaker, London; p. 298-376.

Sumner A.T. (1972). A simple technique of demonstrating centromeric heterochromatin. **Experimental Cell Research** 75:304-306.

Telenius H., Carter N.P., Bebb C.E., Nordenskjold M., Ponder B.A.J., Tunnacliffe A. (1992). Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. **Genomics** 46:718–725.

Tate G. (1939). The mammals of the Guiana region. **Bulletin Americano Museum Natural History** 76:151–229.

Thomas O. (1908). A new deer of the brocket group from Venezuela. **Annals and Magazine of Natural History** 1 (Series 8):349–350.

Thomas O. (1913). The smaller south American 83ervidae. **Annals and Magazine of Natural History** (Series 8) 11: 587

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. (1997). The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. **Nucleic Acids Research** 25(24):4876–4882.

Valeri M.P., Tomazella I.M., Duarte J.M.B. (2018). Intrapopulation chromosomal polymorphism in *Mazama gouazoubira* (Cetartiodactyla; Cervidae): The emergence of a new species? **Cytogenetic and Genome Research** 154:147–152.

Verma R.S., Babu A. (1995). Human chromosomes: principles and techniques. 2nd ed. McGraw-Hill, Inc., New York.

Von den Driesch A. (1976). A guide to the measurement of animal bones from archaeological sites. Peabody Museum Bulletin 1, Harvard University, Peabody Museum of Archaeology and Ethnology,

Vozdova M., Kubickova S., Cernohorska H., Fröhlich J., Vodicka R., Rubes J. (2019) Comparative Study of the Bush Dog (*Speothos venaticus*) Karyotype and analysis of satellite DNA sequences and their chromosome distribution in six species of Canidae. **Cytogenetic and Genome Research** 159:88–96.

Whitehead G. K. (1994). The whitehead encyclopedia of deer. Shrewsbury: Swan Hill Press; p. 493.

# SUPPLEMENTARY MATERIAL

**S1 Table**. Body measurements of a male *Mazama americana citus* Osgood, 1912, and other Neotropical deer in milimeters (cm) following Driesch (1976).

| Species | Voucher ID | Head Large | Head Width | Ear Length | Between eyes | Mandible | Metacarpus length | Height | Body Length | Tail length | Metatarsus length | Neck diameter | Thorax diameter | Abdomen diameter | Mass (in Kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mazama americana citus* | **T410** | 22 | 8.5 | 11 | 5.3 | 7.3 | 13.5 | 56 | 85.5 | 10 | 22 | 46 | 60 | | 23 |
| *Subulo gouazoubira* | **T377** | 19 | 7,8 | 10,3 | 4,8 | 7,15 | 13,5 | 52 | 70 | 10,8 | 21 | 23 | 54 | 61 | 15,5 |
| *Subulo gouazoubira* | **T209** | 20,5 | 7,8 | 11 | 5,05 | 5,9 | 13 | 58 | 81 | 11 | 22 | 25,5 | 55 | | 17 |
| *Subulo gouazoubira* | **T26** | 20 | 7,55 | 10,5 | 4,76 | 6,15 | 14 | 51,5 | 71 | 10 | 21,5 | 20,5 | 47 | 57 | 13,5 |
| *Subulo gouazoubira* | **T372** | 20,5 | 6,4 | 10,5 | 4,4 | 6,9 | 12,5 | 55,8 | 82 | 9,6 | 19,4 | 22,5 | 58,5 | 57,8 | 19 |
| *Subulo gouazoubira* | **T323** | 20,5 | 7,78 | 10,4 | 3,94 | 6,45 | 12 | | 73,5 | 12,5 | 21 | 26,5 | 54,5 | 62,5 | 17,1 |
| *Subulo gouazoubira* | **T355** | 21 | 8 | 11,3 | 4,8 | 6,4 | 13,5 | 56,5 | 72,5 | 12,1 | 21,3 | 26 | 55 | 59,5 | 17,9 |
| *Subulo gouazoubira* | **T82** | 21 | 8,05 | 9,5 | 4,75 | 6,3 | 14,5 | 54,5 | 82 | 8 | 23 | 28 | 60 | 56,5 | 18,5 |
| *Subulo gouazoubira* | **T314** | 29 | 7,5 | 10,5 | 4,4 | 6,05 | 13 | 50,7 | 68 | 12,5 | 21 | 20,5 | 51,5 | 62 | 16,7 |
| *Subulo gouazoubira* | **T386** | 20 | 7,2 | 9,8 | 3,9 | 5,3 | 12 | 50,5 | 71,5 | 10 | 20 | 20 | 47 | 47 | 10,4 |
| *Subulo gouazoubira* | **T347B** | 19 | 7 | 9 | 4,15 | 5,35 | 12 | 46,5 | 61 | 10 | 19,2 | 19,5 | 48 | 53 | 11 |
| *Subulo gouazoubira* | **T403** | 21 | 8,35 | 11,3 | 4,9 | 6,8 | 14 | 57 | 76,5 | | 22 | 25 | 59,5 | 58,5 | 19 |
| *Subulo gouazoubira* | **T113** | 19,5 | 7,8 | 10 | 4,45 | 6,7 | 13,5 | 54 | 71,5 | 11 | 20,5 | 23,5 | 51,5 | 58 | 16 |
| *Mazama nemorivaga* | **T24** | 19,5 | 7,35 | 8,5 | 4,15 | 6,3 | 11 | 48,5 | 63 | 10 | 19,5 | 23 | 48 | 55 | 12,5 |
| *Mazama nemorivaga* | **T346** | 19,5 | 7,45 | 9 | 4,4 | 6 | 12,5 | 48 | 67,5 | 10 | 20 | 19,5 | 48 | 53,5 | 10,5 |
| *Mazama nemorivaga* | **T264** | 19 | 7,85 | 8,5 | 4,5 | 5,25 | 12 | 48,5 | 66 | 8,5 | 18,5 | 25 | 49 | | 12,5 |
| *Mazama nemorivaga* | **T371** | 20 | 7,5 | | 4 | 6,1 | 12 | 48,5 | 64,5 | 9,8 | 19 | 21,5 | 52 | 56 | |
| *Mazama nemorivaga* | **T20** | 22,3 | 8,15 | 8,7 | 5,3 | 6,5 | 11,5 | 49 | 81 | 9 | 20,5 | | 60 | 64,5 | |
| *Mazama nemorivaga* | **T359** | 20,5 | 7,55 | 8,6 | 4,5 | 5,3 | 12,5 | 48 | 70 | 9 | 21 | 21 | 54 | 63 | 14,5 |
| *Mazama nemorivaga* | **T295** | 20 | 8,45 | 9,3 | 4,75 | 3,1 | 12,5 | 52,5 | 77 | 12 | 30,5 | 28 | 55,5 | 57 | 16 |
| *Mazama nemorivaga* | **T322** | 21,5 | 7,75 | 8,7 | 5,03 | 6,07 | 12 | 49 | 75 | 9,2 | 19 | 21 | 52 | 59,5 | 14,8 |
| *Mazama nemorivaga* | **T321** | 21 | 7,9 | 9 | 4,35 | 6,1 | 13 | 50,5 | 67 | 9,3 | 21 | 21 | 54 | 59 | 14,5 |
| *Mazama nemorivaga* | **Catfap4** | 20 | 8 | 9 | 4,5 | 6 | 13 | 51,5 | 72,5 | 12,5 | 20 | 23 | 53 | 58 | |
| *Mazama nemorivaga* | **T263** | 19 | 7,65 | 8,3 | 4,35 | 5,95 | 12 | 50,5 | 72,5 | 11 | 20,5 | 23 | 54 | 63 | 16 |

**S2 Table**. Skull measurements of a male *Mazama americana citus* Osgood, 1912, and other Neotropical deer, in milimeters (mm) following Driesch (1976).

| Species | NPC | TESE | CT | CCB | CB | CCC | PP | EBC | EBF | CVC | CFM | LN | LR | LP | ACR | MCN | CCFL | CPO | CLP | DCD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mazama americana citus* | 121 | 410 | 189.39 | 185.60 | 166.29 | 112.69 | 54.82 | 38.34 | 132.43 | 91.33 | 110.89 | 98.57 | 154.82 | 181.59 | 133.97 | 58.74 | 98.54 | 81.54 | 44.83 | 58.16 |
| *Mazama nemorivaga* | 88 | 295 | 175.63 | 171.01 | 155.66 | 103.42 | 49.80 | 22.38 | 130.08 | 83.94 | 97.04 | 86.28 | 136.21 | 165.99 | 127.57 | 48.71 | 85.28 | 75.63 | 37.31 | 56.90 |
| *Mazama nemorivaga* | 124 | 322 | 183.02 | 176.02 | 162.28 | 101.61 | 54.36 | 22.57 | 139.42 | 81.65 | 115.02 | 101.32 | 140.43 | 176.21 | 129.21 | 42.91 | 94.92 | 79.42 | 46.98 | 52.51 |
| *Mazama nemorivaga* | 165 | CATFAP4 | 178.55 | 173.37 | 155.97 | 101.31 | 55.39 | 21.31 | 129.50 | 83.22 | 104.70 | 91.81 | 139.98 | 168.21 | 126.89 | 50.60 | 89.43 | 76.15 | 44.61 | 52.09 |
| *Mazama nemorivaga* | 62 | 266 | 172.20 | 162.09 | 143.2 | 100.04 | 58.94 | 22.43 | 126.42 | 77.43 | 102.74 | 91.56 | 145.06 | 177.87 | 127.75 | 53.54 | 95.06 | 73.65 | 40.43 | 41.12 |
| *Mazama nemorivaga* | 4 | 275 | 166.01 | 162.94 | 147.06 | 102.18 | 42.69 | 21.30 | 124.93 | 72.18 | 103.87 | 93.55 | 137.43 | 168.49 | 122.33 | 47.63 | 67.59 | 77.03 | 42.33 | 53.98 |
| *Mazama nemorivaga* | 5 | 263 | 168.21 | 158.80 | 145.93 | 100.21 | 51.39 | 27.96 | 119.50 | 73.33 | 113.14 | 100.06 | 129.29 | 169.09 | 121.34 |  | 83.60 | 75.69 | 37.32 | 45.12 |
| *Mazama nemorivaga* | 108 | 371 | 171.07 | 165.22 | 149.92 | 100.62 | 50.17 | 23.70 | 124.99 | 79.59 | 102.34 | 89.09 | 137.71 | 171.55 | 120.79 | 51.78 | 78.28 | 78.40 | 42.30 | 48.29 |
| *Mazama nemorivaga* | 64 | 346 | 178.40 | 170.17 | 155.25 | 102.40 | 53.38 | 29.29 | 127.69 | 84.44 | 103.32 | 92.60 | 139.03 | 171.89 | 123.20 | 48.48 | 85.97 | 76.69 | 41.93 | 51.67 |
| *Mazama nemorivaga* | 80 | 359 | 172.41 | 165.86 | 148.85 | 99.59 | 48.84 | 26.41 | 120.65 | 77.87 | 105.33 | 92.01 | 140.00 | 172.45 | 111.22 | 50.16 | 80.44 | 75.43 | 38.35 | 50.78 |
| *Mazama nemorivaga* | 10 | 321 | 180.78 | 170.47 | 157.22 | 103.43 | 54.16 | 31.24 | 133.64 | 90.80 | 104.92 | 92.44 | 146.52 | 173.88 | 124.31 | 57.32 | 73.45 | 75.66 | 49.62 |  |
| *Mazama nemorivaga* | 6 | 264 | 162.87 | 158.10 | 142.34 | 103.63 | 48.30 | 27.61 | 129.20 | 85.53 | 101.8 | 107.81 | 137.40 | 172.38 | 126.55 | 45.36 | 80.30 | 76.81 | 46.81 | 55.15 |
| *Mazama nemorivaga* | 53 | 265 | 182.92 | 172.08 | 160.40 | 102.19 | 57.25 | 29.41 | 130.34 | 81.46 | 101.93 | 103.74 | 142.5 | 172.93 | 125.84 |  | 84.01 | 76.18 | 41.88 | 57.18 |
| *Subulo gouazoubira* | 57 | 347B | 177.90 | 170.04 | 157.18 | 104.88 | 55.01 | 24.82 | 134.67 | 78.59 | 108.10 | 96.52 | 145.37 | 168.93 | 126.34 | 51.38 | 88.12 | 71.12 | 41.05 | 51.03 |
| *Subulo gouazoubira* | 154 | 403 | 180.68 | 172.86 | 154.24 | 99.92 | 55.21 | 24.71 | 130.25 | 77.07 | 116.27 | 106.67 | 147.80 | 174.95 | 129.59 | 47.88 | 90.05 | 76.05 | 40.91 | 51.61 |
| *Subulo gouazoubira* | 125 | 354 | 163.67 | 155.12 | 140.90 | 94.27 | 49.60 | 24.00 | 115.49 | 67.59 | 104.91 | 95.13 | 133.20 | 155.97 | 113.80 | 43.50 | 62.52 | 66.53 | 38.91 | 46.34 |
| *Subulo gouazoubira* | 61 | 323 | 176.02 | 162.93 | 142.31 | 103.33 | 54.02 | 22.31 | 122.65 | 75.03 | 102.34 | 99.93 | 141.13 | 175.51 | 123.24 | 41.57 | 89.07 | 73.65 | 39.91 | 40.19 |
| *Subulo gouazoubira* | 113 | 377 | 165.91 | 157.14 | 147.02 | 102.44 | 46.25 | 19.67 | 127.19 | 75.68 | 99.87 | 91.09 | 128.70 | 156.21 | 117.51 | 44.20 | 87.65 | 66.62 | 41.30 | 43.13 |
| *Subulo gouazoubira* | 89 | 113 | 180.75 | 172.76 | 155.10 | 106.50 | 51.35 | 28.04 | 130.06 | 85.33 | 106.04 | 95.67 | 146.99 | 173.70 | 127.61 | 54.06 | 83.74 | 75.77 | 40.61 | 56.22 |
| *Subulo gouazoubira* | 104 | 273 | 171.25 | 165.57 | 152.60 | 100.93 | 50.13 | 25.32 | 125.86 | 76.63 | 103.99 | 91.69 | 135.47 | 161.58 | 120.30 | 45.72 | 79.88 | 80.93 | 41.09 |  |
| *Subulo gouazoubira* | 112 | 372 | 178.50 | 175.66 | 155.86 | 94.49 | 56.44 | 25.19 | 123.70 | 85.17 |  | 90.89 | 141.93 | 168.95 | 123.23 | 54.01 | 88.91 | 76.67 | 45.73 | 57.35 |
| *Subulo gouazoubira* | 119 | 389 | 170.52 | 162.91 | 141.39 | 94.59 | 56.38 | 25.47 | 126.76 | 67.68 | 105.26 | 95.68 | 143.97 | 163.75 | 123.40 | 49.93 | 74.65 | 80.71 | 47.99 | 54.77 |

| Species | NPC | TESE | CLM | CLP | MCIO | MAIO | MLM | MLCO | MLPP | MLFM | AFM | MLNC | LFM | MLEO | LMEO | LZ | MLEN | MLPM | MLP | BCNS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mazama americana citus* | 121 | 410 | 33.40 | 25.98 | 31.13 | 30.37 | 57.73 | 35.01 | 53.72 | 15.29 | 16.05 | 56.20 | 60.19 | 69.32 | 52.58 | 81.27 | 20.13 | 22.49 | 56.80 | 62.98 |
| *Mazama nemorivaga* | 88 | 295 | 32.96 | 26.65 | 32.97 | 33.62 | 56.55 | 32.38 | 51.47 | 16.65 | 17.52 | 58.37 | 58.06 | 72.27 | 52.09 | 76.57 | 21.60 | 23.48 | 81.71 | 43.57 |
| *Mazama nemorivaga* | 124 | 322 | 26.70 | 21.90 | 31.62 | 32.79 | 56.82 | 32.44 | 45.85 | 15.68 | 15.12 | 58.24 | 49.44 | 66.48 | 50.29 | 76.17 | 16.75 | 22.10 | 74.68 | 42.56 |
| *Mazama nemorivaga* | 165 | CATFAP4 | 29.27 | 24.68 | 32.56 | 32.20 | 57.56 | 30.83 | 47.93 | 15.16 | 18.40 | 57.94 | 57.06 | 63.87 | 49.78 | 76.45 | 19.85 | 21.75 | 76.44 | 40.70 |
| *Mazama nemorivaga* | 62 | 266 | 26.04 | 21.76 | 31.90 | 30.32 | 52.63 | 31.42 | 46.02 | 17.09 | 14.75 | 55.6 | 45.54 | 66.62 | 45.51 | 72.09 | 18.07 | 20.93 | 52.23 | 39.92 |
| *Mazama nemorivaga* | 4 | 275 | 29.85 | 26.03 | 30.29 | 32.44 | 64.72 | 31.34 | 52.80 | 14.44 | 14.55 | 54.98 | 37.12 | 61.63 | 43.57 | 71.14 | 18.82 | 18.04 | 49.78 | 43.41 |
| *Mazama nemorivaga* | 5 | 263 | 25.48 | 21.79 | 30.88 | 32.51 | 67.42 | 32.49 | 48.64 | 14.37 |  |  | 43.08 | 61.68 | 49.57 | 77.14 |  | 21.24 | 48.14 | 44.42 |
| *Mazama nemorivaga* | 108 | 371 | 27.54 | 20.81 | 30.50 | 31.63 | 64.73 | 29.56 | 43.10 | 15.34 | 14.80 | 52.31 | 42.61 | 64.37 | 45.16 | 68.03 | 20.83 | 17.82 | 49.62 | 37.52 |
| *Mazama nemorivaga* | 64 | 346 | 30.25 | 23.32 | 32.05 | 37.53 | 69.43 | 32.13 | 45.98 | 16.09 |  |  | 46.17 | 65.74 | 47.04 | 72.62 | 20.51 | 22.61 | 51.69 | 37.25 |
| *Mazama nemorivaga* | 80 | 359 | 28.77 | 24.21 | 31.59 | 32.54 |  | 31.56 | 45.97 | 13.50 |  |  | 43.25 | 66.24 | 44.55 | 71.67 | 15.97 | 16.45 | 49.88 | 41.21 |
| *Mazama nemorivaga* | 10 | 321 |  | 22.74 | 34.02 | 34.11 | 66.24 | 30.06 | 46.72 | 15.81 |  |  | 45.81 | 66.52 | 42.76 | 71.35 | 16.66 | 20.26 |  | 37.48 |
| *Mazama nemorivaga* | 6 | 264 | 35.71 | 23.39 | 32.75 | 35.68 |  | 33.71 | 46.79 |  | 17.93 | 54.36 | 43.18 | 60.05 | 45.04 | 72.91 |  | 18.33 | 46.98 | 42.62 |
| *Mazama nemorivaga* | 53 | 265 | 43.21 |  | 35.77 |  |  | 34.21 | 48.78 |  | 15.11 | 51.94 | 48.66 | 68.08 | 49.10 | 71.71 | 20.16 |  | 52.79 | 40.94 |
| *Subulo gouazoubira* | 57 | 347B | 27.57 | 22.53 | 26.78 | 28.59 | 52.60 | 32.37 | 46.89 | 15.03 | 14.22 | 51.67 | 50.83 | 62.73 | 43.72 | 74.00 | 17.39 | 20.50 | 51.81 | 46.99 |
| *Subulo gouazoubira* | 154 | 403 | 27.90 | 22.46 | 28.91 | 28.90 | 58.91 | 35.67 | 53.22 | 15.62 | 17.07 | 56.74 | 62.79 | 70.19 | 48.22 | 79.58 | 21.96 | 23.60 | 50.93 | 46.32 |
| *Subulo gouazoubira* | 125 | 354 | 25.56 | 21.92 | 33.18 | 27.36 | 52.98 | 29.50 | 45.79 | 13.20 | 19.8 | 55.65 | 46.76 | 53.78 | 43.29 | 67.59 | 18.03 | 17.01 | 48.63 | 37.97 |
| *Subulo gouazoubira* | 61 | 323 | 26.57 | 22.9 | 29.51 | 30.51 | 58.69 | 35.04 | 47.42 | 19.14 | 16.62 | 52.14 | 43.80 | 70.09 | 46.52 | 78.09 | 18.02 | 22.03 | 53.02 | 43.35 |
| *Subulo gouazoubira* | 113 | 377 | 26.86 | 27.02 | 28.46 | 26.55 | 55.98 | 33.15 | 37.99 | 14.37 | 15.05 | 51.04 | 43.87 | 54.29 | 40.49 | 73.67 | 15.90 | 20.83 | 52.18 | 38.31 |
| *Subulo gouazoubira* | 89 | 113 | 29.79 | 26.10 | 29.80 | 29.85 | 66.98 | 33.02 | 44.70 | 14.89 |  |  | 44.13 | 63.94 | 47.02 | 73.79 | 19.62 | 20.14 | 53.82 | 38.79 |
| *Subulo gouazoubira* | 104 | 273 |  |  | 30.95 | 29.47 | 71.18 | 32.63 | 51.59 | 15.62 |  |  | 44.00 | 60.51 | 43.29 | 69.51 | 17.11 | 22.04 |  | 38.58 |
| *Subulo gouazoubira* | 112 | 372 | 32.69 | 26.25 | 28.65 | 29.25 | 53.18 | 34.43 | 49.66 | 15.85 | 17.35 | 54.33 | 43.70 | 64.25 | 46.08 | 75.38 | 19.65 | 20.39 | 60.95 | 41.06 |
| *Subulo gouazoubira* | 119 | 389 | 31.72 | 24.56 | 30.38 | 29.02 | 53.66 | 36.77 | 50.19 | 13.58 | 14.07 | 53.45 | 48.12 | 63.78 | 45.16 | 75.96 | 21.02 | 24.10 | 54.09 | 47.13 |

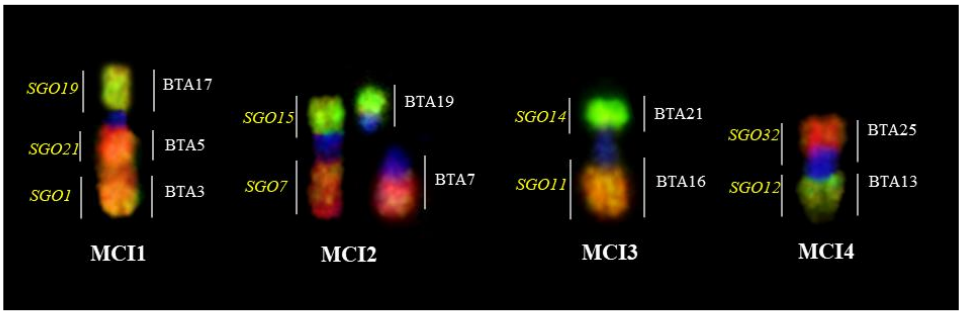
TL= total length, CBL= condilobasal length, BL= basal length, SSL= short skull length, PR= premolare – prostion, BCA= basecranial axis, BFA= basefacial axis, VL= viscerocranium length, MFL= median frontal length, LN= lambda – nasal, LR= lambda – Rhinion, LP= lambda – prostion , AK= akrokranium, GLN= greatest length of the nasals, SLFL= short lateral facial length, OPL= oral palatal length, LLP= lateral length of the premaxilla, LCR= length of the cheektooth row , LMR= length of the molar row , LPR= length of the premolar row , GILO= greatest inner length of the orbit, GIHO= greatest inner height of the orbit, GMB= greatest mastoid breadth, GBOC= greatest breadth of the occipital condyles, GBBP= greatest breadth at the bases of the paraoccipital, GBFM= greatest breadth of the foramen magnum, HFM= Height of the foramen magnum: Basiom - Opisthion, GNB= Greatest neurocranium breadth, LFB= Least frontal breadth, GBAO= greatest breadth across the orbits, LBBO= least breadth between the orbits, ZB= zygomatic breadth, GBAN= greatest breadth across the nasals, GBAP= greatest breadth across the premaxillae, GPB= greatest palatal breadth, BHPSN= Basion – The highest point of the superior nuchal crest.

**S3 Table**. Bovine BAC clones used for the chromosome X analysis in *Mazama americana citus* Osgood, 1912, and their position in the cattle genome (ARS-UCD1.2 Assembly).

| BAC | Position (Mb) |
|---|---|
| 316D2 | 68.49-68.68 |
| 159O16 | 23.04-23.22 |
| 40H2 | 74.95-75.12 |
| 453C5 | 144.43-144.62 |
| 326C13 | 148.27-148.47 |

**Figure S1.** Whole chromosomes probes (WCP). (A) Homologies of chromosomes 1 to 4 between *Mazama americana citus* (MCI) and *B. taurus* (BTA). (B) Chromosomal correspondence between *Mazama americana citus* (MCI), *Subulo gouazoubira* (SGO) and *B. taurus* (BTA).

A




B

| **Classification MCI** | SGO homology | BTA homology | **Classification MCI** | SGO homology | BTA homology |
|---|---|---|---|---|---|
| **1a (braço p)** | 19 | 17 | **14** | 16 | 1prox1t1d |
| **1b (braço q proximal)** | 21 | 5prox | **15** | 17 | 18 |
| **1c (braço q medial)** | 1 | 3 | **16** | 18 | 20 |
| **2 (braço p)** | 15 | 19 | **17** | 20 | 6prox |
| **2 (braço q)** | 7 | 7 | **18** | 22 | 9prox |
| **3 (braço p)** | 14 | 21 | **19** | 23 | 5dist |
| **3 (braço q)** | 11 | 16 | **20** | 24 | 6dist |
| **4 (braço p)** | 32 | 25 | **21** | 25 | 22 |
| **4 (braço q)** | 12 | 13 | **22** | 26 | 8prox |
| **5** | 2 | 4 | **23** | 27 | 23 |
| **6** | 3 | 2prox | **24** | 28 | 24 |
| **7** | 4 | 1c | **25** | 29 | 29 |
| **8** | 5 | 28/26 | **26** | 30 | 2dist |
| **9** | 6 | 10 | **27** | 31 | 27 |
| **10** | 8 | 11 | **28** | 33 | 8dist |
| **11** | 9 | 12 | **29** | 34 | 9dist |
| **12** | 10 | 14 | | X | X |
| **13** | 13 | 15 | | Y | - |

# CAPÍTULO 4 – SPECIES-STATUS OF A SMALL RED BROCKET INHABITING ARGENTINA, *Mazama toba* LÖNNBERG, 1919 (ARTIODACTYLA: CERVIDAE)


## ABSTRACT

*Mazama rufa toba* is a red brocket that was described in Chaco Central of Argentina by its smaller dimension compared with *M. rufa rufa* from Paraguay. The morphological similarities allowed the designation of the taxon as a synonym of *M. americana* species. As no further analyses had been performed to clarify the genetic identity of the Argentinean red brocket, we aimed to review its taxonomy through an integrative assessment using morphological, cytogenetic, and molecular data from a current topotype. We analyzed an individual from the Dique Escaba in Tucuman Province of Argentina, and characterized the morphology (general characterization and morphometry), cytogenetic (conventional staining, Ag-NOR, G and C- banding, and fluorescence *in situ* hybridization), and phylogenetic (complete mitochondrion and partial 224bp CYTB) approaches. In addition, we included the male holotype used for the species description. The morphometric results of body and skull measurement grouped the specimens within the small red brocket species. Classic chromosomal banding revealed a differentiated karyotype with 2n=50 FN=64, corresponding to the first karyotypic description of a *Mazama* collected in Argentina. Using an entire set of cattle whole chromosome painting and BAC probes, was evidenced at least 15 chromosome rearrangements between *M. rufa toba* and *M. rufa* species. The Bayesian Inference recovered the monophyletic condition of *M. rufa toba* within Odocoileina. Thus, the results supported to elevate to species-status the Argentinean small red brocket to *M. toba*.


**KEY-WORDS:** integrative taxonomy, mitogenome, molecular cytogenetic, new species

## 1. INTRODUCTION

The species of the genus *Mazama* Rafinesque (1817) inhabits from southeastern Mexico to northern Argentina, characterized by the medium to small size, hind limbs larger than the forelimbs and simple antlers in males (Merino and Rossi, 2010). Since recent studies had proven that species of the genus share a very similar morphology with a great karyotypic diversity, morphology have been considered as a not useful tool to discriminate among species (Peres et al. 2021; Cifuentes-Rincón et al. 2020). Also, the revealed polyphyly of mitochondrial and nuclear phylogenies has been suggesting the need of an integrative analysis in order to elucidate the complex taxonomy of *Mazama* (Gutiérrez et al. 2017; Heckeberg et al. 2020; Bernegossi et al. 2022).

Recently, a neotype of *M. americana* Erlexben, 1777, was characterized and compared with other south American red brocket from Brazil, evidencing that the specimen was very similar morphologically but different genetically from the Brazilian cytotypes, corroborating the existence of a complex of cryptic species under the name *M. americana* (Cifuentes-Rincón et al. 2020; Abril et al. 2020). Peres et al. (2021) confirmed the chromosomal divergence between this neotype of *M. americana* and individuals from Parana Cytotype in Brazil, indicating at least fifteen rearrangement that were considered as important reproductive barrier (Galindo et al. 2021; Peres et al. 2021). In addition, the phylogenetic analysis supported the monophyly of Parana cytotype for which authors proposed the revalidation of *M. rufa* (Illiger,1815) for the red brocket of Paraguay-southern Brazil (Peres et al. 2021).

This proposal of a new species of *Mazama*, confirms the importance of reviewing the taxonomy of each species of the genus and also raise doubts about the identity of other descriptions of species or subspecies that were considered synonym of both *M. americana* Erlexben (1777) and *M. rufa* (Illiger, 1815). This is the case of Argentinean red brockets of Lönnberg (1919) *Mazama rufa toba* and *Mazama rufa rosii* (Lönnberg, 1919),

Lönnberg (1919) described *M. rufa toba* from an adult male collected in Chaco Central, Rio de Oro, Argentina. The author designed this male as type specimen and deposited in the Swedish Museum of Natural History. The specimen had general color rich rufous, tipped blackish in the withers and upper

neck, whitish hairs ventrally, nape hairs with normal direction, red foreleg, dark brown hind legs, head dark brown in the nose and muffle with absences of the white upper lip (Lönnberg, 1919). The author compared the specimen with the citation of Allen (1915) about the *M. rufa rufa* (Illiger, 1815) describing the shortness of the preorbital region, the maxillary tooth-row and the premaxillae in *M. rufa toba* as the main characters to differentiated from Illiger´s red brocket (Lönnberg,1919). Also, the specimen was compared with the *M. rufa jucunda* (Thomas, 1913) from Paraná, highlighting the longer condylobasal length and smaller breadth of the animal from the Chaco Central (Lönnberg, 1919).

In the same study Lönnberg (1919) described another red brocket from Argentina, that the author noted as larger than *M. rufa* of Illiger (1815), naming *M. rufa rosii* based in an adult male and female, collected in the Chaco Austral, Río de Oro (Lönnberg, 1919). The great difference in size, reversed hair of the upper side of the neck, white muffle and upper lip, were the mean characters that the author pointed out to differentiate this description with the *M. rufa toba* from Chaco Central (Lönnberg, 1919).

The nomination of the Lönnberg´s descriptions as subspecies of *M. rufa* (Illiger, 1815) was maintained by Thomas (1925). After this, it was considered a subspecies of *M. americana*, naming *M. a. toba* (Cabrera, 1961), until the reorganization of the *Mazama* species performed by Grubb (2005) who allocated *M. rufa* Illiger, 1815, *M. rufa toba* Lönnberg, 1919 and *M. rufa rosii* Lönnberg, 1919 as subspecies *of M. americana* (Erlexben, 1777).

Besides some authors had cited the presence of *M. americana* species at north of the Chaco Province, Formosa and also, at 600 to 900 m.a.s.l. of the Yungas, northwest of Argentina (Cabrera, 1961; Richard,2008; Richard e Julia, 2001), there was no other taxonomic study of red brocket occurring in Argentina nor clarification of the genetic identity of the described *M. rufa toba* in relation to other *Mazama* in Latin America.

In addition, the absence of cytogenetic data and molecular phylogeny reveals the need of genetic analyses in order to described the karyotype of *Mazama rufa toba* and understand the phylogenetic relationship with other neotropical deer species. The present study provides much of this information through morphological, phylogenetic (mitochondrial genes), classical cytogenetic (conventional staining, G-banding, C-banding and Ag-NOR), and molecular

cytogenetic (fluorescent in situ hybridization, FISH) analyses of *M. rufa toba* (Lönnberg, 1919).

## 2. MATERIAL AND METHODS

### 2.1 Obtaining animals and samples

We photographed and collected samples of tissue (blood and skin fragment) from an adult female in the Reserva Experimental Horco Molle, with known origin in the Dique Escaba, El Corralito, Tucumán, in the northwest Argentina, part of the geographic reference from Central Chaco region in early 20$^{th}$ century. Due to the geographical proximity, morphological similarity and molecular results that will be showed in the next topics, the samples were associated to the morphotype described by Lönnberg (1919) as *Mazama rufa toba* and received an identification number NUPECCE (T451) at the Deer Research and Conservation Center (NUPECCE) where it remains deposited. In addition, the type specimen of *M. rufa toba* (NRM630636) (adult female) was assessed ed in the Swedish Museum of Natural History for molecular and craniometrical analysis, and a tissue of a red brocket individual (identified ARG01) from El Perdido, Chaco Province in Argentina (coordinates 26°33’55.78’’ O x 58°57’49.871’’ S) was collected for molecular analysis.

### 2.2 Morphological characterization

The *M. rufa toba* T451 specimen was photographed and morphological aspects were analyzed considering the general coat color, chromogenetic fields of the body (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, rounded hair tufts in the tarsal region and the chromogenetic fields of the head (Hershkovitz 1982). External body measurements were taken using a pendulum scale, measuring tape and digital caliper 0.05 mm. The following were measured: width and length of the head, ear length, between eyes, mandible, neck circumference, thorax circumference, height of the body,

metacarpus length, tail length, mass in kilograms, metatarsus length, abdomen circumference, and total body length.

Different angles of the skulls of *M. rufa toba* (adult female) (NRM630636) and *M. rufa rosii* (adult male) (NRM630631) were photographed to complement the documentation and description of the species. We took 36 cranial measurements following the criteria for the standard measurements for cervids (Driesch 1976).

We performed a cluster analysis based on 14 body measurements of the *M. rufa toba* T451 specimen, and separated, we performed a cluster analysis on 36 cranial measurements of *M. rufa toba* (adult female) (NRM630636) and *M. rufa rosii* (adult male) (NRM630631). Both analyses were performed by the Unweighted Pair Group Method with Arithmetic mean method (UPGMA) to position and compared these specimens with adult individuals from the NUPECCE database belonging to the red brocket species *M. americana*, *M. jucunda*, *M. rufa*, *M. temama*, and *M. nana*.

### 2.3 Cytogenetic analysis

After chemical restraint of the specimen (7 mg/Kg ketamine and 1 mg/kg xylazine intravenously), 5 x 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 (Howell and Black, 1980). The chromosomes were classified as acrocentric, submetacentric, or metacentric according to their arm ratio (Levan et al. 1964) and separated into groups according to their relative lengths (Cifuentes-Rincón et al. 2020).

### 2.4 FISH

Fluorescence *in situ* hybridization was performed using cattle whole chromosome painting (WCP) probes and BAC probes on the karyotype of the

*Mazama rufa toba* (MTO) specimen. The whole cattle chromosome probes were selected considering their proven efficiency in karyotypic studies of the Cervidae family (Frohlich et al. 2017). For probe preparation, whole cattle chromosomes were isolated by microdissection in the PALM Microlases system (Carl Zeiss MicroImaging GmbH, Munich, Germany) or by flow cytometry using MoFlo XDP Cell Sorter (Beckman Coulter, USA) as described in Frohlich et al. (2017). The amplification was performed using degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) (Telenius et al. 1992) and probe labeling, which was done during the second PCR with Green-dUTP or Orange-dUTP (Abbott Park, IL, USA) (Kubickova et al. 2002). BAC clones were selected from the CHORI-240 library based on the NCBI ARS-UCD1.2 Assembly data and obtained from BACPAC Genomics, Emeryville, CA, USA. For DNA extraction, we use a protocol adapted from the method included in Wizard® 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® Array CGH Genomic Labeling (Invitrogen, Carlsbad, CA, USA). The clones were selected from the 29 pairs of cattle acrocentric autosomes and from the submetacentric sex chromosome X, comprising the q and p arms regions. For the pseudoautosomal region (PAR) probe, two BAC clones were selected and used together as a single probe.

FISH was performed as described in Vozdova et al. (2019). The hybridization signals were examined using the Zeiss Axio Imager Z2 fluorescent microscope and digital images were captured with ISIS software (Meta-Systems, Altlussheim, Germany).

**2.5 DNA extraction and sequencing**

Genomic DNA extraction from tissue samples of *Mazama rufa toba* T451 and sample ARG01 from Argentina, were performed using the Qiagen Dneasy® Blood Qiamp Tissue kit following the manufacturer's instructions. Approximately 1 µg of DNA from each specimen was sent to NGS Soluções Genômicas (https://ngsgenomica.com.br) for massive parallel DNA sequencing. First, the DNA samples were fragmented by Covaris sonication to an average size of 300 base pairs. Then, these fragmented DNA samples were used as input for

genomic library preparation using the TruSeq Nano DNA sample preparation kit (Illumina, USA) following the manufacturer's instructions. All genomic libraries were pooled and sequenced on the Illumina HiSeq 2500 Platform (Illumina, San Diego, CA, USA), 100 bp paired-end sequencing. Unmerged reads were aligned to the *Mazama americana* mitogenome (GenBank accession number JN632656) as a reference using Bowtie2 v2.4.1 (Langmead and Salzberg, 2012) on the Galaxy online server (Afgan et al. 2018). Annotation was performed using the MITOS (Bernt et al. 2013) web-based platform (http://mitos.bioinf.uni-leipzig.de/index.py) and manually adjusted based on comparisons to the reference mitogenome using GENEIOUS (Biomatters; http://www.geneious.com/).

DNA was extracted from nasal bone of *Mazama rufa toba* holotype using a modified protocol based on the methodology described by Medrano et al. (1990). The samples were quantified by spectrophotometry and analyzed by agarose gel analysis, and then diluted in a solution for use. The amplification was performed with real time PCR using a Rotor Gene (Qiagen) of the cytochrome b partial gene (Cytb 224pb) (González et al., 2009). The reaction was completed with 5 µL of SensiFASTTM 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 segments / 54 °C for 10 segments (20 cycles) / 53 °C for 10 segments (20 cycles) / 52 °C for 10 segments (20 cycles) / 72ºC for 20 segments.

The PCR product was analyzed by electrophoresis in 2% agarose and purified with the Wizard SV gel and PCR Clean-Up System kit (Promega TM), followed by sequencing using the ABI BigDye Terminator kit (Applied Biosystems) in an ABI 3130xl automatic sequencer (Applied Biosystems).

### 2.6 Phylogenetic analysis

We generated 1 Cytb partial gene (224 bp) from *M. rufa toba* type specimen and 2 complete mitochondrion sequences (one from *M. rufa toba* T451 captive female in Reserva Horco Molle and another from tissue sample ARG01 from Chaco central) (see Table 1). We obtained sequences of *S. gouazoubira*, *M. nemorivaga*, *M. americana*, *M. nana*, *M. jucunda*, *M. temama*, *Odocoileus*

*virginianus*, *O. hemionus*, *Blastocerus dichotomus*, *M. rufa*, *O. bezoarticus*, and *Hippocamelus antisensis* from GenBank to perform the phylogenetic analyses. We use sequence of *Alces alces* as the outgroup (Table 1).

Both datasets, complete mitogenome and partial Cytb gene, were aligned separated using the Clustal X program (Thompson et al. 1997). For the mitochondrion genome, we excluded the control region from the alignment because of the tandem repeat located inside them and the high incidence of potential homoplasy. The optimal partitioning scheme was selected by the Partitionfinder 2.0 tool (Cognato and Vogler, 2001) under the Bayesian information criterion. For the Cytb data set we selected the best molecular evolution model using the jModelTest v. 0.1.1 (Posada and Crandall, 1998), following the corrected Akaike information criterion, AICc (Akaike, 1973).

Bayesian inference (BI) analysis was performed using multiple four chain Metropolis-coupled analysis (with default heating) using MRBAYES 3 (Huelsenbeck and Ronquist, 2001) with 10,000,000 generations, sampling every 1,000 generations, until a variance of <0.01 was obtained. The first 25% of the sampled trees were discarded as burn-in and the remaining trees were used to construct a 50% majority-rule consensus tree. The consensus tree was edited using FigTree v.1.4.0 (Rambaut, 2012).

**Table 1**. – Mitochondrial sequences used in the phylogenetic analysis of *Mazama rufa toba* Lönnberg, 1919 and other neotropical cervids.

| SPECIES | MITOGENOME | CYTB 224 | ORIGIN |
|---|---|---|---|
| *Mazama rufa toba NRM630636* | | This study | Chaco Central, Argentina. Lönnberg, 1919. |
| *Mazama rufa toba T451* | This study | | Dique Escaba, Tucuman, Argentina. |
| *Mazama rufa ARG01* | This study | | El Perdido. Chaco, Argentina. |
| *Mazama americana T358* | MZ350857 | MZ350857 | Cayenne, French Guiana |
| *Mazama americana T255* | | MZ488890 | Juina, Brazil |
| *Mazama americana T253* | MZ350856 | MZ350856 | Juina, Brazil |
| *Mazama americana T274* | MZ488862 | MZ488878 | Carajás, Pará, Brazil |
| *Mazama americana TEM9472* | JN632656 | JN632656 | French Guiana |
| *Mazama rufa T268* | MZ488857 | MZ488893 | Paraná, Brazil |

| | | | |
|---|---|---|---|
| *Mazama rufa T385* | MZ488852 | MZ488894 | Foz de Iguaçu, Paraná, Brazil |
| *Mazama nana T107* | MZ350863 | MZ350863 | Paraguay |
| *Mazama bororo T215* | MZ350859 | MZ350859 | P. E. Intervales-SP, Brazil |
| *Mazama temama T366* | MZ350864 | MZ350864 | Veracruz, México |
| *Mazama temama T362* | MZ362858 | MZ362858 | Campeche, México |
| *Odocoileus hemionus* | JN632670 | JN632670 | Arizona, USA |
| *Subulo gouazoubira T377* | MZ350858 | MZ350858 | Puerto Galileo, Paraguay |
| *Mazama nemorivaga T346* | MZ350867 | MZ350867 | Lupar-PA, Brazil |
| *Mazama nemorivaga MRGMa36* | JN632659 | JN632659 | Peru |
| *Alces alces* | MF784602 | MF784602 | Poland, USA |
| *Ozotoceros bezoarticus* | JN632691 | JN632681 | Bolivia |
| *Ozotoceros. bezoarticus* | FNMA40 | FNMA40 | Brazil |
| *Blastocerus dichotomus CV21* | OM543539 | OM543540 | Brazil |
| *Blastocerus dichotomus MRGBd8* | JN632603 | | Brazil |
| *Subulo gouazoubira 082* | MZ350858 | MZ350858 | Brazil |
| *Subulo gouazoubira T386* | MZ350865 | | Brazil |
| *Hippocamelus antisensis MRGHa9* | JN632646 | | Argentina |
| *Pudu puda M92144* | JN632692 | | Chile |
| *Odocoileus virginianus* | KM6122278 | | Mexico |
| *Odocoileus virginianus* | JN632671 | | French Guiana |
| *Mazama americana T310* | This study | | Rondonia, Brazil |
| *Mazama americana T424* | This study | | Roraima, Brazil |

## 3. RESULTS

### 3.1 Morphological characterization

The specimen showed a reddish general body coloration, with blackish hairs in the neck and head, and reddish fore and hind limbs with a whitish color

in the inguinal region. The head has a white narial patch, a blackish rostral band, an inferior orbital band with dark gray over an orange band and a reddish-brown color above the orbit. The mental, buccal and gular patch are white with presence of a dark mandibular band and long hairs in dorsal head, lateral and ventral body, tail and inguinal region. The female had a white scar in the middle of the frontal region of the head, produced during the emergency situation that preceded the rescue and captivity of the individual (Figure 1).


**Figure 1.** Specimen *Mazama rufa toba* Lönnberg, 1919, collected in Tucuman, Argentina. A) Lateral view of the body; B) rostral view; C) Ventral view of the body; D) Dorsal view of the body.

The UPGMA distance tree of 14 body measurements shows two groups of red brocket individuals (Figure 2). One group characterizing the large red brocket specimens divided in two subgroups, one allocated *M. jucunda* species and one individual of *M. americana* from Acre, Brazil, separated for the other subgroup that was formed by the other *M. americana* individuals and *M. rufa*. The

other group characterized the small red brocket group formed by the species *M. nana M. temama* and *Mazama rufa toba* (Figure 2A).

Despite it was no possible to collect the skull of a recent *M. rufa toba* individual, we accessed the cranial measurements of Lönnberg´s holotypes from *M. rufa toba*. The cluster analysis of 36 craniometrical characters showed a similar result than body measurement analysis with the holotype NRM630363 *M. rufa toba* grouped with the small brocket species *M. temama* and *M. nana,* separated from a second group with *M. americana, M. jucunda* and *M. rufa* (Figure 2B).

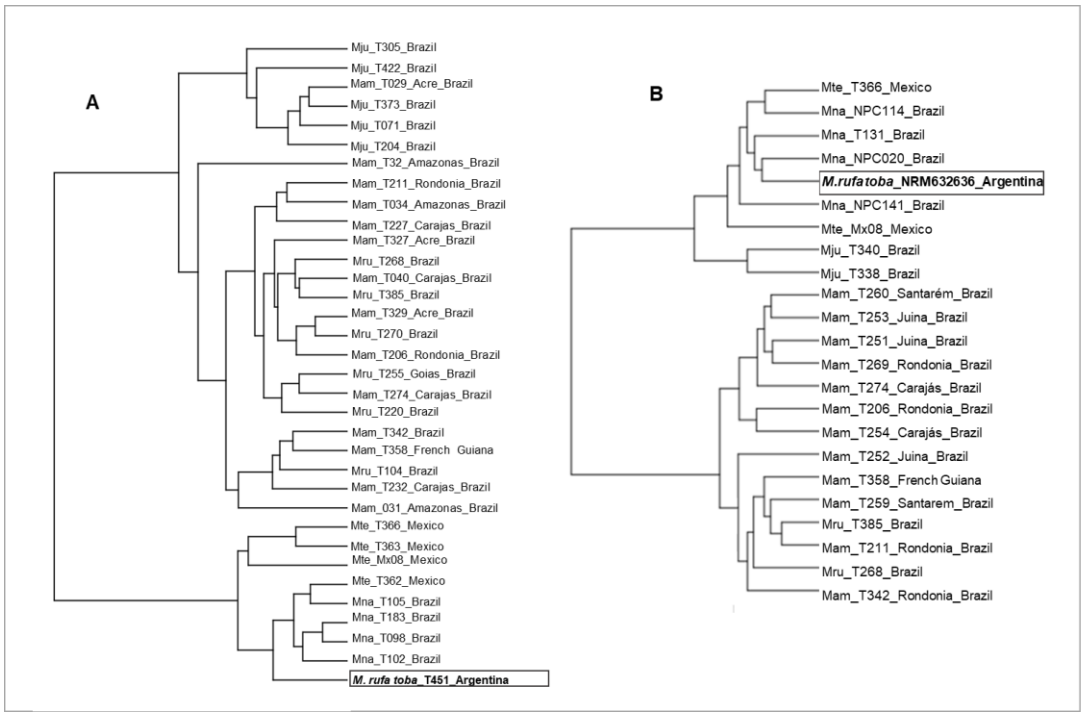



**Figure 2.** UPGMA distance tree of red brocket species. A) Body measurement B) Skull measurement. Square = *M. rufa toba*; Mna = *M. nana;* Tem = *M. temama*; Mju = *M. jucunda*; Mam = *M. americana*; Mru = *M. rufa*.

### 3.2 Cytogenetic description

The *M. rufa toba* T451 specimen showed a karyotype with 2n = 50 and FN=64, 24 pairs of autosomal chromosomes and a simple sexual system XX (XY) (Figure 3). Both X chromosome appeared as a submetacentric chromosome. The chromosomal biometry classified pairs 1 to 3 in the group A (large bi-armed chromosomes), pairs 4 and 5 in the group C (small bi-armed chromosomes), pair

6 in the group D (large one arm chromosomes) and pairs from 7 to 24 in the group E (small one arm chromosomes). B chromosomes appeared as small acrocentric chromosomes that varied from 1 to 5 in the different metaphases of the topotype (Figure 3).

The Ag-NOR staining showed three nucleolus organizing regions in the telomeric area of the long arms of the two chromosomes of pair 7, and in the telomeric region of the long arms of one chromosomes of pair 8 (Figure 4). C-banding showed constitutive heterochromatin blocks in the pericentromeric region of all autosomal and X chromosomes (Figure 4). Pair 1 showed two slight interstitials heterochromatin marking in the long arms of both chromosomes. A similar marking was observed in long arms of pairs 2 and 3, and in the distal region of pairs 6 and 7 (Figure 4).

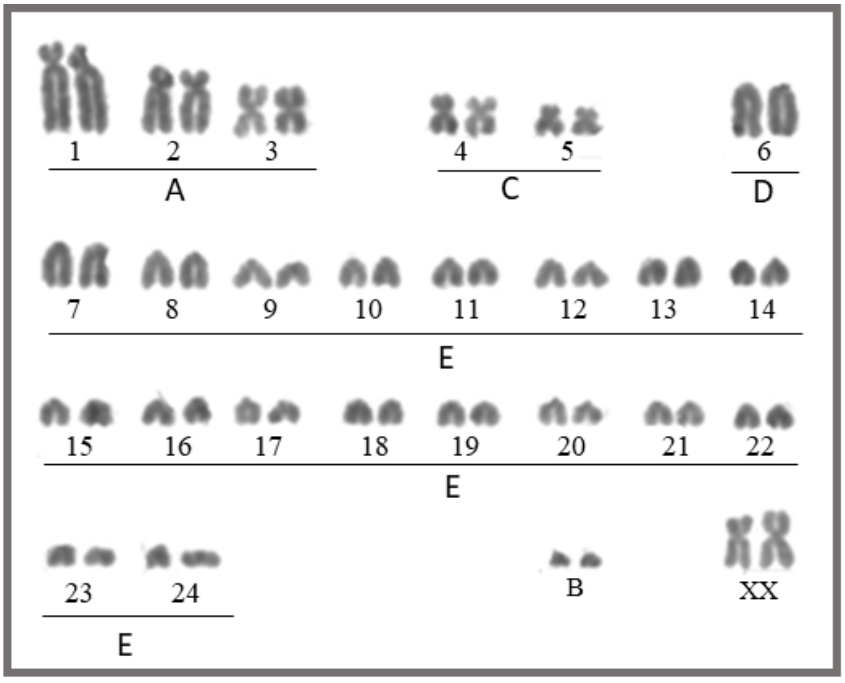



**Figure 3**. Karyotype of a female of *M. rufa toba* Lönnberg, 1919, 2n = 50 FN = 64. Conventional Giemsa staining.

G-Banding associated with bovine WCP (BTA) chromosomal painting and BAC probes (Figure 4) demonstrated that *Mazama rufa toba* (MTO) underwent chromosomal rearrangements compared to the karyotype of *Mazama rufa* species (MRU, 2n =52 and FN=56).

The p arm of the submetacentric MTO1 is homologous to the proximal region of MRU2 and the q arm to the MRU chromosomes 5, 7 and 11. The p arm

of the submetacentric MTO2 is homologous to the MRU 12, and the q arm to the MRU 4. The p arm of the submetacentric MTO3 is homologous to the proximal region of MRU9 and the q arm to the MRU chromosomes 19 and distal region of X chromosome. The p arm of the metacentric MTO4 is homologous to the medial region of MRUX chromosome and the q arm to the medial region of MRU 1. The submetacentric MTO5 is homologous to the proximal region of MRU1. The MTO6 pair corresponded to the pair MRU8 and the medial regions of MRU2, and the MRU7 is homologous to the distal region of MRU3 and chromosome MRU21. The composition of sex chromosomes also differed between the two species. The individual *M. rufa toba* have a simple sex chromosome system XY while the *M. rufa* species have a multiple sex chromosome system XY1Y2 formed by X-autosomal fusions during the divergence from a common ancestor. Thus, at least 9 rearrangements separate *M. rufa* and *M. rufa toba* karyotypes (Figure 4 and Figure S2).

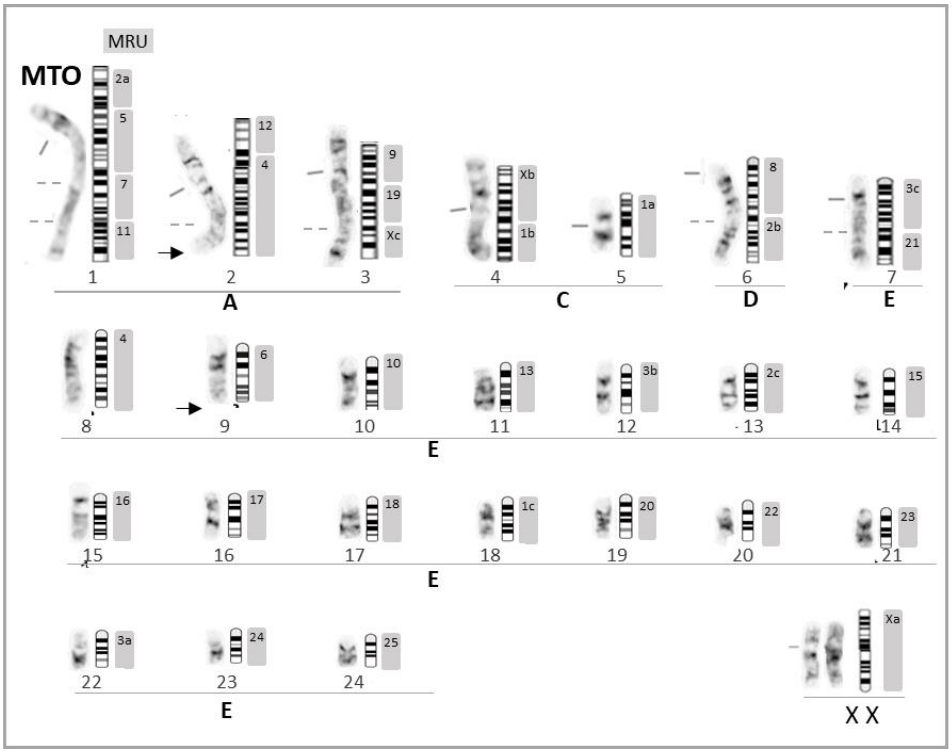



**Figure 4**. Karyotype of a female topotype of *M. rufa toba* Lönnberg, 1919. MTO= *Mazama rufa toba*. G-banding and schematic representation. The gray square indicates the homologies with MRU (*Mazama rufa*) chromosomes. The letters a, b, and c represent regions of the chromosome that were homologous to *M. rufa toba*. For the submetacentric pairs MRU1, MRU2, MRU3 and MRUX: a = p arm, b = proximal q arm, and c = terminal q arm. Lines: C-banding marking (centromere), pairs 8 to 24 idem to pair 7; Dashed lines: interstitial c-banding marking; Arrow= Ag-Nor staining.

### 3.3 Phylogenetic analysis

The Bayesian Inference (BI) analysis of the complete mitogenome (Figure 5) recovered all the Argentinean red brockets within the Odocoileina subtribe. The *M. rufa toba* was recovered in a clade separated from the other taxa of the subtribe, with *M. jucunda*, *M. nana* and one individual *M. americana* from Juína locality in Brazil as sister taxa (100% posterior probability). The ARG01 sample from Chaco Austral was observed within the monophyletic *M. rufa*, with *M. americana* from Jari in Brazil as sister taxa. *Mazama americana* from French Guiana was observed in a monophyletic clade that includes the specimen from Roraima, north Brazil with 100% posterior probability. *M. temama* was monophyletic. *Mazama rufina* was the first species that diverged within Odocoileina.

The phylogenetic tree of partial Cytb (224 bp) recovered a similar result that the complete mitogenome tree with all red brockets allocated in the Odocoileina subtribe. The *M. rufa toba* individuals were the first branch that diverged within Odocoileina, with moderated branch value (90%). The ARG01 individual was recovered in a clade formed by the *M. rufa* species and *M. americana* specimens from Carajás in Brazil and French Guiana. The species *M. nana*, *M. jucunda* and one individual *M. americana* from Juina in Brazil were recovered in a separated clade. In addition, the tree showed the separated monophyletic clades of *M. temama* and individuals of the genus *Odocoileus*. The species *S. gouazoubira*, *M. nemorivaga*, *B. dichotomus* and *O. bezoarticus* were recovered within the Blastocerina subtribe in both analyses (Figure 5).

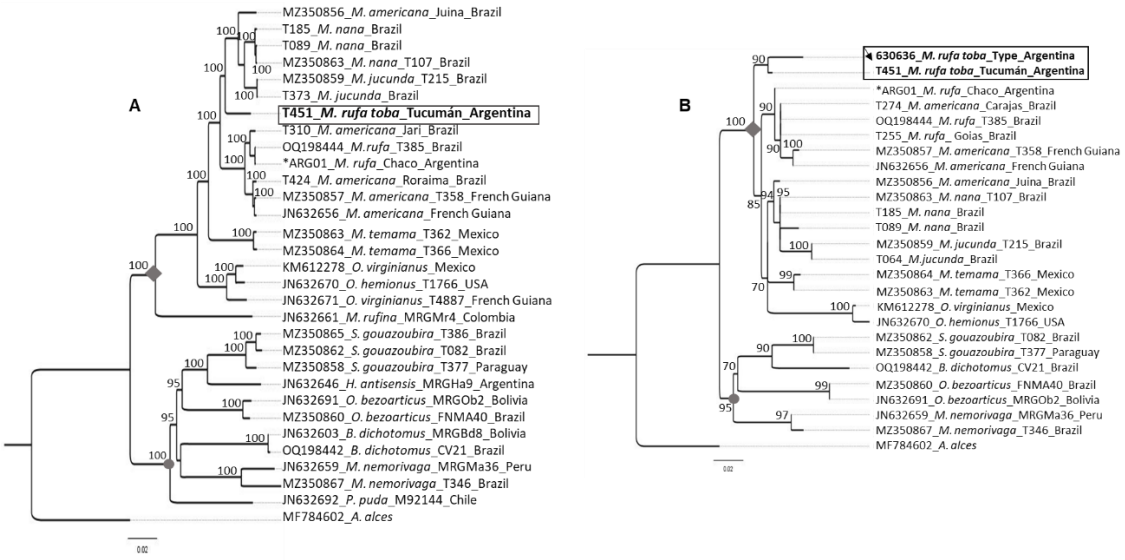



**Figure 5**. Bayesian Inference (BI) tree of neotropical deer. A) Complete mitochondrion B) Partial CYTB 224bp. Square: *Mazama rufa toba* individuals; Arrow: Lönnberg (1919) holotype *Mazama rufa toba*. *Sample ARG01 from El Perdido, Argentina. Gray diamond: Odocoileina subtribe; Gray circle: Blastocerina subtribe.

## 4. DISCUSSION

### 4.1 Morphological characterization

The analysis of body coloration and morphometric characters of the *M. rufa toba* T451 specimen agrees with the descriptions of Lönnberg, 1919 The general reddish color, blackish in the upper neck and ventral hairs, and dark hairs in the head are the mean features in common between these descriptions (Lönnberg, 1919).

Specifically, comparing the *M. rufa toba* T451 specimen and the Lönnberg (1919) holotype, we observed that both individuals show a reddish-brown color superior to the orbit that Lönnberg, 1919, considered dull rufous color blend with the cinnamon of the head. The only difference was the presence of a white narial patch in our analyzed *M. rufa toba* specimen when Lönnberg (1919) described the muffle with absence of the white upper lip in the *M. rufa toba* holotype. All these characters could have an intraspecific variability as showed by Rossi (2000) with other brocket deer, after analyzing head patches, hairs direction and bands colors of *Mazama* individuals.

The UPGMA distance analyses demonstrated that *M. rufa toba* grouped with others small red brockets confirming the smaller measurements that Lönnberg (1919) noted when compared with the *M. rufa* (Illiger, 1815) and *M. jucunda* (Thomas, 1913) species, and also separated *M. rufa toba* from *M. americana* species from which currently is considered as synonym (Lönnberg, 1919; Allen, 1915). These results confirm that size difference is the main morphological character to separate *M. rufa toba* from the large red brocket. Within the small red brocket group, the analyses based on skull and body measurement showed the similarities between *M. rufa toba* and the species *M. temama* (Kerr, 1792) and *M. nana* (Hensel, 1872). These previous species were described having a reddish color homogeneity of body and head, contrasting with the grayish and blackish hairs of the head, neck and nape of *M. rufa toba* species (Sandoval et al. 2022; Rossi, 2000). Although we did not access body measurement of *M. rufina* species, the literature cited the species restricted to the Andes with a small bright red coat, with long hairs all over the body and black color in the head, forelimb and hindlimb (Bisbal, 1991; Pucheran and Bourcier, 1851). This reveal that aspects of color distribution in different region of the body and length of hairs may distinguish morphologically *M. rufa toba* from *M. temama*, *M. nana* and *M. rufina*.

## 4.2 Cytogenetic description

The cytogenetic results revealed the unique karyotype of *Mazama rufa toba* (Lönnberg, 1919) with 2n=50 and NF=64. Besides the diploid number of the species varied only by two chromosomes with the *M. rufa* karyotype (2n=52 FN=54) (Peres et al. 2021), the great difference of fundamental number between *M. rufa toba* and *M. rufa* evidenced a completely different chromosome composition. Classic cytogenetic banding (Giemsa staining, C-banding, G-banding and Ag-NOR) showed the morphologic features of *M. rufa toba* with five bi-armed chromosomes pairs contrasting with the *M. rufa* karyotype with only one pair of bi-armed chromosome (Peres et al. 2022; Abril et al. 2010).

The G-banding associated with FISH were important tools for the comparative analysis between *M. rufa toba* and *M. rufa*. Different chromosomal rearrangements were observed, revealing that the species diverged during their

karyotypic evolutions in at least 15 rearrangements. These rearrangements included seven pairs of autosome chromosome and the simple sexual system compared to the XY1Y2 sexual system in *M. rufa* (Abril et al. 2010; Peres et al. 2022). Previous studies have showed that accumulation of central and tandem fusion leads to error in meiosis, chromosome pairing, meiotic segregation, recombination suppression and subsequent zygote death (Dobigny et al. 2017). Therefore, chromosomal rearrangements are efficient reproductive barriers that could potentially isolate sympatric populations (Cursino et al. 2014; Salviano et al. 2017; Galindo et al. 2022).

With base on that, cytogenetics studies in other red brocket species had revealed a chromosomal polymorphism with high levels of intra and interspecific variation (Abril et al. 2010). This is the case of *M. americana* Erlexben, 1777, which the description of a neotype for the species showed a diploid number 2n=45 FN= 51 (Cifuentes-Rincón et al. 2020), and allowed the confirmation of 15 rearrangement differencing *M. rufa* from *M. americana* senso stricto (Peres et al. 2022). Despite this, there is no homology between the chromosomal rearrangements of *M. rufa toba* and *M. americana* described by Cifuentes-Rincón et al. (2020) and Peres et al. (2022).

Regarding with other small red brocket species, the differences in the karyotypic descriptions revealed a great discrepancy between the diploid and fundamental number of *M. nana* (2n= 36-40, FN = 58), *M. jucunda* (2n= 32-34, FN = 46), *M. temama* (2n =44-48, FN=70) and *M. rufa toba* (2n=50, FN= 64), demonstrating the divergence of chromosomal evolution that may involve centric fusions, tandem fusions and chromosomal inversions (Abril and Duarte, 2008; Duarte and Jorge, 1996; Sandoval et al. 2022; Jorge and Bernishke, 1977).

### 4.3 Phylogenetic characterization

The result of the complete mitogenome and separated partial CYTB (224bp) was consistent with previous phylogeny of *Mazama* demonstrating the polyphyletic condition of the genus that recovered the species into two subtribes, Blastocerina and Odocoileina (Gutiérrez et al. 2017; Bernegossi et al. 2022; Peres et al. 2021).

Within Odocoileina, the Bayesian Inference tree showed the presence of two species of red brocket in north Argentina, the *M. rufa toba* analyzed here and the individual ARG01 recovered within the recently validated *M. rufa* clade evidencing that the species occurs in Chaco regions of Argentina and Paraguay (Peres et al. 2021). The complete mitochondrion tree also revealed that the individual *M. rufa toba* diverged in a separated from the other species of *Mazama* and genus *Odocoileus*. Thus, the BI tree showed that *Mazama rufa toba*, originally described as a subspecies of *M. rufa*, is not related with the Illiger´s red brocket, nor with the *M. americana* species which was considered synonymous by some authors (Cabrera, 1961; Grubb, 2005).

The partial CYTB tree confirmed the close relationship between the Lönnberg´s holotype and the T451 individuals from Tucuman, Argentina, evidencing that *M. rufa toba* clade is a full and separated species that may be considered valid within *Mazama*.

### 4.4 Species-status of *Mazama toba* (Lönnberg, 1919)

The Chaco is a geographical region composed by a set of wetland macrosystems of fluvial origin that extends in 1.280.000 km² distributed among Paraguay, Bolivia, Brazil and Argentina (Demarchi and Garcia, 2008). Specifically, 40% of the Chaco total territory is in Argentina, subdivided in two ecoregions, the humid Chaco formed by the Province of Chaco, Formosa and north of Santiago del Estero, and the dry Chaco that also includes the Province of Tucumán, north of Córdoba and north of La Rioja (Adamoli et al. 2004; Adamoli et al. 1990). This dry Chaco, is surrounded by the rivers Pilcomayo and Bermejo (Adamoli et al. 1990), cited in the last century as central Chaco (Demarchi and Garcia, 2008), where Lönnberg (1919) described a red brocket male smaller than the *M. rufa* from Paraguay (Illiger, 1815) under the name *Mazama rufa toba.*

The analysis of a female specimen from the Dique Escaba, Tucumán in Argentina, agreed with the Lönnberg´s description, and revealed morphological and cytogenetic features that differentiated this specimen from other red brockets. The phylogenetic results from complete mitochondrion analysis, and also, partial CYTB 224bp including *M. rufa toba* holotype revealed these specimens as a separated clade within Odocoileina subtribe.

It is important to consider that Lönnberg (1919) described the red brocket from Argentina as a variation of the *Mazama rufa* from Paraguay. With the analysis of this holotype and a recently collected specimen from north Argentina, it was evidenced that this initially described subspecies should be elevated to species status by the morphological, cytogenetic and molecular distinction from *M. rufa* species (Illiger, 1815). This is based on the smaller size of *M. rufa toba*, body coloration pattern, exclusive karyotype with at least 15 rearrangements between *M. rufa toba* and *M. rufa*, and the monophyletic clade within Odocoileina subtribe, separated from the other species of the genus with high posterior probability. Regarding the other species *Mazama*, including *M. americana*, there are no morphological correspondence nor chromosomal homology with Lönnberg´s (1919) description, indicating that *Mazama toba* (Lönnberg, 1919), is a valid species occurring in northern Argentina.

## 5. CONCLUSION

Our phylogenetic and morphologic analysis of a recently collected small red brocket from Argentina agrees with the description of *Mazama rufa toba.* Through accessing this specimen and also, the holotype of this subspecies, the integrative characterization indicates the differences of this small red brocket with other species of *Mazama*. Thus, we suggest the species-status elevation as a first step to clarify its taxonomy and recommend further studies through sampling voucher of wild living specimens for cytogenetic characterization and a multi-locus molecular analysis, including more samples of red brocket from the countries that formed the Chaco Region in south America such as Argentina, Bolivia and Paraguay.

## 6. REFERENCES

Abril V.V., Duarte J.M.B. 2008. Chromosome polymorphism in the Brazilian dwarf brocket deer, *Mazama nana* (Mammalia, Cervidae). **Genetics and Molecular Biology** 31: 53–57.

Abril V.V., Carnelossi E.A.G., Gonzalez S., Duarte J.M.B., 2010. Elucidating the evolution of the red brocket deer *Mazama americana* complex (Artiodactyla; Cervidae). **Cytogenetic and Genome Research** 128: 177–187.

Adámoli J., Sennhauser E., Acero J.., Rescia A. 1990. Stress and disturbance: vegetation dinamics in the dry Chaco region of Argentina. **Journal of Biogeography** 17: 491–500.

Adámoli J., Ginzburg R., Torrella S., Herrera P. 2004. Expansión de la frontera agraria en la región chaqueña: el ordenamiento territorial como herramienta para la sustentabilidad. **Gerencia Ambiental** 11:810–823.

Allen J.A. 1915. Notes on American deer of the genus *Mazama.* Bulletin of the **American Museum of Natural History** 34:521–553.

Bernegossi A, M., Borges C., Sandoval E. D. P., Cernohorska H., Svatava K., Vozdova, M., Caparroz R., González S., Duarte, J. M. B. 2022. Resurrection of the genus *Subulo* Smith, 1827 for the gray brocket deer, with designation of a neotype. **Journal of Mammalogy.** doi: 10.1093/jmammal/gyac068

Bisbal F. 1991. Distribución y taxonomía del venado matacán (*Mazama* sp.) en Venezuela. **Acta Biológica Venezuélica** 13:89–104.

Cabrera A. 1961. Catálogo de los mamíferos de America der Sur. Revista del Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”. **Zoologia** 4(2):309–732.

Caparroz R., Mantellatto A M.B., Bertioli D.J., Figueiredo M. G., Duarte J.M.B. 2015. Characterization of the complete mitochondrial genome and a set of polymorphic microsatellite markers through next-generation sequencing for the brown brocket deer *Mazama gouazoubira*. **Genetics and Molecular Biology** 38(3):338–345.

Cifuentes-Rincón A., Morales-Donoso J.A., Sandoval E.D.P., Tomazella I.M., Mantellatto A.M.B., de Thoisy B., Duarte J.M.B. 2020. Designation of a neotype for *Mazama americana* (Artiodactyla, Cervidae) reveals a cryptic new complex of brocket deer species. **ZooKeys** 958: 143–164.

Cursino M., Salviano M., Abril V., Zanetti E. D., Duarte, J. M. 2014. The Role of Chromosome Variation in the Speciation of the Red Brocket Deer Complex: The Study of Reproductive Isolation in Females. **BMC Evolutionary Biology** 14, 40. Doi:10.1186/1471-2148-14-40

Demarchi D. A., Garcia A. 2008. Genetic Structure of Native Population from the Gran Chaco Region, South America. **International Journal of Human Genetic** 8(1-2): 131–141.

Dobigny G., Britton-Davidian J., Robinson T. J. 2017. Chromosomal Polymorphism in Mammals: an Evolutionary Perspective. **Biological Reviews** 92: 1–21.

Duarte J.M.B., Jorge W. 1996. Chromosomal polymorphism in several populations of deer (genus *Mazama*) from Brazil. **Archivos de Zootecnia** 45: 281–287.

Duarte J.M.B., González S., Maldonado J.E. 2008. The surprising evolutionary history of South American deer. **Molecular Phylogenetics and Evolution** 49:17–22.

Duarte J.M.B., Boer J.A., Sandoval E.D.P., Bernegossi A.M., Tomazella I.M. 2021. Skin Freezing Technique for Living Cell Bank. GenProtocols. Available

from https://genprotocols.genengnews.com/protocols/skin-freezing-technique-for-living-cell-bank/1041

Erxleben J.C.P. 1777. Systema regni animalis per classes, ordines, genera, species, varietates com synonymia et history animalium, Classis 1. Mammalia. Imprensis Weygandianis.

Escobedo-Morales L.A., Mandujano S., Eguiarte L.E., Rodríguez-Rodríguez M.A., Maldonado J.E. 2016. First phylogenetic analysis of Mesoamerican brocket deer *Mazama pandora* and *Mazama temama* (Cetartiodactyla:Cervidae) based on mitochondrial sequences: Implications on Neotropical deer evolution. **Mammalian Biology** 81: 303–313.

Frohlich J., Kubickova S., Musilova P., Cernohorska H., Muskova H., Vodicka R., Rubes J. 2017. Karyotype relationships among selected deer species and cattle revealed by bovine FISH probes. **PLOSone** 12(11): e0187559.

Galindo D.J., Martins G.S., Vozdova M., Cernohorska H., Kubickova S., Bernegossi A.M., Kadlcikova D., Rubes J., Duarte J.M.B. 2021. Chromosomal Polymorphism and Speciation: The Case of the Genus *Mazama* (Cetartiodactyla; Cervidae). **Genes** 12(2):165.

González S., Maldonado J. E., Ortega J., Talarico A. C., Bidegaray-Batista, L., Garcia, J. E., et al. 2009. Identification of the Endangered Small Red Brocket Deer (*Mazama bororo*) Using Noninvasive Genetic Techniques (Mammalia; Cervidae). **Molecular Ecology Resources** 9, 754–758.

Grubb P. 2005. Order Artiodactyla. In: Wilson DE, Reeder DM. (Eds.) **Mammal species of the world**. 3rded. Baltimore: Johns Hopkins University Press. pp: 629–722.

Gutiérrez E.E., Helgen K.M., McDonough M.M., Bauer F., Hawkins M.T.R.,

Escobedo-Morales L.A., Patterson B.D., Maldonado J.E. 2017. A gene-tree test of the traditional taxonomy of American deer: The importance of voucher specimens, geographic data, and dense sampling. **ZooKeys** 697:87–131.

Hall T.A. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. **Nucleic Acids Symposium Series** 41: 95–98.

Hammer O., Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. **Palaeontologia electronica** 4(1):9.

Heckeberg N.S. 2020. The systematics of the Cervidae: A total evidence approach. **PeerJ** 8:e8114.

Hensel R. 1872. Beiträge zur Kenntniss der Säugethiere Süd-Brasiliens. Abhandl. König. **Sitzungsberichte der Bayerischen Akademie der Wissenschaften** 1–130.

Hershkovitz P. 1982. Neotropical deer (Cervidae). Part 1. Pudus, genus *Pudu* Gray. **Fieldiana Zoology, New Series** 11:1–86.

Howell W., Black D. A. 1980. Controlled silver-staining of nucleous organizer regions with a protective 109ervidae109 developer. **Experientia** 36:1014–1015.

Huelsenbeck J., Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. **Bioinformatics** 17:754–755

Illiger J.K.W. 1815. Uebelblick der Säugethiere nach ihrer Vertheilung über die Welttheile. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlim 39–159.

Jorge W., Benirschke K. 1977. Centromeric heterochromatin and G-banding of the red brocket deer, *Mazama americana temama* (Cervoidea, Artiodactyla) with a probable non-Robertsonian translocation. **Cytologia**. **42**:711–721.

Kerr R. 1792. The animal kingdom; p. 303.

Kubickova S., Cernohorska H., Musilova P., Rubes J. 2002. The use of laser microdissection for the preparation of chromosome-specific painting probes in farm animals. **Chromosome Research** 46:143–147.

Levan A., Fredga K., Sandberg A. 1964. Nomenclature for centromeric position of chromosomes. **Hereditas** 52:201–219.

Lönnberg, E. 1919. On some mammals from the Argentine Chaco. **Arkiv för Zoologi** 12:1–20.

Merino M.L., Rossi R.V. 2010. Origin, Systematics, and Morphological Radiation. *In:* J.M.B. Duarte and S. Gonzalez S. (Eds.) **Neotropical Cervidology, Biology and Medicine of Latin American Deer.** Funep/IUCN, Jaboticabal/Gland. pp. 2–11.

Peres P.H. F., Luduvério D. J., Bernegossi A.M., Galindo D.J., Nascimento G.B., Oliveira M.L., Sandoval E.D.P., Vozdova M., Kubickova S., Cernohorska H., Duarte J.M.B. 2021. Revalidation of *Mazama rufa* (Illiger 1815) (Artiodactyla: Cervidae) as a Distinct Species out of the Complex *Mazama americana* (Erxleben 1777). **Frontiers in Genetics**. 12: 1–18. Doi: 10.3389/fgene.2021.742870.

Posada D., Crandall K.A. 1998. Modeltest: testing the modelo f DNA substitution. **Bioinformatics** 14:817–818.

Pucheran J. 1851. Note sur une espèce nouvelle de Cerf (Cervus rufinus Bourc. Et Puch). **Revue et Magasin de Zoologie** 2(III): 561–565.

Rafinesque G.S. 1817. No title. **American Monthly Magazine** 1:363–364.

Rambaut A. 2012. FigTree version 1.4.0. Computer program and documentation. University of Oxford. United Kingdom. http://tree.bio.ed.ac.uk/software/figtree/.

Richard E. 2008. Aspectos ecobiológicos de la corzuela parda (*Mazama gouazoubira*) en Argentina, aplicables al manejo de la especie en toda su área de distribución. En: Fontúrbel, F. (ed.) **Manejo y conservación de fauna silvestre: Un enfoque conceptual, metodológico y práctico para el tercer milenio**. CD-ROM interactivo, Ed. Publicaciones Integrales, La Paz, Bolivia. ISBN 99905-0- 490-3.

Richard E., Julia J. P., Samaniego J., Aceñolaza, P. 2008. La corzuela parda. **Serie monográfica y didáctica** 22: 7–35.

Rossi R.V. 2000. Taxonomia de *Mazama* Rafinesque, 1817 do Brasil (Artiodactyla, Cervidae). Dissertação de Mestrado – Universidade de São Paulo. São Paulo, São Paulo, Brasil.

Sambrook J., Fritsch E., Maniatis T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Press 16.

Salviano M. B., Cursino M. S., Zanetti E. D. S. S., Abril V. V., Duarte J. M. B. 2017. Intraspecific Chromosome Polymorphisms Can lead to Reproductive Isolation and Speciation: An Example in Red Brocket Deer (*Mazama* A). **Biology of Reproduction** 96, 1279–1287. Doi:10.1093/biolre/iox041

Sandoval E.D.P., Rola L.D., Morales-Donoso J.A., Gallina S., Reyna-Hurtado R., Duarte J.M.B. 2021. Integrative analysis of *Mazama temama* (Artiodactyla; Cervidae) and designation of a neotype for the species. **Journal of Mammalogy 103**:447–458.

Seabright M. 1971. A rapid banding technique for human chromosomes. **Lancet** 11:971–972.

Sneath P.H.A., Sokal R.R. 1973. Numerical taxonomy: the principles and practice of numerical classification. San Francisco, W. H. Freeman and Co.

Sumner A.T. 1972. A simple technique of demonstrating centromeric heterochromatin. **Experimental Cell Research** 75:304-306.

Telenius H., Carter N.P., Bebb C.E., Nordenskjold M., Ponder B.A.J., Tunnacliffe A. 1992. Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. **Genomics** 46:718–725.

Thomas O. 1913. The smaller south American 112ervidae. **Annual Magazine of Natural History** 8(11): 587

Thomas O. 1925. The Spedan Lewis South American exploration. On mammals from Southern Bolivia. **Annual Magazine of Natural History** 9: 581–582.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. 1997. The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. **Nucleic Acids Research** 25(24):4876–4882.

Verma R.S., Babu A. 1995. Human chromosomes: principles and techniques. 2nd ed. McGraw-Hill, Inc., New York.

Vozdova M., Kubickova S., Cernohorska H., Fröhlich J., Vodicka R., Rubes J. 2019 Comparative Study of the Bush Dog (*Speothos venaticus*) Karyotype and analysis of satellite DNA sequences and their chromosome distribution in six species of Canidae. **Cytogenetic and Genome Research** 159:88–96.

## SUPPLEMENTARY MATERIAL

**Figure S1.** Red brocket holotypes of Lönnberg, 1919. Macerated skull of the type specimen of *Mazama rufa toba* NRM6303601) Dorsal view. 2) Ventral view 3) Right lateral view 4) Left lateral view.

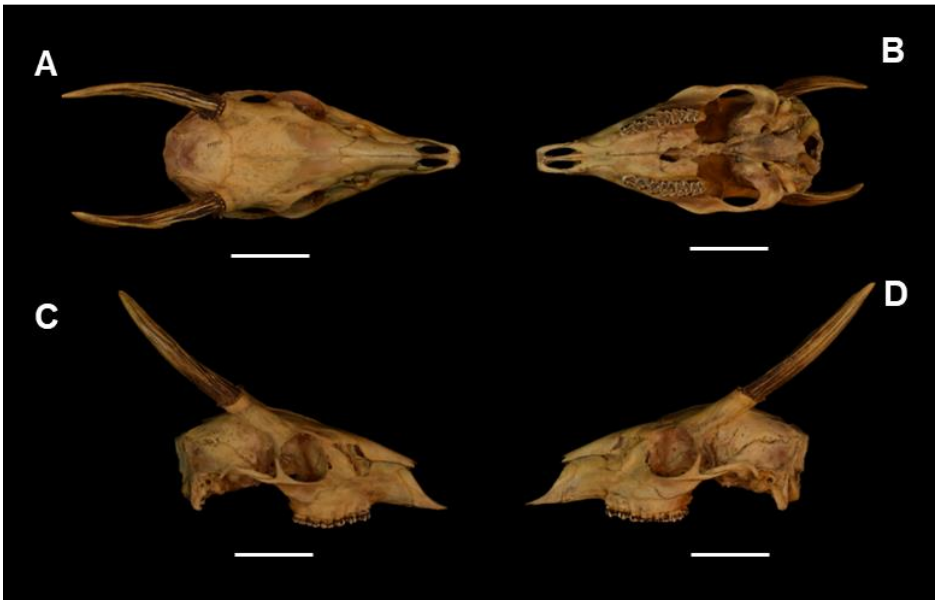



**Figure S2.** Chromosomal correspondence between *Mazama rufa toba* (MTO), *Mazama rufa* (MRU) and *B. taurus* (BTA), pairs 1 to 7.

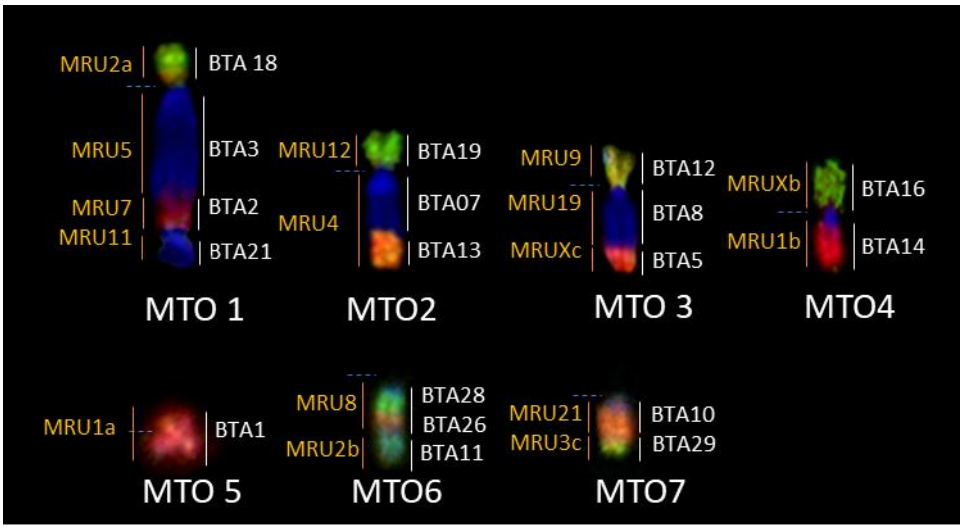


**Tabela S1.** Body measurements of a male topotype of *Mazama rufa toba* Lönnberg, 1919 (T451) in milimeters (mm). Mass in kilogram (kg).

| CHARACTER in mm | T451 | CHARACTER in mm | T451 |
|---|---|---|---|
| **Head length** | 20.5 | **Body length** | 77 |
| **Head width** | 8.55 | **Neck diameter** | 26 |
| **Ear length** | 10.4 | **Thorax diameter** | 60 |
| **Between eyes** | 4.85 | **Abdomen diameter** | 60 |
| **Mandible** | 6.95 | **Metatarsus length** | 20.5 |
| **Metacarpus length** | 13 | **tail Length** | 10.5 |
| **Height** | 57 | **Weight** | 19.7 |

**Tabela S2.** Skull measurements of *Mazama rufa toba* Lönnberg, 1919 in milimeters (mm) following DRIESCH (1976). Mass in kilogram (kg).

| Character in mm | NRM 630363 *M. rufa toba* | Character in mm | NRM 630363 *M. rufa toba* |
|---|---|---|---|
| **CT** | 136.87 | **CLP** | 25.00 |
| **CCB** | 186.92 | **MCIO** | 35.93 |
| **CB** | 175.96 | **MAIO** | 34.2 |
| **CCC** | 102.46 | **MLM** | 62.16 |
| **PP** | 75.17 | **MLCO** | 39.81 |
| **CVC** | 83.52 | **MLPP** | 61.99 |
| **CFM** | 124.62 | **MLFM** | 20.35 |
| **LR** | 158.94 | **MLNC** | 59.47 |
| **LP** | 188.52 | **LFM** | 67.64 |
| **ACR** | 139.35 | **MLEO** | 58.64 |
| **MCN** | 48.41 | **LMEO** | 45.18 |
| **CCFL** | 91.62 | **LZ** | 84.36 |
| **CPO** | 78.87 | **MLEN** | 22.99 |
| **CLP** | 46.98 | **MLPM** | 24.48 |
| **DCD** | 52.47 | **MLP** | 56.98 |
| **CLM** | 27.93 | **BCNS** | 45.07 |

**Table S3.** Chromosomal correspondence between *Mazama rufa toba* (MTO), and *B. taurus* (BTA)

| Classification MTO | BTA homology | Classification MTO | BTA homology |
|---|---|---|---|
| **1a (braço p)** | 18 | **9** | 15 |
| **1b (braço q proximal)** | 3 | **10** | 1c |
| **1c (braço q medial)** | 2prox | **11** | 20 |
| **1d (braço q distal)** | 21 | **12** | 17 |
| **2a (braço p)** | 19 | **13** | 6prox |
| **2b (braço q proximal)** | 7 | **14** | 5prox |
| **2c (braço q distal)** | 13 | **15** | 9prox |
| **3ª (braço p)** | 12 | **16** | 6dist |
| **3b (braço q proximal)** | 8prox | **17** | 22 |
| **3c (braço q distal)** | 5dist | **18** | 23 |
| **4ª (braço p)** | 16 | **19** | 24 |
| **4b (braço q)** | 14 | **20** | 2dist |
| **5 (inversão)** | 1prox1t1d | **21** | 27 |
| **6ª (proximal)** | 28/26 | **22** | 25 |
| **6b (distal)** | 11 | **23** | 8dist |
| **7ª (proximal)** | 10 | **24** | 9dist |
| **7b (distal)** | 29 | X | X |
| **8** | 4 | - | - |

# CAPÍTULO 5 – Assessing the taxonomic status of *Mazama simplicicornis argentina* Lönnberg, 1919 (Artiodactyla: Cervidae)

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## ABSTRACT

*Mazama simplicicornis argentina* is the name that was given to describe a gray brocket collected by Lönnberg in 1919 in the central Chaco region of Argentina. Subsequent authors, based on morphological similarities, considered this name to be a synonym for 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 corresponded with 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 revealing 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 a branch value of 100%. These results show that there is no discontinuity between the Argentinian and Paraguayan gray brockets. Therefore, the individuals originally described as *M. simplicicornis argentina* should be recognized as *S. gouazoubira* species.

**Key-word:** Animal cytogenetic, Cervidae, gray brocket, mitochondrial DNA, morphology

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## 1. 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 identified individuals from Paraguay and named them Gouazoubira, a name of Guarani 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) and subsequently transferred to the genus *Mazama* by Rafinesque (1817) due to 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 2020).

Authors have discussed this polyphyletic condition of the genus *Mazama*, in which the species are recovered in two subtribes, the Blastocerina and Odocoileina (Heckeberg 2020). In this sense, the Azara´s individuals would be recovered within the Blastocerina subtribe, separated from *M. americana* (Erxleben, 1777), the type species of the genus *Mazama,* that is recovered within the Odocoileina subtribe (Gutiérrez et al. 2017; Heckeberg 2020; Bernegossi et al. 2022). A recent study of the complete mitochondrial phylogeny confirmed the relationship and reviewed the applicability of the name-group *Subulo* Smith, 1827, to describe the gray brocket deer, resulting in the 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 for 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) suggested 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, which was considered absent in the individual from Paraguay (Lönnberg 1919).

During the 20th 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 region 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. Some have defended the absence of morphological differences between *S. gouazoubira* subspecies, and Azara´s description as the only gray brocket present in Argentina (Cabrera 1960; Czernay 1987). In this context, cytogenetic data is an important tool to clarify taxonomic status. This was demonstrated in studies analyzing subspecies of *Mazama americana,* which identified karyotypic divergences that 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.

## 2. MATERIALS AND METHODS

### 2.1 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 iss 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.

### 2.2 Morphological characterization

After collecting, the topotype was photographed, and 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 and basion (defined as the highest point of the superior nuchal crest).

We used the “Paleontological Statistics” PAST program (Hammer et al. 2001) to perform a cluster analysis by the Unweighted Pair Group Method with Arithmetic mean method (UPGMA) based on Euclidean 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.*

### 2.3 Cytogenetic analysis

After collecting the topotype, 5 x 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 characterize 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® 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® Array CGH Genomic Labeling (Invitrogen, Carlsbad, CA, USA). FISH was performed as described in Vozdova et al. (2019). A Zeiss Axio Imager Z2 (Carl Zeiss Microimaging GmbH, Jena, Germany) fluorescence microscope, equipped with appropriate fluorescence filters for the visualization of FISH results, was used.

**2.4 DNA extraction and sequencing**

The genomic DNA was extracted from the 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 of 5 µL of SensiFASTTM 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) / 72ºC for 20 seconds.

Purification of the PCR products was performed with the Wizard SV gel and PCR Clean-Up System kit (Promega TM), followed by sequencing using the

ABI BigDye Terminator kit (Applied Biosystems) in an ABI 3130xl automatic sequencer (Applied Biosystems).

### 2.5 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* 691pb partial gene and *ND5* 688 bp partial gene) (Table 1). From the GenBank, we obtained available sequences of the 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 the ends were manually replaced 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 MRBAYES 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 |
|---|---|---|---|---|
| *Mazama simplicicornis argentina* (NRM-MA620394) | OP627522 | OP627524 OP627525 | Chaco Province, Argentina. Lönnberg 1919. | This study |
| *Mazama simplicicornis argentina* (T450) | OP627521 | OP627523 | Chaco Province, Presidencia de la Plaza. Argentina | This study |
| *Subulo gouazoubira* (T377) | MZ350858 | MZ350858 | Puerto Galileo, Paraguay | Bernegossi et al. 2022 |
| *Subulo gouazoubira* (T389) | MZ350866 | MZ350866 | Puerto Arecutacuá, Paraguay | Bernegossi et al. 2022 |
| *Subulo gouazoubira* (T082) | MZ350862 | MZ350862 | Camobi-RS, Brazil | Bernegossi et al. 2022 |

| | | | | |
|---|---|---|---|---|
| *Subulo gouazoubira* | KJ772514 | KJ772514 | Pantanal, Brazil | Caparroz et al. 2015 |
| *Subulo gouazoubira* (MRGsp2) | JN632658 | JN632658 | Colombia | Hassanin et al. 2012 |
| *Mazama nemorivaga* (T24) | MZ350861 | MZ350861 | Porto Velho-RO, Brazil | Bernegossi et al. 2022 |
| *Mazama nemorivaga* (T346) | MZ350867 | MZ350867 | Lupar-PA, Brazil | Bernegossi et al. 2022 |
| *Mazama nemorivaga* | JN632659 | JN632659 | Peru | Hassanin et al. 2012 |
| *Mazama nemorivaga* | JN632660 | JN632660 | French Guiana | Hassanin et al. 2012 |
| *Mazama americana* (MAZ9472) | JN632656 | JN632656 | French Guiana | Hassanin et al. 2012 |
| *Mazama americana* (T358) | MZ350857 | MZ350857 | Cayenne, French Guiana | Bernegossi et al. 2022 |
| *Mazama americana* (T255*)* | MN726909 | MZ488890 | Juina, Brazil | Cifuentes-Rincon et al. 2020/ Peres et al. 2021 |
| *Mazama americana* (T253) | MZ350856 | MZ350856 | Juina, Brazil | Bernegossi et al. 2022 |
| *Mazama rufa* (T385) | MZ488852 | MZ488894 | Foz de Iguaçu, Paraná, Brazil | Peres et al. 2021 |
| *Mazama nana* (T107) | MZ350863 | MZ350863 | Paraguay | Bernegossi et al. 2022 |
| *Mazama jucunda* (T071) | DQ789231 | MZ488899 | Paraná, Brazil | Duarte et al. 2008/ Peres et al. 2021 |
| *Mazama jucunda* (T215) | MZ350859 | MZ350859 | P. E. Intervales-SP, Brazil | Bernegossi et al. 2022 |
| *Mazama temama* (T366) | MZ350864 | MZ350864 | Veracruz, México | Bernegossi et al. 2022 |
| *Hippocamelus antisensis* | JN632646 | JN632646 | Argentina | Hassanin et al. 2012 |
| *Alces alces* | MF784602 | MF784602 | Poland | Swisloca et al. 2020 |
| *Ozotoceros. bezoarticus* (FNMA40) | MZ350860 | MZ350860 | Brazil | Bernegossi et al. 2022 |
| *Blastocerus dichotomus* (CV21) | OM543539 | OM543540 | Brazil | Sandoval et al. 2022 |

## 3. RESULTS

### 3.1 Morphological characterization

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).


**Figure 1.** Lateral view of the adult male topotype of *Mazama simplicicornis argentina* (Lönnberg 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.

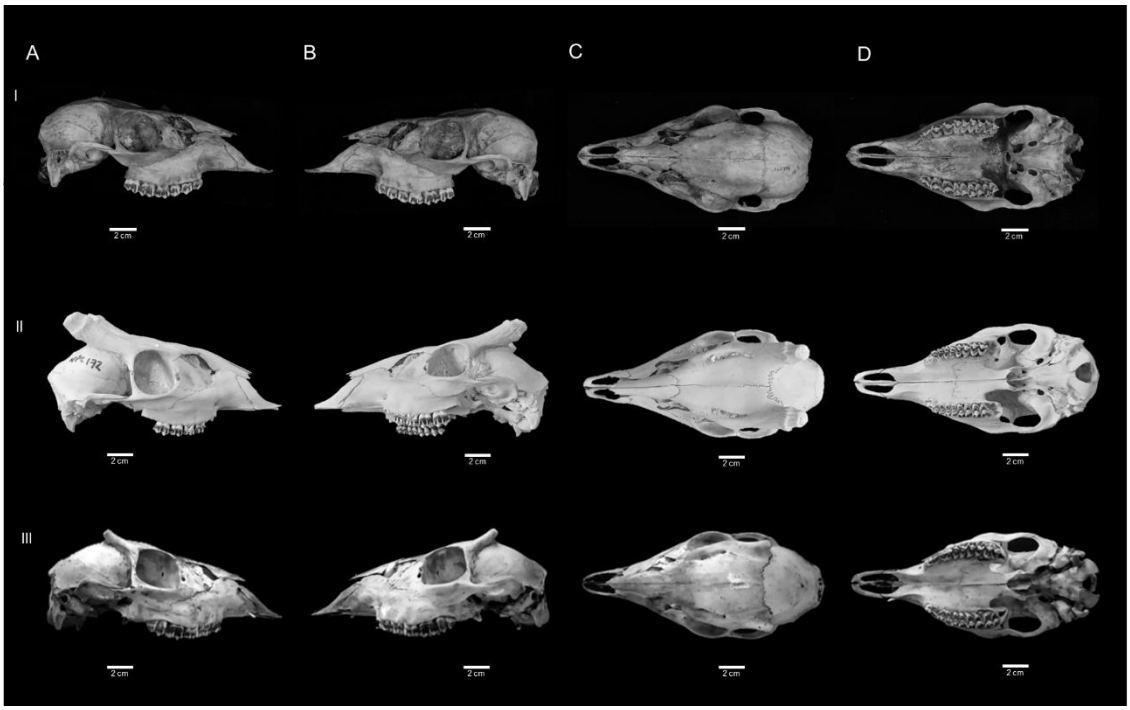


**Figure 2.** Skull views of *Mazama simplicicornis argentina* (I) Holotype and (II) topotype, compared with (III) the skull of *Subulo gouazoubira* neotype (Bernegossi et al. 2022, adapted). A= right lateral view, B= left lateral view, C= dorsal view and D= ventral view.

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.

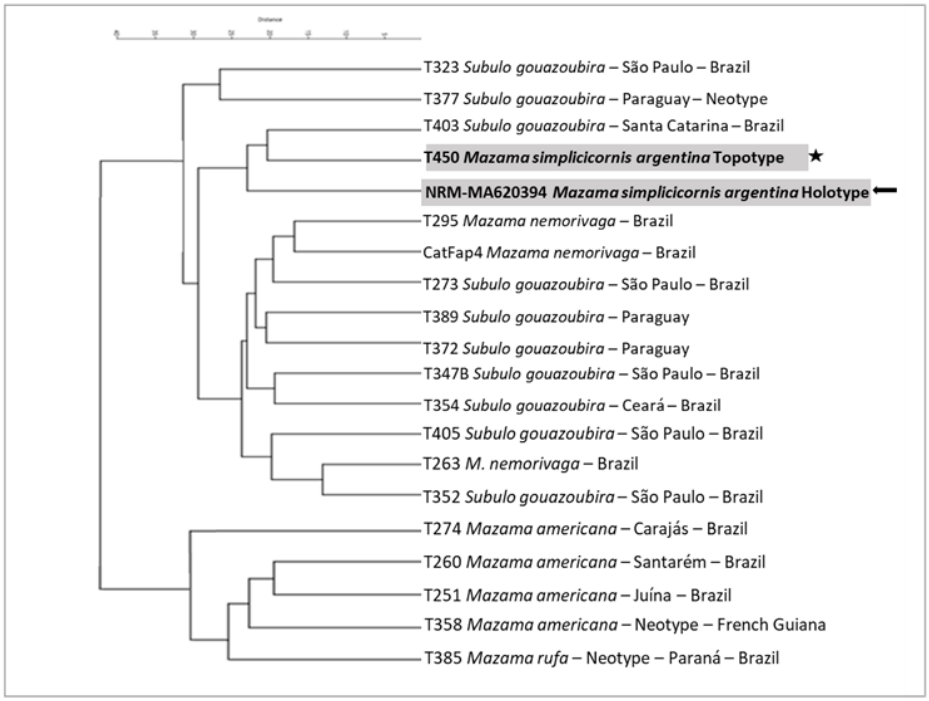


**Figure 3.** Cluster analysis of skull measurements of *Mazama simplicicornis argentina* (MSA) (Lönnberg 1919) compared with *Subulo gouazoubira*, *Mazama nemorivaga*, *Mazama americana* and *Mazama rufa*; star= collected adult male topotype; arrow= adult female holotype.

### 3.2 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 (Figure 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).

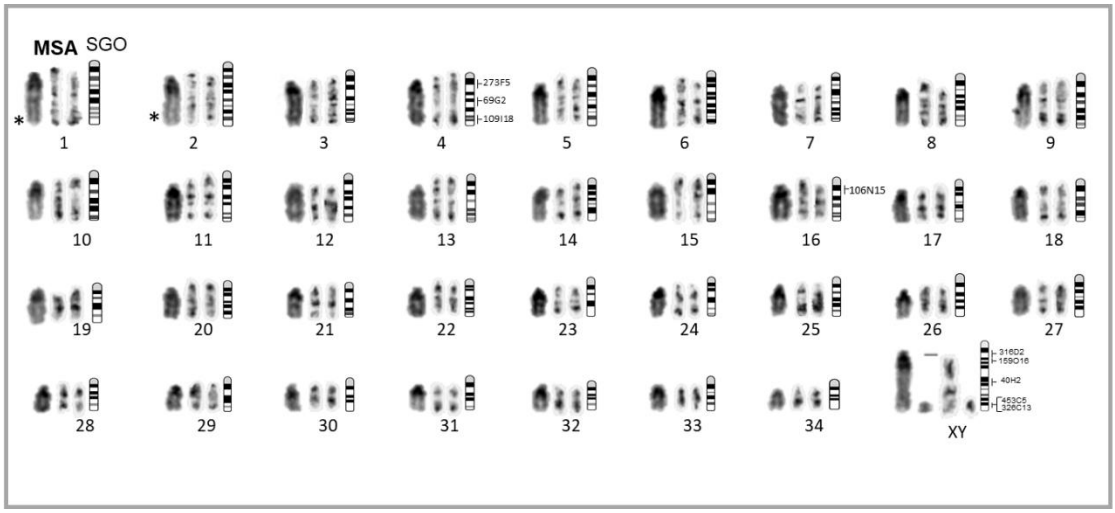


**Figure 4.** Karyotype of a male topotype of *Mazama simplicicornis argentina* (MSA) (Lönnberg 1919) collected in the Chaco Province of Argentina. From left to the right: chromosomes under C-banding, G-banding and ideogram of *Subulo gouazoubira* (SGO) showing correspondence to MSA chromosomes (adapted 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.

### 3.3 Phylogenetic analysis

The Bayesian Inference tree of concatenated fragments of *Cytb* and *ND5* showed two major clades. The first clade consisted of the Odocoileina subtribe, which included the species *M. jucunda*, *M. nana*, *M. temama*, *M. rufa*, and the paraphyletic *M. americana*. The second clade comprised the Blastocerina subtribe, encompassing the species *S. gouazoubira*, *M. nemorivaga*, *H. antisensis*, *B. dichotomus*, and *O. bezoarticus* (Fig. 5). Both the holotype and topotype of *M. simplicicorins 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 of *S. gouazoubira* from Paraguay and the topotype was recovered in a subclade with one individual from Brazil.

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

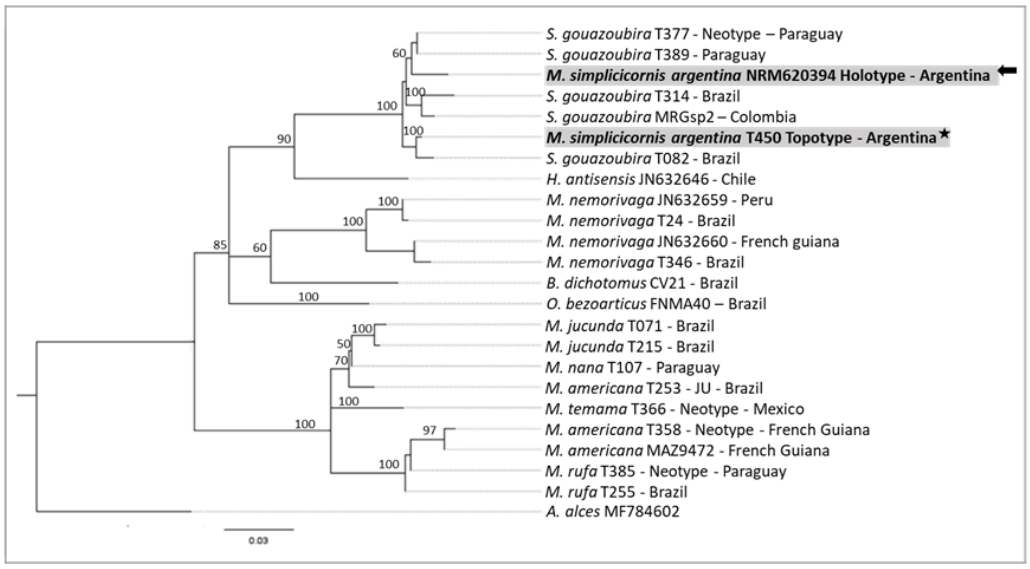


**Figure 5.** Bayesian Inference of concatenated mitochondrial genes *Cytb* (partial 224bp) and *ND5* (partial 224bp and partial 249bp) of neotropical deer species including *Mazama simplicicornis argentina* topotype (star) and holotype (arrow). Outgroup= *A. alces.*

## 4. 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 with 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 can be allocated in the same small brocket group as *S. gouazoubira* individuals. It is important to note that *M. nemorivaga* individuals were also recovered in the same group, which contrasts with previous studies that found them to be morphometrically different from *S. gouazoubir*a due to smaller skull measurements (González et al. 2018; Rossi 2000). The diverging results may be related to the different sampling numbers 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 in distinguishing *S. gouazoubira* from *M. nemorivaga* with larger orbital cavities, and narrow auditory bulla (Rossi 2000). 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. The diploid number of the *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 the *M. simplicicornis argentina* topotype. In addition, the B chromosomes were not observed in the metaphases of the topotype, which are commonly reported in *S. gouazoubira* individuals. The descriptions had shown 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 there is 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 a branch value of 100%. 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 the *S. gouazoubira* clade to be within the Blastocerina subtribe as previously described (Gutiérrez et al. 2017; Heckeberg 2020), further supporting 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 did not detected differentiation between the *M. simplicicornis argentina* holotype, topotype and *S. gouazoubira* neotype, and the other *S. gouazoubira* specimens. In conclusion, our analysis supports the idea that *M. simplicicornis argentina* should be considered as junior synonym of *Subulo gouazoubira*. This confirmation, in addition to revealing genetic data of gray brocket individuals from Chaco Argentina, contributes to the taxonomic

clarification of the Lönnberg´s (1919) description in the context of the 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 OP627525.

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## 5. REFERENCES


Allen JA. 1915. Notes on American deer of the genus. Bull. of the Am. Mus. Nat. Hist. **34**:521–553.

Avila-Pires FD. 1959. As formas sul-americanas do veado-virá. An. Acad. Bras. Ciên. **31(4)**:547–556.

Azara F. 1801. Essais sur l'histoire naturelle des quadrupedes de la province du Paraguay. Traduits sur le manuscrit inédit de l'auteur par M. L. E. Moreau-Saint-Méry. Charles Pougens, Paris.

Bernegossi AM, Borges C, Sandoval EDP, Cernohorska H, Svatava K, Vozdova M, Caparroz R, González S, Duarte JMB. 2022. Resurrection of the genus *Subulo* Smith, 1827 for the gray brocket deer, with designation of a neotype. J. Mammal. doi: 10.1093/jmammal/gyac068

Black-Décima P, Rossi RV, Vogliotti A, Cartes JL, Maffei L, Duarte JMB, Gonzalez S, Juliá JP. 2010. Brown Brocket Deer *Mazama gouazoubira* (Fischer 1814). *In*: Duarte J.M.B., Gonzalez S. (eds.) Neotropical Cervidology, Biology and Medicine of Latin American Deer. Funep/IUCN, Jaboticabal/Gland, p. 190–201.

Cabrera A. 1960. Catálogo de los mamíferos de América del Sur. Rev. Mus. Argent. Cienc. Nat. **4**:309–732.

Caparroz R, Mantellatto AM, Bertioli DJ, Figueiredo MG, Duarte JMB. 2015. Characterization of the complete mitochondrial genome and a set of polymorphic microsatellite markers through next-generation sequencing for the brown brocket deer *Mazama gouazoubira*. Gen. Mol. Biol. **38**:338–345. doi:10.1590/S1415-475738320140344

Caraballo CF. 2009. Patrón de uso de hábitat del guazuncho (*M. gouazoupira*, Artiodactyla, Cervidae) durante un ciclo anual, en bosques nativos y exóticos del Centro-Oeste de Entre Ríos. APRONA, Bol. Cient. **41**:1–15.

Cifuentes-Rincón A, Morales-Donoso JA, Sandoval EDP, Tomazella IM, Mantellatto AMB, de Thoisy B, Duarte JMB. 2020. Designation of a neotype for *Mazama americana* (Artiodactyla, Cervidae) reveals a cryptic new complex of brocket deer species. ZooKeys **958**:143–164. doi: 10.3897/zookeys.958.50300

Czernay S. 1987. Die Spiesshirsche und Pudus: die Gattungen *Mazama* und *Pudu*. A. Ziemsen, Wittenberg Lutherstadt. Die Neue Brehm-Bucherei Heft **581**:84.

Driesch A. 1976. A Guide to the Measurement of Animal Bones from Archaeological Sites. Massachusetts: Peabody Museum Harvard Univ. Massachusetts, p. 27–57.

Duarte JMB, Jorge W. 1998. Análise citotaxonômica dos *Mazama* cinzas do Brasil (*Mazama gouazoubira* e *Mazama rondoni*). *In*: Duarte, JMB, (Eds.). Análise citogenética e taxonômica do gênero *Mazama* (Cervidae; Artiodactyla) no Brasil. Ph.D. Dissertation, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil.

Duarte JMB, Boer JA, Sandoval EDP, Bernegossi AM, Tomazella IM. 2021. Skin Freezing Technique for Living Cell Bank. GenProtocols. Available from https://genprotocols.genengnews.com/protocols/skin-freezing-technique-for-living-cell-bank/1041

Duarte JMB, González S, Maldonado JE. 2008. The surprising evolutionary history of South American deer. Mol. Phylogenet. and Evol. **49**:17–22. doi:10.1016/j.ympev.2008.07.009

Duarte JMB, Jorge W. 2003. Morphologic and cytogenetic description of the small red brocket (*Mazama bororo* Duarte, 1996) in Brazil. Mammalia. **67**: 403–410. doi:10.1515/mamm.2003.67.3.403

Erxleben JCP. 1777. Systema regni animalis per classes, ordines, genera, species, varietates cum synonymia et history animalium, Classis 1. Mammalia. Impensis Weygandianis.

Fischer G. 1814. Zoognosia Tabulis Synopticis Illustrata. Typis Nicolai Sergeidis Vsevolozky. Mosquae **3**:17–32.

Frohlich J, Kubickova S, Musilova P, Cernohorska H, Muskova H, Vodicka R, Rubes J. 2017. Karyotype relationships among selected deer species and cattle revealed by cattle FISH probes. PLOSone 1–17. doi: 10.1371/journal.pone.0187559

Galindo DJ, Martins GS, Vozdova M, Cernohorska H, Kubickova S, Bernegossi AM, Kadlcikova D, Rubes J, Duarte JMB. 2021. Chromosomal Polymorphism and Speciation: The Case of the Genus *Mazama* (Cetartiodactyla; Cervidae). Genes **12**:165. doi: 10.3390/genes12020165

Gilbert C, Ropiquet A, Hassanin A. 2006. Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): systematics, morphology, and biogeography. Mol. Phylogenet. Evol. **40**:101–117. doi: 10.1016/j.ympev.2006.02.017

González S. 1997. Estudio de la variabilidad morfológica, genética y molecular de poblaciones relictuales de venado de campo (*Ozotoceros bezoarticus* L. 1758) y sus consecuencias para la conservación. Dissertation, PEDECIBA.

González S, Maldonado JE, Ortega J, Talarico AC, Bidegaray-Batista L, Garcia JE, Duarte JMB. 2009. Identification of the Endangered Small Red Brocket Deer (*Mazama bororo*) Using Noninvasive Genetic Techniques (Mammalia; Cervidae). Mol. Ecol. Resour. **9**:754–758. doi: 10.1111/j.1755-0998.2008.02390.x

González S, Mantellatto AMB, Duarte JMB. 2018. Craniometrical differentiation of gray brocket deer species from Brazil. Rev. Mus. Argen. Cienc. Nat. **20(1)**:179–193. doi: 10.22179/REVMACN.20.561

Gray JE. 1850. Synopsis of the species of deer (Cervina), with the description of a new species in the Gardens of the Society. Proc. Zool. Soc. Lond. **18**:222–242.

Gutiérrez EE, Helgen KM, McDonough Bauer F, Hawkins MTR, Escobedo-Morales LA, Patterson BD, Maldonado JE. 2017. A Gene-Tree Test of the Traditional Taxonomy of American Deer: The Importance of Voucher Specimens, Geographic Data, and Dense Sampling. Zookeys **697**:87–131. doi:10.3897/ zookeys.697.15124

Hall TA. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**:95–98.

Hammer O, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software 845 package for education and data analysis. Palaeontol. eléctron. **4(1)**:9.

Hassanin A, Pasquet E, Vignes JD. 1998. Molecular systematics of the subfamily Caprinae (Artiodactyla, Bovidae) as determined from cytochrome b sequences. J. Mammal. Evol. **5**:217–236. doi: 10.1023/A:1020560412929

Hassanin A, Delsuc F, Ropiquet A, Hammer C, van Vuuren BJ, Matthee C, Ruiz-Garcia M, Catzeflis F, Areskoug V, Nguyen TT, Couloux A. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes Rendus. Biologies **335**:32–50. doi: 10.1016/j.crvi.2011.11.002

Heckeberg NS. 2020. The Systematics of the Cervidae: A Total Evidence Approach. PeerJ 8, e8114. doi:10.7717/peerj.8114

Hershkovitz P. 1982. Neotropical Deer (Cervidae): Part I. Pudus, Genus *Pudu* Gray. 11th ed. Chicago: Field Museum of Natural History.

Howell W, Black DA. 1980. Controlled silver-staining of nucleous organizer regions with a protective coloidal developer. Experientia **36**:1014–1015. doi:10.1007/BF01953855

Huelsenbeck J, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics **17**:754–755. doi:10.1093/bioinformatics/17.8.754

ICZN (International Commission on Zoological Nomenclature). 1999. International code of zoological nomenclature. The International Trust for Zoological Nomenclature (c/o The Natural History Museum), London, United Kingdom.

ICZN (International Commission on Zoological Nomenclature). 2001. Opinion 1985 (Case 3018). *Cervus gouazoubira* Fischer, 1814 (currently *Mazama gouazoupira*; Mammalia, Artiodactyla): specific name conserved as the correct original spelling. Bull. Zool. Nomencl. **58:**247

Illiger A. 1815. Überblick der S äugethiere nach ihrer Vertheilung über die Welttheile. Akademie der Wissenchaften, 1804–1811.

Jorge W, Benirschke K. 1977. Centromeric heterochromatin and G-banding of the red brocket deer, *Mazama americana temama* (Cervoidea, Artiodactyla) with a probable non-Robertsonian translocation. Cytologia. **42**:711–721. doi:10.1508/cytologia.42.711

Kerr R. 1792. The Animal Kingdom. *Cervus temama* 303.

Leandro SFS. 2019. Prospecção de oligonucleotídeos iniciadores para DNA mitocondrial e amplificação em amostras fecais de veado-campeiro (*Ozotoceros bezoarticus*). Final Scientific Report FAPESP n° 2017/26529-9.

Lesson RP. 1842. Nouveau tableau de règne animal. Mammifères. Arthus-Bertrand, Paris.

Lönnberg E. 1919. On some mammals from the Argentine Chaco. Arkiv för zoologi **12**:1-20.

Lydekker R. 1915. Catalogue of the ungulate mammals in the British Museum (Natural History). British Museum, London.

Medrano JF, Aasen E, Sharrow L. 1990. DNA extraction from nucleated red blood cells. Biotechniques **8(1)**:43.

Miranda-Ribeiro A. 1919. Os veados do Brasil segundo as coleções Rondon e de vários museus nacionais e estrangeiros. Rev. Mus. Paul. **11**:209–308.

Neitzel H. 1987. Chromosome evolution of Cervidae: karyotypic and molecular aspects. *In*: Obe G, Basler A, (eds.) Cytogenetics, Basic and applied aspects. Springer Verlag. Berlin. p. 90–112.

Pelzeln AV. 1883. Brasilische Säugethiere. Resultate von Johann Natterer's Reisen in den Jahren 1817 bis 1835. Verhandlungen der kaiserlich-königlichen zoologisch- botanischen Gesellschaft **33**:1–140.

Peres PHF, Luduvério DJ, Bernegossi AM, Galindo DJ, Nascimento GB, Oliveira ML, Sandoval EDP, Vozdova M, Kubickova S, Cernohorska H, Duarte JMB. 2021. Revalidation of *Mazama rufa* (Illiger 1815) (Artiodactyla: Cervidae) as a Distinct Species out of the Complex *Mazama americana* (Erxleben 1777). Front. Genet. **12**:1–18. doi: 10.3389/fgene.2021.742870

Periago ME, Leynaud GC. 2009. Density estimates of *Mazama gouazoubira* (Cervidae) using the pellet count technique in the arid Chaco (Argentina). Ecol. Austral **19**:73–77.

Pinder L, Leeuwenberg F. 1997. Veado-catingueiro (*Mazama gouazoubira*, Fischer 1814). *In*: Duarte, JMB, (ed) Biologia e conservação de cervídeos sul-americanos: *Blastocerus*, *Ozotoceros* e *Mazama*. FUNEP, Jaboticabal, p.60–68.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics **14**:817–818. doi:10.3389/fgene.2017.00010

Rafinesque GS. 1817. No title. Am. Mon. Mag. **1**:363–364

Rambaut A. 2012. FigTree version 1.4.0. Computer program and documentation. University of Oxford. United Kingdom. http://tree.bio.ed.ac.uk/software/figtree/.

Richard E, Juliá JP. 2001. La corzuela parda. *In*: Dellafiori, C., Maceira, N. (eds.) Los ciervos autóctonos de la argentina y la acción del hombre. Secretaría de

Desarrollo Sustentable y Política Ambiental. Ministerio de Desarrollo Social y Medio Ambiente. Buenos Aires, Argentina, p. 35– 46.

Rossi RV. 2000. Taxonomia de *Mazama* Rafinesque, 1817 do Brasil (Artiodactyla, Cervidae). M.S. thesis, Universidade de São Paulo. São Paulo, Brazil.

Sambrook J, Fritsch E, Maniatis T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Press 16.

Sandoval EDP, Rola LD, Morales-Donoso JA, Gallina S, Reyna-Hurtado R, Duarte JMB. 2022. Integrative analysis of *Mazama temama* (Artiodactyla: Cervidae) and designation of a neotype for the species. J. Mammal. **103**(2):447–458. doi: 10.1093/jmammal/gyab169

Seabright M. 1971. A rapid banding technique for human chromosomes. Lancet **11**:971–972. doi:10.1016/S0140-6736(71)90287-X

Sumner AT. 1972. A simple technique of demonstrating centromeric heterochromatin. Exp. Cell Res. **75**:304–306. doi:10.1016/0014-4827(72) 90558-7

Smith HC. 1827. The seventh order of the Mammalia. The Ruminantia. *In*: Griffith E, Smith CH, Pidgeon E, (eds.) The animal kingdom arranged in conformity with its organization, by the Baron Cuvier, with additional descriptions of all the species hitherto named, and many not before noticed, by Edward Griffith and others. Geo B. Whitaker, London. **5**:298–376.

Świsłocka M, Matosiuk M, Ratkiewicz M, Borkowska A, Czajkowska M, Mackiewicz P. 2020. Phylogeny and diversity of moose (*Alces alces*, Cervidae, Mammalia) revealed by complete mitochondrial genomes. Hystrix **31**(1): 1–9. doi: https://doi.org/10.4404/hystrix-00252-2019

Thomas O. 1913. The smaller south American cervidae. Ann Mag Nat Hist (Series 8) **11**:587

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. Nucleic Acids Res. **25(24)**:4876–4882. doi:10.1093/nar/25.24.4876

Tomazella IM. 2016. Análise do polimorfismo cromossômico em *Mazama gouazoubira* (Artiodactyla; Cervidae): implicações para a evolução cariotípica

em Cervidae. Dissertation, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista. Jaboticabal, São Paulo, Brazil.

Valeri MP, Tomazella IM, Duarte JMB. 2018. Intrapopulation chromosomal polymorphism in *Mazama gouazoubira* (Cetartiodactyla; Cervidae): The emergence of a new species? Cytogenet. Genome Res. **154**:147–152. doi: 10.1159/000488377

Verma RS, Babu A. 1995. Human chromosomes: principles and techniques. 2nd ed. McGraw-Hill, Inc., New York.

Vozdova M, Kubickova S, Cernohorska H, Fröhlich J, Vodicka R, Rubes J. 2019. Comparative Study of the Bush Dog (*Speothos venaticus*) Karyotype and analysis of satellite DNA sequences and their chromosome distribution in six species of Canidae. Cytogenet. Genome Res. **159**:88–96. doi:10.1159/000503082

## SUPPLEMENTARY MATERIAL

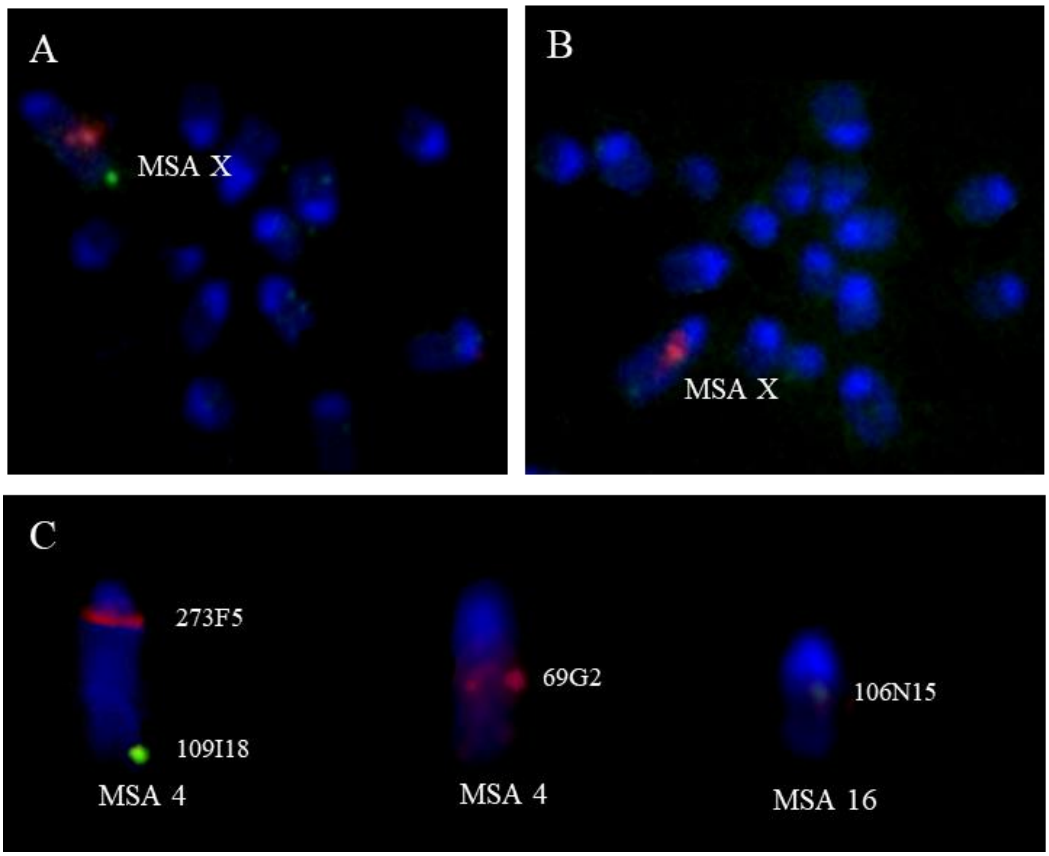


**Fig. S1.** FISH results using BAC clones derived from cattle on *Mazama simplicicornis argentina* (MSA) chromosomes. (A) X chromosome labeled with BAC40H2 (red) and BACs 453C5 and 326C13 (green). (B) X chromosome labeled with BAC159O16 (red). (C) Hybridization with the BAC clones derived from the BTA1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

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

| CHARACTER | T450 | CHARACTER | T450 |
|---|---|---|---|
| **Head length** | 225 | **Right testicle length** | 55 |
| **Head width** | 87 | **Left testicle length** | 60 |
| **Ear length** | 110 | **Right testicle diameter** | 30 |
| **Distance between eyes** | 55 | **Left testicle diameter** | 26 |
| **Mandible total length** | 65 | **Metatarsus length** | 224 |
| **Neck circumference** | 255 | **Tail length** | 125 |
| **Thorax circumference** | 590 | **Mass** | 20 |
| **Height** | 580 | **Body length** | 727 |
| **Metacarpus length** | 140 | **Abdomen circumference** | 600 |

**Table S2.** Skull measurement of a male topotype of *Mazama simplicicornis argentina* Lönnberg, 1919, and other neotropical cervids.

| ID | TL | CBL | BK | SSL | PR | CVC | GLN | SLFL | CPO | LLP | LCR | LMR0 | LPR | GILO | GIHO | GMB | GBBP | GBFM | HFM | GNB | LFB | LBBO | GBAO | ZB | GBAN | GBAP | GBP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T450 Topotype | 181.02 | 173.73 | 157.98 | 103.63 | 52.44 | 79.05 | 49.73 | 95.30 | 71.65 | 45.16 | 59.42 | 35.24 | 29.15 | 30.66 | 29.93 | 59.72 | 37.42 | 46.71 | 13.91 | 56.45 | 58.94 | 66.21 | 52.44 | 81.99 | 14.86 | 22.58 | 52.18 |
| NRM-MA 620394 Holotype | | 172.99 | 162.14 | 107.53 | 54.93 | 79.55 | 44.39 | 96.69 | 75.01 | 45.44 | 54.93 | 32.15 | 24.62 | 32.39 | 32.78 | 58.12 | 36.11 | 54.2 | 18.32 | 55.74 | 53.22 | 57.27 | 42.37 | 79.67 | 20.14 | 23.51 | 55.94 |
| T377_Sgo Neotype | 165.91 | 157.14 | 147.02 | 102.44 | 46.25 | 75.68 | 44.20 | 87.65 | 66.62 | 41.30 | 43.13 | 26.86 | 27.02 | 28.46 | 26.55 | 55.98 | 33.15 | 37.99 | 14.37 | 51.04 | 43.87 | 54.29 | 40.49 | 73.67 | 15.90 | 20.83 | 52.18 |
| T389_Sgo | 160.52 | 152.91 | 141.39 | 82.58 | 56.38 | 77.68 | 49.93 | 84.65 | 80.71 | 47.99 | 54.77 | 31.72 | 24.56 | 30.38 | 29.02 | 53.66 | 36.77 | 50.19 | 13.58 | 53.45 | 48.12 | 73.78 | 41.16 | 75.96 | 21.02 | 24.10 | 54.09 |
| T273_Sgo | 171.25 | 165.57 | 152.60 | 100.93 | 50.13 | 76.63 | 45.72 | 79.88 | 80.93 | 41.09 | | | | 30.95 | 29.47 | 71.18 | 32.63 | 51.59 | 15.62 | 54.87 | 44.00 | 60.51 | 43.29 | 69.51 | 17.11 | 22.04 | 59.25 |
| T372_Sgo | 178.50 | 175.66 | 155.86 | 94.49 | 56.44 | 85.17 | 54.01 | 88.91 | 76.67 | 45.73 | 57.35 | 32.69 | 26.25 | 28.65 | 29.25 | 53.18 | 34.43 | 49.66 | 15.85 | 54.33 | 43.70 | 64.25 | 46.08 | 75.38 | 19.65 | 20.39 | 60.95 |
| T354_Sgo | 163.67 | 155.12 | 140.90 | 94.27 | 49.60 | 67.59 | 43.50 | 62.52 | 66.53 | 38.91 | 46.34 | 25.56 | 21.92 | 33.18 | 27.36 | 52.98 | 29.50 | 45.79 | 13.20 | 55.65 | 46.76 | 53.78 | 43.29 | 67.59 | 18.03 | 17.01 | 48.63 |
| T352_Sgo | 180.75 | 172.76 | 155.10 | 106.50 | 51.35 | 83.74 | 29.80 | 29.85 | 66.98 | 33.02 | 44.70 | 14.15 | 53.22 | 44.13 | 63.94 | 47.02 | 73.79 | 19.62 | 20.14 | 55.78 | 45.18 | 59.05 | 48.12 | 68.29 | 19.89 | 18.25 | 47.78 |
| T405_Sgo | 181,00 | 175.22 | 150.2 | 100.22 | 50.17 | 74.59 | 54.78 | 75.28 | 76.4 | 41.2 | 47.29 | 28.54 | 24.51 | 30.52 | 33.63 | 66.74 | 28.56 | 45.1 | 17.34 | 54.58 | 43.61 | 63.35 | 44.13 | 67.02 | 20.01 | 18.18 | 45.62 |
| 347B_Mgo | 177.90 | 170.04 | 157.18 | 104.88 | 55.01 | 78.59 | 51.38 | 88.12 | 71.12 | 41.05 | 51.03 | 27.57 | 22.53 | 26.78 | 28.59 | 52.60 | 32.37 | 46.89 | 15.03 | 51.67 | 50.83 | 62.73 | 43.72 | 74.00 | 17.39 | 20.50 | 51.81 |
| T403_Mgo | 180.68 | 172.86 | 154.24 | 99.92 | 55.21 | 77.07 | 47.88 | 90.05 | 76.05 | 40.91 | 51.61 | 27.90 | 22.46 | 28.91 | 28.90 | 58.91 | 35.67 | 53.22 | 15.62 | 56.74 | 62.79 | 70.19 | 48.22 | 79.58 | 21.96 | 23.60 | 50.93 |
| T323_Mgo | 176.02 | 162.93 | 142.31 | 103.33 | 54.02 | 75.03 | 41.57 | 89.07 | 73.65 | 39.91 | 40.19 | 26.57 | 22.9 | 29.51 | 30.51 | 58.69 | 35.04 | 47.42 | 19.14 | 52.14 | 43.80 | 70.09 | 46.52 | 78.09 | 18.02 | 22.03 | 53.02 |
| T295_Mne | 175.63 | 171.01 | 155.66 | 103.42 | 49.80 | 83.94 | 48.71 | 85.28 | 75.63 | 37.31 | 56.90 | 32.96 | 26.65 | 32.97 | 33.62 | 56.55 | 32.38 | 51.47 | 16.65 | 58.37 | 58.06 | 72.27 | 52.09 | 76.57 | 21.60 | 23.48 | 81.71 |
| Catfap4_Mne | 178.55 | 173.37 | 155.97 | 101.31 | 55.39 | 83.22 | 50.60 | 89.43 | 76.15 | 44.61 | 52.09 | 29.27 | 24.68 | 32.56 | 32.20 | 57.56 | 30.83 | 47.93 | 15.16 | 57.94 | 57.06 | 63.87 | 49.78 | 76.45 | 19.85 | 21.75 | 76.44 |
| T263_Mne | 168.21 | 158.80 | 145.93 | 100.21 | 51.39 | 73.33 | | 83.60 | 75.69 | 37.32 | 45.12 | 25.48 | 21.79 | 30.88 | 32.51 | 67.42 | 32.49 | 48.64 | 14.37 | 53.24 | 43.08 | 61.68 | 49.57 | 77.14 | | 21.24 | 48.14 |
| T371_Mne | 171.07 | 165.22 | 149.92 | 100.62 | 50.17 | 79.59 | 51.78 | 78.28 | 78.40 | 42.30 | 48.29 | 27.54 | 20.81 | 30.50 | 31.63 | 64.73 | 29.56 | 43.10 | 15.34 | 52.31 | 42.61 | 64.37 | 45.16 | 68.03 | 20.83 | 17.82 | 49.62 |
| T358_Mam Neotype | 223.19 | 222.99 | 197.33 | 137.02 | 61.87 | 119.03 | 71.00 | 116.10 | 99.14 | 61.17 | 68.87 | 37.77 | 30.16 | 30.22 | 37.31 | 66.28 | 41.46 | 60.14 | 18.18 | 63.13 | 66.65 | 84.59 | 46.16 | 100.97 | 21.35 | 28.10 | 70.44 |
| T274_Mam | 220.74 | 219.45 | 187.43 | 121.52 | 68.13 | 104.72 | 65.07 | 116.10 | 102.61 | 61.17 | 63.42 | 34.39 | 30.86 | 31.44 | 39.67 | 69.32 | 37.92 | 57.62 | 18.73 | 59.80 | 68.34 | 80.93 | 57.12 | 97.93 | 23.06 | 27.67 | 61.27 |
| T260_Mam | 205.90 | 210.68 | 187.21 | 122.07 | 63.91 | 104.22 | 60.63 | 101.07 | 98.18 | 58.23 | 54.29 | 32.8 | 26.77 | 32.75 | 36.07 | 67.34 | 43.91 | 59.29 | 15.96 | 62.17 | 63.9 | 81.53 | 51.89 | 87.46 | 20.36 | 24.05 | 60.89 |
| T251_Mam | 210.53 | 209.02 | 189.66 | 125.24 | 66.41 | 104.62 | 66.18 | 111.23 | 87.92 | 59.10 | 65.40 | 38.23 | 30.34 | 35.36 | 36.12 | 64.33 | 41.92 | 62.12 | 19.53 | 62.10 | 68.98 | 82.65 | 54.92 | 91.78 | 17.44 | 22.61 | 60.79 |
| T385_Mru Neotype | 200.63 | 199.37 | 184.46 | 122.72 | 62.39 | 97.64 | 69.19 | 106.88 | 121.87 | 51.33 | 63.2 | 32.17 | 28.73 | 33.58 | 32.55 | 64.91 | 41.55 | 55.6 | 20.54 | 58.98 | 56.6 | 71.82 | 54.22 | 88.9 | 22.94 | 25.47 | 61.02 |

*M. nemorivaga* – Mne; *S. gouazoubira* – Sgo; *M. rufa* – Mru; *M. americana* – Mam; TL= Total length. CBL= condylobasal length, BL= basal length, SSL= short skull length, PR= premolar 1 – prostion, BCA= basecranial axis, BFA= basefacial axis, VL= viscerocranium length, MFL= median frontal length, LN= lambda – nasal, LR= lambda – Rhinion, LP= lambda – prostion , AK= akrokranion GLN= greatest length of the nasals, SLFL= short lateral facial length, OPL= oral palatal length, LLP= lateral length of the premaxilla, LCR= length of the cheektooth row, LMR= length of the molar row, LPR= length of the premolar row, GILO= greatest inner length of the orbit, GIHO= greatest inner height of the orbit, GMB= greatest mastoid breadth, GBOC= greatest breadth of the occipital condyles, GBBP= greatest breadth at the bases of the paraoccipital, GBFM= greatest breadth of the foramen magnum, HFM= height of the foramen magnum: Basiom - Opisthion, GNB= Greatest neurocranium breadth, LFB= Least frontal breadth, GBAO= greatest breadth across the orbits, LBBO= least breadth between the orbits, ZB= zygomatic breadth, GBAN= greatest breadth across the nasals, GBAP= greatest breadth across the premaxillae, GPB= greatest palatal breadth, BHPSN= Basion – The highest point of the superior nuchal crest.

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

| Chromosome | BAC ID | Position in BTA (Mb) |
|---|---|---|
| BTA1 | 106N15 | 2.08-2.25 |
| | 69G2 | 57.29-57.48 |
| | 109I18 | 116.68-116.91 |
| | 273F5 | 154.36-154.55 |
| | | |
| BTAX | 316D2 | 68.49-68.68 |
| | 159O16 | 23.04-23.22 |
| | 40H2 | 74.95-75.12 |
| | 453C5 | 144.43-144.62 |
| | 326C13 | 148.27-148.47 |

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

| **MSA chromosome** | **SGO homology** | **BTA homology** |
|---|---|---|
| 1 | 1 | 3 |
| 2 | 2 | 4 |
| 3 | 3 | 2prox |
| 4 | 4 | 1dist |
| 5 | 5 | 28/26 |
| 6 | 6 | 10 |
| 7 | 7 | 7 |
| 8 | 8 | 11 |
| 9 | 9 | 12 |
| 10 | 10 | 14 |
| 11 | 11 | 16 |
| 12 | 12 | 13 |
| 13 | 13 | 15 |
| 14 | 14 | 21 |
| 15 | 15 | 19 |
| 16 | 16 | 1prox |
| 17 | 17 | 18 |
| 18 | 18 | 20 |
| 19 | 19 | 17 |
| 20 | 20 | 6prox |
| 21 | 21 | 5prox |
| 22 | 22 | 9prox |
| 23 | 23 | 5dist |
| 24 | 24 | 6dist |
| 25 | 25 | 22 |
| 26 | 26 | 8prox |
| 27 | 27 | 23 |
| 28 | 28 | 24 |
| 29 | 29 | 29 |
| 30 | 30 | 2dist |
| 31 | 31 | 27 |
| 32 | 32 | 25 |
| 33 | 33 | 8dist |
| 34 | 34 | 9dist |
| X | X | X |

## CAPÍTULO 6 – Considerações finais

O uso de topótipos atuais de táxons descritos em países da América Latina como México, Venezuela e Argentina foi muito importante para acessar aos dados morfológicos, citogenéticos e moleculares dos mesmos e comparar com as outras espécies de cervídeos neotropicais. A partir destes, é possível concluir que:

a. O veado-cinza da Pensinsula de Yucatán, México, não possui ancestral comum exclusivo com as espécies do gênero *Mazama*, uma vez que as análises filogenéticas recuperam o mesmo dentro do clado monofilético do gênero *Odocoileus*. A espécie atualmente reconhecida como *Odocoileus pandora* (Merriam, 1901) possui características morfológicas e cariotípicas diferenciadas dos demais gêneros de cervídeos neotropicais. Portanto, estudos com uma amostragem mais ampla devem ser realizados, para considerar a alocação deste táxon em um novo gênero.

b. O veado-cinza do noroeste da Venezuela corresponde morfologicamente e geograficamente com a descrição de *Mazama americana citus* Osgood, 1912, realizada a partir de espécimes da costa oriental do Lago de Maracaibo, Venezuela. O táxon é grupo irmão das espécies da subtribo Blastocerina, revelando que além de não haver correspondência com o gênero *Mazama* da subtribo Odocoileina, não está relacionado com os demais gêneros de cervídeos neotropicais. A evidência dos espécimes recuperados filogeneticamente como linhagem basal dentro de Blastocerina e a exclusividade dos dados morfológicos e cariotípicos sustentam a consideração do táxon ao nível específico e a necessidade de designar um novo nome genérico para alocar a espécie.

c. A análise de um indivíduo de veado-mateiro em cativeiro com origem conhecido em Duque Escaba, Tucumán, Argentina, revelou um menor tamanho em comparação com os veado-mateiros correspondentes as espécies *Mazama americana* e *Mazama rufa*. Também, mostrou um cariótipo diferenciado das outras espécies do gênero *Mazama* e a recuperação em um clado monofilético com o espécime tipo de *Mazama rufa toba* descrito Lönnberg, 1919. De tal forma, o resultado filogenético permite considerar o indivíduo de Tucumán como *M. rufa*

*toba* cuja caracterização integrativa sugere que o táxon seja elevado a espécie válida dentro do gênero *Mazama*.

d. A análise do topótipo de *M. simplicornis argentina* Lönnberg, 1919, confirmou a similaridade morfológica com *Subulo gouazoubira*, e evidenciou que não há distinção citogenética nem molecular com a espécie do Paraguai. Portanto, os dados confirmam que a espécie *Subulo gouazoubira* ocorre também no norte da Argentina, especificamente na região do Chaco.

e. Observou-se que o uso dos holótipos de *Mazama rufa toba* e *Mazama simplicicornis argentina* permitiu confirmar a correspondência dos espécimes coletados recentemente através da análise do DNA mitocondrial extraído do material de museu. Esse resultado é de grande relevância apresentando o acesso a espécimes de museus e a consequente análise de DNA antigo como ferramenta eficiente para comparar com topótipos atuais e assim auxiliar no esclarecimento taxonômico do gênero *Mazama*.

Este estudo contribui com as primeiras descrições citogenéticas de cada espécie e subespécie acessada. Também, constitui a primeira análise genética de cervídeos do gênero *Mazama* na Venezuela e na Argentina. As diferentes técnicas utilizadas neste trabalho geraram importantes informações a respeito dos táxons avaliados, contribuindo para o entendimento da sua taxonomia e história evolutiva. Assim como a citogenética se apresenta como uma técnica essencial para definição de espécie, pelo acúmulo de rearranjos cromossômicos entre os táxons, a filogenia mitocondrial revelou o posicionamento dos animais analisados em relação às outras espécies e gêneros de cervídeos. Desta forma, os cervídeos neotropicais, pertencentes à tribo Odocoileini, estão organizados em duas subtribos, com da seguinte maneira (Figura 1):

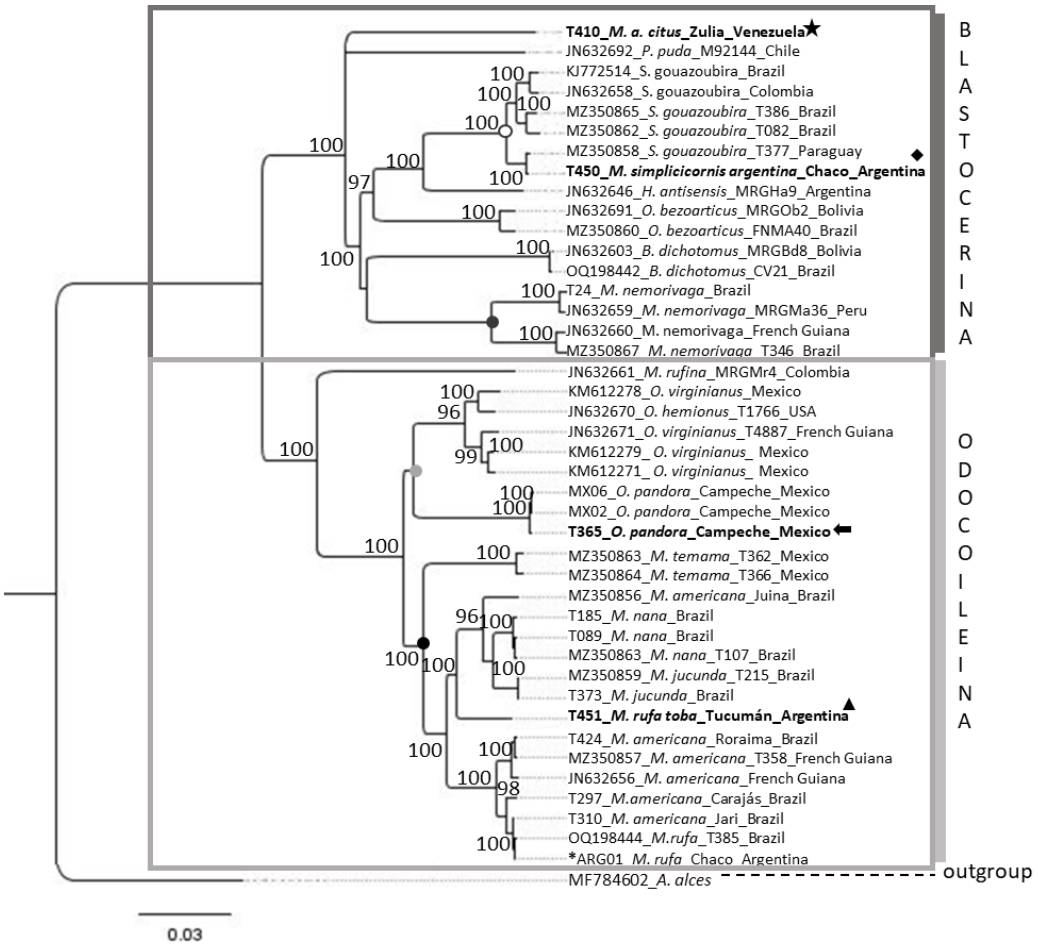


**Figura 1.** Árvore de Inferência Bayesiana (IB) do mitogenoma completo das espécies de cervídeos neotropicais (Tribo Odocoileini). Subtribo Blastocerina (Cinza escuro); Subtribo Odocoileina (Cinza claro). Topótipos coletados: *Mazama americana citus* Osgood, 1912 (Estrela), *Mazama simplicicornis argentina* Lönnberg, 1919 (diamante), *Odocoileus pandora* (Merriam, 1901) (flecha) e *Mazama rufa toba* (Lönnberg, 1919) (triângulo). Valores indicam a probabilidade posterior. Clados dos gêneros *Mazama* Rafinesque (1817) (círculo preto), *Odocoileus* Rafinesque (1832) (círculo cinza claro), *Subulo* Smith (1827) (círculo branco) e *Mazama nemorivaga* - atualizando ao gênero *Passalites* Gloger (1941) (círculo cinza escuro).

Há necessidade de um investimento na continuidade da amostragem de cada espécie que ocorre na América Latina para refinar a diagnose morfológica de cada grupo, entender a variação cariotípica das populações, acessar aos demais espécimes tipos coletados e realizar uma abordagem multi-locus que inclua genes mitocondriais e nucleares. Dessa forma, o esclarecimento da complexa taxonomia dos cervídeos neotropicais, especificamente do gênero *Mazama*, possa ser alcançado e assim avançar no estabelecimento de ações para conservação e manejo das populações no ambiente in-situ e ex-situ.

## APÊNDICE A

PERMISSION FOR COLLECTION IN MEXICO

SECRETARÍA DE MEDIO AMBIENTE
Y RECURSOS NATURALES

SUBSECRETARÍA DE GESTIÓN
PARA LA PROTECCIÓN AMBIENTAL
DIRECCIÓN GENERAL DE VIDA SILVESTRE

**OFICIO NÚM. SGPA/DGVS/** 06821 **/14**

**MÉXICO, D. F., A** 25 JUL 2014

***"2014, Año de Octavio Paz".***

**DRA. GRISELDA ESCALONA SEGURA**
**DIVISIÓN DE BIOLOGÍA DE LA CONSERVACIÓN**
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**AV. RANCHO POLÍGONO No. 2 – A**
**CIUDAD INDUSTRIAL, C.P. 24500**
**CAMPECHE, CAMPECHE**
**TEL. (981)127 3720, gescalon@ ecosur.mx**

Considerando que ha dado cumplimiento a los requisitos establecidos para efectuar investigación y colecta científica de flora y fauna silvestres en territorio mexicano y con fundamento en el Artículo 32 Bis fracciones I, III, XX, XXXIX de la Ley Orgánica de la Administración Pública Federal; Artículos 19 fracción XXV y 32 fracciones VI, XVIII, XXI, XXIV del Reglamento Interior de la Secretaría de Medio Ambiente y Recursos Naturales, publicado en el Diario Oficial de la Federación el 26 de noviembre de 2012; 79, 80 fracción I, 82, 83 y 87 párrafo cuarto de la Ley General del Equilibrio Ecológico y la Protección al Ambiente; Artículos 9º. Fracción XII, 97 y 98 de la Ley General de Vida Silvestre; 12, 123 Fracción III y 126 del Reglamento de la Ley General de Vida Silvestre; Artículo 85, Artículo 88, fracciones I y II, Artículo 105, fracciones II y III del Reglamento de la Ley General del Equilibrio Ecológico y la Protección al Ambiente en Materia de Áreas Naturales Protegidas (ANP's); las disposiciones relativas de la Norma Oficial Mexicana NOM-126-SEMARNAT-2000, por la que se establecen las especificaciones para la realización de actividades de colecta científica de material biológico de especies de flora y fauna silvestres y otros recursos biológicos en el territorio nacional; la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, protección ambiental-especies nativas de México de flora y fauna silvestres-categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-lista de especies en riesgo, la Dirección General de Vida Silvestre **autoriza** la Colecta Científica por proyecto sobre especies o poblaciones en riesgo o sobre hábitat crítico, para realizar las siguientes actividades inherente al Proyecto denominado **"Monitoreo de especies prioritarias (aves y mamíferos) en la Reserva de la Biosfera de Calakmul/Estado de conservación de las aves y mamíferos en la Península de Yucatán"**:

- Colecta, recolecta y liberación de ejemplares de las especies pertenecientes a las **Clases: Aves y Mammalia (mamíferos pequeños)** para toma de medidas morfométricas, obtención de muestras de sangre, colecta de ecto y endoparásitos y su liberación inmediata.
- Colecta definitiva de hasta tres (03) ejemplares por especie de las **Clases: Aves y Mammalia (mamíferos pequeños)** de las especies que no hayan sido colectadas anteriormente y que no se encuentren listadas en la Norma Oficial Mexicana NOM-059-SEMARNAT-2010.
- Observación directa y grabación de cantos de aves y registro de huellas de mamíferos medianos y grandes.
- Colecta definitiva de hasta cinco (05) muestras para herbario por especie de flora silvestre que se encuentre listada en la Norma Oficial Mexicana NOM-059-SEMARNAT-2010 y que no haya sido colectada anteriormente.
- De ejemplares de "pavo ocelado" ***Meleagris ocellata*** aprovechados legalmente en UMA se obtendrán muestras de contenido estomacal y muestras de tejidos.

La autorización de colecta de Plantas vasculares que no se encuentren listadas en la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, de conformidad con el artículo 1°, de la Ley General de Vida Silvestre, quedà fuera del àmbito de competencia de esta Dirección General de Vida Silvestre, motivo por el cual, deberà dirigirse a la Dirección General de Gestión Forestal y Suelos, ubicada en Av. Progreso No. 3, Edificio 3, Planta Alta, Colonia del Carmen Coyoacán, Delegación Coyoacán, C.P. 04100, México, D.F., Tels. 5484 35 68 y 5484 35 69, (dgforestal@semarnat.gob.mx), para que le informen el trámite que deberá realizar.

Las actividades de colecta se llevarán a cabo en **la Reserva de la Biosfera Calakmul, Reserva de la Biosfera Los Petenes y Área de Protección de Flora y Fauna Laguna de Términos, Estado de Campeche; Reserva de la Biosfera Ría Lagartos, Reserva de la Biosfera Ria Celestún y Parque Nacional Arrecife Alacranes, Estado de Yucatán; Reserva de la Biosfera Sian Ka'an, Área de Protección de Flora y Fauna Bala 'an K' aax, Área de Protección de Flora y Fauna Uaymil y Área de Protección de Flora y Fauna Yum Balam, Estado de Quintana Roo; Reserva de la Biosfera Pantanos de Centla, Estado de Tabasco.** Esta autorización tendrá una vigencia de un (01) año a partir de la expedición de la presente.

Las actividades se realizarán con el Aval del El Colegio de la Frontera Sur-Unidad Campeche, con la colaboración de la Dra. Sophie Calmé Delande, Dr. Manuel Weber, Biol. Enrique Escobedo y C. Guillermo E. Castillo Vela, todos de la Institución antes mencionada; M. en C. Sergio Padilla, Biol. Rodolfo Noriega, Biol. Jesús Vargas Soriano y Biol. David Enrique Simá Pante de la Universidad Autónoma de Campeche; Dr. Hugo Ruiz Piña, Dr. Jorge Eduardo Zavala Castro, Dr. José Arturo Farfán Alé, Dr. Ignacio Vado Solis, Dr. Carlos R. Ávila Buenfil y Dra. Rosani Flores Canul del Laboratorio de Parasitología del Centro de Investigaciones Regionales "Dr. Hideyo Noguchi" de la Universidad de Yucatán; Jorge A. Vargas Contreras de la Universidad Nacional Autónoma de México y los estudiantes José del C. Puc Cabrera, José D. Cú Vizcarra, Alma de La Gala Quijano, Rosalía Puch Chávez, Tammy Esperanza Chi Coyoc; Dr. Armando Alayón Gamboa; MVZ. Ana Romero Calderón, MVZ. Adriana Vallarino Moncada, C. Alexis Plasencia del Colegio de la Frontera Sur; Jon T. McRoberts de Texas Tech Univ y Pas. de Biól. Ana Catzim Uc; MsC. Anay Serrano Rodríguez; M. en B. Moisés Andrade Herrera, Biól. Rodríguez Macedo de El Colegio de la Frontera Sur **y el titular y colaboradores quedan sujetos a las siguientes condicionantes**:

1.- Deberá cumplir con las disposiciones Administrativa, Fiscales y de Sanidad exigibles por las autoridades competentes en la Materia, sean Federales, Estatales ó Municipales, así como con las disposiciones establecidas en la Ley General del Equilibrio Ecológico y la Protección al Ambiente y su Reglamento en Materia de Áreas Naturales Protegidas y demás disposiciones legales aplicables.

2. - **Obligatoriamente y previo al inicio de las actividades de campo en el Área Natural Protegida, deberá contactar a su Director correspondiente: C. José Adalberto Zúñiga Morales, Director de la Reserva de la Biosfera de Calakmul (Xpugil, Calakmul, Campeche (Av. Insurgentes No. 445 entre Tecnológico de Chetumal y Tecnológico de Mérida, C.P. 77039, Chetumal Q. Roo. Tel(s). 01 (983) 285 4623 e-mail: calakmul@conanp.gob.mx); C. César Uriel Romero Herrera, Subdirector de la Reserva de la Biosfera Los Petenes (Calle 10 No. 329, entre calles 59 y 61 Edificio San Ignacio, 1er Nivel, L 4 y L 7, Col. Centro, C. P. 24000, San Francisco de Campeche, Campeche). Tel. 01 (981) 811 7156, e-mail: petenes@campeche.semarnat.gob.mx); C. José Hernández Nava, Director del Área de Protección de Flora y Fauna Laguna de Términos (Av. Adolfo López Mateos s/n esq. Héroes del 21 de Abril, Prolongación Playa Norte, C.P. 24140, Ciudad del Carmen, Campeche, Tel. 01 (938) 382 6270, e-mail: jhernandez@conanp.gob.mx); C. Rafael Robles de Benito, Director de la Reserva de la Biosfera Ría Lagartos (Estación de Campo Ría Lagartos: Crucero Ría Lagartos-Tizimín, Ría Lagartos, Yuc. y Calle 18, No 120 por Av. Pérez Ponce, Col. Itzimná, C.P. 97100, Mérida, Yuc., Tel. 01 (986) 861 14 30, e-mail: rafael.robles@conanp.gob.mx, lagartos@conanp.gob.mx y rbrialagartos@prodigy.net.mx); C. René Humberto Kantu Palma, Director de la Reserva de la Biosfera Ría Celestún y del Parque Nacional Arrecife Alacranes (Calle 18, No. 120 por Av. Pérez Ponce, Col. Itzimná, C.P. 97100, Mérida, Yuc., Tel. 01 (999) 926 42 18 y 938 07 08, e-mail: celestun@conanp.gob.mx y rkantu@conanp.gob.mx); C. Ángel Omar Ortiz Moreno, Director de la RB Sian Ka'an y el APFF Uaymil (Calle Venado No. 71, Segundo Piso, Supermanzana 20, Manzana 18, C. P. 77500, Cancún, Benito Juárez, Quintana Roo., Tel. 01 (998) 892 15 67, e-mail: siankaan@conanp.gob.mx y omortiz@conanp.gob.mx); C. José Juan Pérez Ramírez, Director del Área de Protección de Flora y Fauna Bala 'an Ka' ax (Venado No. 71, 2do Nivel, Sm 20 Mz. 18, C.P. 77500, Cancún, Q. Roo., Tel. 01 (998) 887 1997, e-mail: jperez@conanp.gob.mx); y al C. José Bernardo Rodríguez de la Gala Méndez, Director del Área de Protección de Flora y Fauna Yum Balam (Calle Venado # 71 y 73, Supermanzana 20, Manzana 18, Retorno 8, Lotes 2 y 4**

SECRETARÍA DE MEDIO AMBIENTE
Y RECURSOS NATURALES

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**OFICIO NÚM. SGPA/DGVS/** 06821 **/14**

**MÉXICO, D. F., A**

25 JUL 2014

*"2014, Año de Octavio Paz".*

**C.P. 77500, Cancún, Benito Juárez, Q. Roo y Avenida Palominos s/n, Edificio SEMARNAT, C.P. 37580, Holbox, Municipio de Lázaro Cárdenas, Q. Roo., Tel. 01 (999) 938 07 08, e-mail: yumbalam@conanp.gob.mx), C. Carlos Agustin Bautista Jiménez, Director de la Reserva de la Biosfera Pantanos de Centla (Av. Paseo de la Sierra 613, 2ª. Planta, Col. Reforma, C.P. 86080, Villahermosa, Tabasco, Tel. (993)313 9362, e-mail: pcentla@conanp.gob.mx; cbautista@conanp.gob.mx), lo anterior para coordinar las actividades de campo con el ANP, presentar su programa de actividades, lista de participantes y fechas en que pretende ingresar al ANP; así mismo se le asignará el personal del ANP que lo acompañará durante los trabajos de campo y deberá acatar las indicaciones, acceso y recomendaciones que le haga dicho personal.**

3. – Obligatoriamente y previo al inicio de actividades en la UMA, deberá contactar al Director ó Propietario de las mismas, para que le permita el acceso, para lo cual deberá indicar del Proyecto que realiza las actividades que realizará y los beneficios que obtendrá al participar el proyecto.

4. - En la realización del proyecto propuesto, se responsabilizará al titular de la investigación de cualquier impacto significativo que resulte sobre las poblaciones de la flora o fauna silvestres y sus hábitats, por lo que deberá considerar el riesgo de perturbación del ecosistema, antes de su ejecución y no llevarlo a cabo si existe algún riesgo.

5. - Previo al inicio de las actividades de campo, deberá enviar obligatoriamente por escrito y utilizando cualquier medio su programa de trabajo a las Delegaciones Federales de la Secretaría de Medio Ambiente y Recursos Naturales en los Estados de **Campeche** (Tel. 01 (981) 811 23 31), **Quintana Roo** (Tel. 01 (983) 835 0222, 835 0226), **Tabasco** (Tel. 01 (993) 910 14 07, 910 1408), **Yucatán** (Tel. 01 (999) 942 13 07), enviando copia del mismo a la Dirección General de Vida Silvestre. De igual manera, al término de dichas actividades lo notificará a esas Delegaciones Federales, enviándole un reporte detallado por escrito.

6.- La totalidad del material colectado deberá destinarse exclusivamente a los fines específicos del proyecto, objeto de la presente autorización. Con base al Capítulo IV, Artículo 98 de la Ley General de Vida Silvestre, el material biológico colectado será depositado en las Instalaciones del Herbario del CEDESU y el Museo de la Biodiversidad Maya, ambos de la Universidad Autónoma de Campeche y en el Museo de Zoología de El Colegio de la Frontera Sur y el titular de la autorización, asume la responsabilidad de remitir a esta Dirección General, copia de la(s) constancia(s) del(os) depósito(s) debidamente firmado(s), especificando la cantidad del material depositado.

7. - Con base al Capítulo IV, Artículo 98 de la Ley General de Vida Silvestre y 126 del Reglamento de la Ley General de Vida Silvestre, el responsable del proyecto deberá someter a la consideración de la Dirección General de Vida Silvestre, en un plazo no mayor de 30 (TREINTA) días de concluida la vigencia de la presente, un informe que describa **detalladamente** las actividades realizadas, los resultados obtenidos, la problemática del área trabajada, las potenciales alternativas de solución y -en su oportunidad-, la(s) publicación(es) y sobre tiros producto de la investigación.

8. - Queda estrictamente **prohibido** efectuar cualquier aprovechamiento de las especies de flora y fauna silvestres, cualesquiera que sea su estatus, excepto lo aquí autorizado, así como realizar actividades en áreas naturales protegidas de México, sean Estatales o Federales, sin previa autorización.

**continúa al reverso.../**


Av. Revolución 1425, Nivel 1, Col. Tlacopac San Ángel
Delegación Álvaro Obregón, C. P. 01040, México, D. F.
Teléfono: 01(55) 56-24-33-09, Fax: 01(55)56-24-56-42

9. - <u>De acuerdo al Artículo 87 de la Ley General del Equilibrio Ecológico y la Protección al Ambiente y al Capítulo IV, Artículo 97 de la Ley General de Vida Silvestre, esta autorización no ampara el aprovechamiento del material biológico colectado para fines comerciales, ni de utilización en biotecnología.</u>

Se recomienda que durante sus actividades de campo, en el caso de observar ejemplares de especies listadas en la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, se notifique de ello (la especie, ubicación geográfica y la fecha) a esta Dirección General, en el informe de actividades antes mencionado.

La presente autorización es personal e intransferible y habrá de mostrarse a las Autoridades Federales, Estatales y Municipales cuantas veces lo soliciten. Así mismo y tomando en consideración lo establecido por el Artículo 87 de la Ley General del Equilibrio Ecológico y la Protección al Ambiente y por el Capítulo IV, Artículo 97 de la Ley General de Vida Silvestre, el titular de la presente deberá contar con el consentimiento previo, expreso e informado de los legítimos propietarios de la(s) tierra(s) donde pretende desarrollar el proyecto.

**El incumplimiento de las condiciones aquí establecidas, dará origen a la instauración de un procedimiento administrativo ante la autoridad competente, para proceder a la cancelación de la autorización y a la aplicación de la legislación correspondiente, según sea el caso.**

**EL DIRECTOR GENERAL**

SEMARNAT

**JORGE MAKSABEDIAN DE LA ROQUETTE**

SECRETARÍA DE MEDIO AMBIENTE Y RECURSOS NATURALES
RECIBIDO
05 AGO 2014
DELEGACIÓN

**C.c.p.** **C. Joel González Moreno**.- Director General de Inspección y Vigilancia de Vida Silvestre, Recursos Marinos y Ecosistemas Costeros, PROFEPA.- Carr. Picacho Ajusco No. 200, 6°, Piso, Col. Jardines de la Montaña, Delegación Tlalpan, C.P. 14210, México D.F. vida_silvestre@profepa.gob.mx; jmejia@profepa.gob.mx
**C. Cesar Murillo Juárez**.- Coordinador de Asesores de la Subsecretaría.- coordinacion.sgpa@semarnat.gob.mx
**C. Enrique Pérez Gómez**.- Delegado Federal de la SEMARNAT en el Estado de Campeche.- delegado@campeche.semarnat.gob.mx
**C. Raúl Omar González Castilla**.- Delegado Federal de la SEMARNAT en el Estado de Quintana Roo.- delegado@qr.semarnat.gob.mx; raulo.gonzalez@qr.semarnat.gob.mx
**C. Luis Alberto López Carbajal**.- Delegado Federal de la SEMARNAT en el Estado de Tabasco.- delegado@tabasco.semarnat.gob.mx
**C. Jorge Carlos Berlín Montero**.- Delegado Federal de la SEMARNAT en el Estado de Yucatán.- delegado@yucatan.semarnat.gob.mx
**C. José Adalberto Zúñiga Morales**.- Director de la Reserva de la Biosfera de Calakmul.- calakmul@conanp.gob.mx; jzuniga@conanp.gob.mx
**C. César Uriel Romero Herrera**.- Director de la Reserva de la Biosfera Los Petenes.- petenes@campeche.semarnat.gob.mx
**C. José Hernández Nava**.- Director del Área de Protección de Flora y Fauna Laguna de Términos.- jhernandez@conanp.gob.mx
**C. Rafael Robles de Benito**.- Director de la Reserva de la Biosfera Ría Lagartos.- rafael.robles@conanp.gob.mx, lagartos@conanp.gob.mx; rbrialagartos@prodigy.net.mx
**C. René Humberto Kantu Palma**.- Director de la Reserva de la Biosfera Ría Celestún y del Parque Nacional Arrecife Alacranes.- celestun@conanp.gob.mx; rkantu@conanp.gob.mx
**C. Ángel Omar Ortiz Moreno**.- Director de la Reserva de la Biosfera Sian Ka'an y Área de Protección de Flora y Fauna Uaymil.- siankaan@conanp.gob.mx; oscortiz@conanp.gob.mx
**C. José Juan Pérez Ramírez**.- Director del Área de Protección de Flora y Fauna Bala'an Ka' ax.- jperez@conanp.gob.mx
**C. José Bernardo Rodríguez de la Gala Méndez**, Director del Área de Protección de Flora y Fauna Yum Balam.- yumbalam@conanp.gob.mx
**C. Carlos Agustín Bautista Jiménez**.- Director de la Reserva de la Biosfera Pantanos de Centla.- pcentla@conanp.gob.mx; cbautista@conanp.gob.mx
**C. David Gutiérrez Carbonell**.- Director General de Manejo para la Conservación de Áreas Naturales Protegidas, Comisión Nacional de Áreas Naturales Protegidas.- Carretera Picacho Ajusco No. 200, 6°, Piso, Col. Jardines de la Montaña, Delegación Tlalpan, C. P. 14210, México, D. F.- dagut@conanp.gob.mx
**C. Fernando Sánchez Camacho**.- Jefe del Departamento de Análisis para el Aprovechamiento de Otras Especies.- Edificio fsanchez@semarnat.gob.mx
Archivo General (04/O1-0077/06/14).

**LEGM/AACC/ColectClient- GriseldaEscalonaSeg-AVE_MAMIFER(18-7-14)**

*"Por una cultura ecológica y el uso eficiente del papel, las copias de conocimiento de este oficio se remiten vía electrónica"*

# APÊNDICE B

SPECIMEN DONATION TO NUPECCE

**INSTITUTO DE ECOLOGIA A.C**

**Carretera Antigua a Coatepec #351**
**Las Hayas, Xalapa, Veracruz, México.**

**Enero, 27 2016**

**A: Autoridades de la Aduana Brasileira.**
**Asunto: Donación de Material Biológico.**

A quien pueda interesar,

Por medio del siguiente documento doy constancia de la donación de material biológico que se encuentra actualmente bajo la responsabilidad de la Profa. Dr. Sonia Gallina Tessaro, Investigador titular del Instituto de Ecología A.C. La presente donación va dirigida al Núcleo de Pesquisa e Conservação de Cervideos (NUPECCE) perteneciente a la Universidad Estadual Paulista "Julio de Mesquita Filho" UNESP. Este material forma parte de la colaboración científica entre el investigador brasilero José Mauricio Barbanti Duarte y los investigadores mexicanos Sonia Gallina Tessaro y Rafael Reyna-Hurtado, que servirá para la realización del proyecto de investigación titulado: "La búsqueda de Topótipos y producción de citótipos y genotipos como la mejor forma para la resolución de la compleja taxonomía del genero *Mazama* (Mammalia;Cervidae)", financiado por el Consejo Nacional de Desarrollo Tecnologico (CNPq) Brasil.

Están siendo donados al Nucleo de Pesquisa e Conservação de Cervideos (NUPECCE): Cráneos, post-cráneos, Pieles abiertas y muestras de tejido conservados en formol y etanol, así como también tejidos conservados en nitrógeno líquido, correspondientes a los siguientes animales:

Un macho de la especie *Mazama pandora*, originario de la Península de Yucatán, colectado bajo el permiso SGPA/DGVS/04214/2015.

Un macho de la especie *Mazama temama*, originario del estado de Veracruz, colectado bajo el permiso SGPA/DGVS/04214/2015.

Un macho y un hembra de la especie *Mazama temama*, originarios de la Península de Yucatán, colectados bajo el permiso SGPA/DGVS/06821/2014 Y donados al Instituto de Ecología A.C. (INECOL) según consta en el oficio anexo.

Una hembra de *Mazama temama*, originaria del estado de Veracruz, donada al INECOL según consta en el oficio redactado por el señor Carlos Ros.

Todos los documentos se encuentran anexados a esta declaración como parte de la justificativa del origen legal de los especimenes.

Sin más que decir, por favor no deje de contactarme para cualquier otra información que pueda ser necesaria.

Dr. Martín Ramón Aluja Schuneman Hofer
Director General
Instituto de Ecología A.C

Prof. Sonia Gallina Tessaro
Investigador titular
Red de Biología y Conservación de Vertebrados
Instituto de Ecología A.C

**APÊNDICE C**

PERMISSION FOR COLLECTION IN VENEZUELA

Gobierno Bolivariano de Venezuela | Ministerio del Poder Popular para Ecosocialismo y Aguas | Dirección General de Diversidad Biológica | ZAMORA

Oficio Nº 0374

SISTRA N°20356

Fecha: 04 ABR 2018

Ciudadana
**ELUZAI DINAI PINTO SANDOVAL**
C.I. V-18.955.547
Instituto Venezolano de Investigaciones Científicas (IVIC)
Presente.-

Tengo a bien dirigirme a usted, de conformidad con lo establecido en los artículos 73 y 75 de la Ley Orgánica de Procedimientos Administrativos, en la oportunidad de notificarle el texto íntegro de la Providencia Administrativa Nº 0026 de fecha 04 ABR 2018 , emanada de este Despacho, mediante la cual se decidió su Solicitud de de Licencia de Caza con Fines Científicos para la colecta de productos de la fauna silvestre, dentro del proyecto denominado: **"Caracterización Morfológica, citogenética y Molecular de las especies del genero Mazama en Venezuela".**

**".....REPÚBLICA BOLIVARIANA DE VENEZUELA MINISTERIO DEL PODER POPULAR PARA ECOSOCIALISMO Y AGUAS VICEMINISTERIO DE ECOSOCIALISMO AMBIENTAL DIRECCIÓN GENERAL DE DIVERSIDAD BIOLÓGICA**
**PROVIDENCIA Nº 0026 Caracas, 04 ABR 2018 207°, 158°, 18°**

**VISTO**

La Planilla para la Renovación de la Solicitud de Licencia de Caza con Fines Científicos, recibida en fecha **23 de junio 2017** y tramitada por la ciudadana **ELUZAI DINAI PINTO SANDOVAL,** titular de la Cédula de Identidad Nro.**V-18.955.547**, para la caza científica de animales de la fauna silvestre, en el marco del proyecto denominado **"Caracterización Morfológica, citogenética y Molecular de las especies del genero Mazama en Venezuela".**

**CONSIDERANDO**

Que consta en el expediente administrativo los siguientes recaudos:

- La Planilla para la renovación de la Solicitud de Licencia de Caza con Fines Científicos, recibida en fecha **23 de junio 2017,** y tramitada por la ciudadana **ELUZAI DINAI PINTO SANDOVAL,** titular de la Cédula de Identidad Nro. **V-18.955.547**, con sus respectivos timbres fiscales.
- Comunicación de fecha **20 de junio de 2017** suscrita por el **Dr. Eloy A. Sira Galíndez**, en su condición de Director del **Instituto Venezolano de Investigaciones Científicas (IVIC)**, mediante la cual otorgan el respaldo institucional al proyecto: **"Caracterización Morfológica, citogenética y Molecular de las especies del genero Mazama en Venezuela".**
- Documento científico titulado: **"Caracterización Morfológica, citogenética y Molecular de las especies del genero Mazama en Venezuela".**
- Documento de evaluación técnica efectuada al Proyecto: **"Caracterización Morfológica, citogenética y Molecular de las especies del genero Mazama en Venezuela",** remitido en el **Memorando Nro. 000121 de fecha 19 de marzo de 2018**, realizada por el grupo de trabajo de

la Dirección General de Diversidad Biológica **Dirección de Conservación de Especies Amenazadas,** donde se recomienda otorgar la referida Licencia.

**CONSIDERANDO**

- *Que el Estado bajo el marco del desarrollo sustentable, tiene como derecho y deber fundamental, el máximo bienestar y seguridad de la población, a través, de su poder reglamentario y de la gestión ambiental, como proceso, para el sostenimiento del país, el planeta y de la humanidad.*
- *Que conforme al artículo 2 de la Ley Orgánica del Ambiente, se entiende por gestión del ambiente el proceso constituido por un conjunto de acciones o medidas orientadas a …(omissis)… aprovechar los ecosistemas, la diversidad biológica y demás recursos naturales y elementos del ambiente, en garantía del desarrollo sustentable.*
- *Que conforme al artículo 18 de la Ley Orgánica del Ambiente, es responsabilidad del Estado a través del Ministerio del Poder Popular para el Ambiente como Autoridad Nacional Ambiental, formular, planificar, dirigir, ejecutar, coordinar, controlar y evaluar las políticas, planes, proyectos y actividades estratégicas para la gestión del ambiente, así como, sobre actividades capaces de degradar el ambiente.*
- *Que conforme al artículo 48 de la Ley Orgánica del Ambiente se establece que "A los fines de la conservación de los ecosistemas, recursos naturales y de la diversidad biológica, serán objeto de medidas prioritarias de protección: …(omissis)…numeral 2. Las especies o poblaciones de animales y plantas particularmente vulnerables, endémicas o que se encuentren amenazadas o en peligro de extinción."*
- *Que según el artículo 80 de la Ley Orgánica del Ambiente, se consideran actividades capaces de degradar el ambiente: numeral "1. Las que directamente o indirectamente contaminen o deterioren la atmósfera, agua, fondos marinos, suelo y subsuelo o incidan desfavorablemente sobre las comunidades biológicas, vegetales o animales…(omissis)…numeral 18. Las que afecten la sobrevivencia de especies amenazadas, vulnerables o en peligro de extinción…(omissis)…numeral 20. Cualesquiera otras que puedan dañar el ambiente o incidir negativamente sobre las comunidades biológicas, la salud humana y el bienestar colectivo."*
- *Que la Ley Orgánica del Ambiente establece en su artículo 82 que "La Autoridad Nacional Ambiental ejercerá el control previo ambiental, a través de los siguientes instrumentos: 1. Autorizaciones., 2. Aprobaciones, 3. Permisos, 4. Licencias, 5. Concesiones, 6.Asignaciones, 7. Contratos, 8. Planes de manejo, 9. Registros, 10. Los demás que establezca la ley.*
- *Que la Ley Orgánica del Ambiente establece en su artículo 83 que "El Estado podrá permitir la realización de actividades capaces de degradar el ambiente, siempre y cuando su uso sea conforme a los planes de ordenación del territorio, sus efectos sean tolerables, generen beneficios socioeconómicos y se cumplan las garantías, procedimientos y normas. En el instrumento de control previo se establecerán las condiciones, limitaciones y restricciones que sean pertinentes.*

| Fauna Silvestre | Media Firma | |
|---|---|---|
| Licencia de Caza con Fines Cientificos de la fauna silvestre | Técnico Evaluador: | Técnico Supervisor: |

**DECIDE**

Otorgar a la ciudadana **ELUZAI DINAI PINTO SANDOVAL**, titular de la Cédula de Identidad **Nro. V-18.955.547**, la **LICENCIA DE CAZA CON FINES CIENTÍFICOS** para realizar la caza de animales de la fauna silvestre que se indican a continuación, en el marco del Proyecto: **"Caracterización Morfológica, citogenética y Molecular de las especies del genero Mazama en Venezuela":**

| DESCRIPCION DE LOS ESPECIMENES AUTORIZADOS | | | |
|---|---|---|---|
| **Nombre común** | **Nombre científico (Familias)** | **Cantidad autorizada** | **Observaciones** |
| Venado matacán andino | ***Mazama bricenii*** | **UNO (01)** | **Colecta: cacería.** |
| Venado matacán rojizo | ***Mazama sheila*** | **UNO (01)** | |
| Venado matacán rojizo | ***Mazama americana juruana*** | **UNO (01)** | |

La presente **LICENCIA** se otorga al titular con base en las siguientes condiciones:

1. Esta Licencia es válida para el estudio de los especímenes autorizados **en el Páramo Sierra de la Culata y la Montaña Limones, estado Mérida, y en el Panorama, Rio Aurare, Costa Oriental del Lago de Maracaibo, estado Zulia.**
2. Esta Licencia es "**PERSONAL E INTRANSFERIBLE** y su presentación es obligatoria ante las autoridades competentes, cuando éstas así lo requieran. Su titular deberá portarla cuando esté realizando las labores para las cuales le fue otorgada.
3. **Queda el Autorizado en la obligación de presentar ante la Dirección General de Diversidad Biológica, un informe de actividades y avances preliminares de las investigaciones que se efectúen al amparo de ésta Licencia y del Proyecto citado.** La no presentación de los mismos prevalecerá al momento del otorgamiento de autorizaciones subsiguientes.
4. Esta Licencia **AUTORIZA, el acceso a los recursos genéticos**, mediante aprobación de la Comisión Intraministerial de Acceso a los Recursos Genéticos, con **Oficio Nº 0350**, de fecha **11 de diciembre de 2017**, respectivamente.
5. Esta Licencia **NO AUTORIZA** la entrada a las Áreas Bajo Régimen de Administración Especial (ABRAE), sin el debido permiso de acceso a dichas áreas, expedido por la autoridad competente.
6. No podrá realizarse traspasos de especímenes que se capturen, sin la aprobación previa de esta Dirección General de Diversidad Biológica.

| Fauna Silvestre | Media Firma | |
|---|---|---|
| Licencia de Caza con Fines Científicos de la fauna silvestre | Técnico Evaluador: | Técnico Supervisor: |

la Dirección General de Diversidad Biológica **Dirección de Conservación de Especies Amenazadas,** donde se recomienda otorgar la referida Licencia.

**CONSIDERANDO**

- *Que el Estado bajo el marco del desarrollo sustentable, tiene como derecho y deber fundamental, el máximo bienestar y seguridad de la población, a través, de su poder reglamentario y de la gestión ambiental, como proceso, para el sostenimiento del país, el planeta y de la humanidad.*
- *Que conforme al artículo 2 de la Ley Orgánica del Ambiente, se entiende por gestión del ambiente el proceso constituido por un conjunto de acciones o medidas orientadas a …(omissis)… aprovechar los ecosistemas, la diversidad biológica y demás recursos naturales y elementos del ambiente, en garantía del desarrollo sustentable.*
- *Que conforme al artículo 18 de la Ley Orgánica del Ambiente, es responsabilidad del Estado a través del Ministerio del Poder Popular para el Ambiente como Autoridad Nacional Ambiental, formular, planificar, dirigir, ejecutar, coordinar, controlar y evaluar las políticas, planes, proyectos y actividades estratégicas para la gestión del ambiente, así como, sobre actividades capaces de degradar el ambiente.*
- *Que conforme al artículo 48 de la Ley Orgánica del Ambiente se establece que "A los fines de la conservación de los ecosistemas, recursos naturales y de la diversidad biológica, serán objeto de medidas prioritarias de protección: …(omissis)…numeral 2. Las especies o poblaciones de animales y plantas particularmente vulnerables, endémicas o que se encuentren amenazadas o en peligro de extinción."*
- *Que según el artículo 80 de la Ley Orgánica del Ambiente, se consideran actividades capaces de degradar el ambiente: numeral "1. Las que directamente o indirectamente contaminen o deterioren la atmósfera, agua, fondos marinos, suelo y subsuelo o incidan desfavorablemente sobre las comunidades biológicas, vegetales o animales…(omissis)…numeral 18. Las que afecten la sobrevivencia de especies amenazadas, vulnerables o en peligro de extinción…(omissis)…numeral 20. Cualesquiera otras que puedan dañar el ambiente o incidir negativamente sobre las comunidades biológicas, la salud humana y el bienestar colectivo."*
- *Que la Ley Orgánica del Ambiente establece en su artículo 82 que "La Autoridad Nacional Ambiental ejercerá el control previo ambiental, a través de los siguientes instrumentos: 1. Autorizaciones., 2. Aprobaciones, 3. Permisos, 4. Licencias, 5. Concesiones, 6.Asignaciones, 7. Contratos, 8. Planes de manejo, 9. Registros, 10. Los demás que establezca la ley.*
- *Que la Ley Orgánica del Ambiente establece en su artículo 83 que "El Estado podrá permitir la realización de actividades capaces de degradar el ambiente, siempre y cuando su uso sea conforme a los planes de ordenación del territorio, sus efectos sean tolerables, generen beneficios socioeconómicos y se cumplan las garantías, procedimientos y normas. En el instrumento de control previo se establecerán las condiciones, limitaciones y restricciones que sean pertinentes.*

## APÊNDICE D

PERMISSION FOR COLLECTION IN ARGENTINA

PROVINCIA DEL CHACO
SUBSECRETARIA DE AMBIENTE Y BIODIVERSIDAD

CONSTANCIA DE AUTORIZACIÓN

----Por la presente se AUTORIZA al Dr. Juan Pablo Julià D.N.I. 20.219.678, Director de la Reserva Experimental Horco Molle, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán así como a Jose Mauricio Barbanti Duarte (RG: 15980869-8) y Eluzai Dinai Pinto Sandoval (pasaporte: 089201613) a realizar actividades relacionadas con captura y colecta de individuos adultos de la especie de "corzuelas" (*Mazama sp*) de las cuales se tomaran muestras de tejido (hígado, riñón, corazón, pulmón y músculo) para extracción de ADN y caracterización molecular; así mismo se conservara el resto del animal para llevar a colecciones de museos. Estos individuos serán colectados en los Departamentos de Libertador General San Martín, Quitilipi (Departamento de Quitilipi) y Charadai (Departamento de Tapenagá); en el marco del Proyecto de Investigación "Caracterización morfológica, citogenética y molecular de las especies del género Mazama en Argentina".

----La presente autorización es válida dentro de los límites de la provincia del Chaco, debiendo informar a esta Dirección de Áreas Protegidas y Biodiversidad, al terminar la campaña de investigación los resultados obtenidos y cualquier traslado de muestras deberá estar acompañado de su guía de transito respectiva.

----A los efectos de ser presentada ante las autoridades que así lo requieran, se extiende la presente a los 18 días de agosto de 2021.-

VENCIMIENTO: 18/08/2022.

Ing. Edgardo Witchiensky
Director de Áreas Protegidas y Biodiversidad
Provincia del Chaco