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Research Article

Craniometric differentiation suggests disruptive selection on body size among sympatric brocket deer

André Magnani Xavier De Lima[1,*], María Martha Torres Martínez[1,2], José Maurício Barbanti Duarte[3], Susana González[4,5]

[1] *Capão da Imbuia Natural History Museum (MHNCI)*
[2] *Universidade Federal da Integração Latino-Americana (UNILA)*
[3] *Universidade Estadual Paulista Júlio de Mesquita Filho*
[4] *Instituto de Investigaciones Biológicas Clemente Estable*
[5] *Deer Specialist Group, IUCN Species Survival Commission*

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Abstract

The value of craniometrics in classifying brocket deer has been a topic of debate, with its effectiveness within this genus being unclear. This study addressed this uncertainty by examining craniometric data from sympatric species of brocket deer. We present a dataset integrating both published and original data, to elucidate the potential species differentiation by analyzing the inter- and intraspecific variation. Leave-one-out cross-validation (LOOCV) yielded >95 % accuracy in species classification. We observed that variation in skull size primarily involves overall size changes rather than specific variation in skull shape among the species. Our findings demonstrate the effectiveness of multivariate craniometric data for taxonomic classifications and offer valuable insights into the evolutionary dynamics of brocket deer species. The observed multidimensional distinction among brocket deer skulls suggests that disruptive selection plays a key role in driving differences in body size across species, while latitude might be an additional important confound factor.

Introduction

Neotropical brocket deer are a group of cryptic species that inhabit tropical and subtropical forests (Gallina-Tessaro et al., 2019; González et al., 2018). The taxonomic status of the group has been updated primarily through karyotype and DNA analysis, revealing that brocket deer are, in fact, a polyphyletic group, with members from three main subtribal clades (Sandoval et al., 2024; Morales-Donoso et al., 2023; Bernegossi et al., 2023; Peres et al., 2021a; Mantellatto et al., 2022; Heckeberg, 2020; Mantellatto et al., 2020). *Mazama* Rafinesque, 1817 remains the most diverse genus among brocket deer, with species belonging to the Odocoileina subtribe (Sandoval et al., 2024). Despite these advances, most of these species are classified in threatened categories, with six species of *Mazama* listed as vulnerable by the IUCN (IUCN, 2024, e.g., Vogliotti et al., 2016; Duarte et al., 2015). However, the data are often outdated due to new taxonomic classifications or insufficient information for accurate categorisations (e.g., *Mazama rufa*, Peres et al., 2021a).

One major issue in improving the understanding of species occurrence, and therefore updating conservation strategies, is the challenge of identifying brocket deer species based on morphological traits, even when voucher specimens are available (Peres et al., 2021b). The lack of more detailed information on voucher specimens has hindered the study of the tribe Odocoileini (Gutiérrez et al., 2017), emphasizing the need for more reliable identification methods. Improving accuracy in species identification among this group is crucial, especially for sympatric species. In particular, in a large portion of non-Amazon South America, five brocket deer species have partially overlapped geographic distributions, mainly at the tropical/subtropical regional transition in southern Brazil (Oliveira et al., 2022; Peres et al., 2021a). These species include four *Mazama* species (*M. rufa, M. americana, M. jucunda*, and *M. nana*) and the revalidated *Subulo gouazoubira*, a member of the subtribe Blastocerina (Sandoval et al., 2023; Bernegossi et al., 2023; Heckeberg, 2020).

Classification based on external morphological traits is suitable for some paired comparisons but often requires skin preservation and body measurements (Gippoliti and Aloise, 2016). Characteristics of body size and hair colour, position, and morphology have been tested for discriminating between deer species locally and globally; however, several limitations are known (Hua et al., 2020; Silva et al., 2020). Previous attempts to group brocket deer species particularly based on craniometrics have yielded inconclusive results (Merino et al., 2005; Rossi, 2000). Also, several morphological-based identifications of voucher specimens in natural history museum collections have been revised following molecular analysis (Mantellatto et al., 2020). This indicates that previous morphological identifications likely included misclassified specimens, impacting taxonomy and conservation planning (Peres et al., 2021b). Given that genetic approaches remain costly, time-consuming, and not always feasible (e.g., due to the lack of properly preserved tissue samples), there is a need for accessible and reliable taxonomic methods to classify brocket deer specimens (Pires and Marinoni, 2010).

*Corresponding author
Email address: andremxlima@gmail.com (André Magnani Xavier De Lima)

More recently, factorial and principal component analyses have shown some success in differentiating *Mazama* species based on craniometrics (González et al., 2018; Peres et al., 2021a), suggesting that multivariate approaches can be effective for species distinction and for exploring the importance of variation in skull traits (Croitor, 2024; Machado and Teta, 2020). However, besides the historical bias from misidentifications (Mantellatto et al., 2020), small sample sizes have been a general issue in previous comparative analyses. Improving the sample size of correctly identified specimens could provide better insights into the potential for distinguishing sympatric brocket deer through predictive multivariate analysis of craniometrics. In an applied context, skulls are often the most available voucher material in museums, mostly from road-killed specimens (Gippoliti and Aloise, 2016). Thus, understanding how to classify brocket deer species based on craniometrics would significantly enhance the accuracy of identifying voucher specimens in natural history collections.

Unravelling potential patterns of species differentiation through craniometrics is also ecologically important for understanding whether these partially sympatric species have undergone distinct evolutionary processes that might explain differences in skull traits (Munkhzul et al., 2018; Mahmoudi et al., 2017). At a macroecological scale, many mammal species, including cervids, show size variations consistent with Bergmann's rule, which links latitudinal variation to body size due to thermoregulatory needs (Clauss et al., 2013; McNab, 2010; Diniz-Filho et al., 2007; Ashton, 2004; Ashton et al., 2000), though findings have been inconsistent for cervids (Gohli and Voje, 2016). The lack of robust evaluations of intra- and interspecific variation in skull size and shape among brocket deer makes this study critical for understanding the evolutionary forces driving speciation and natural selection within this group (González et al., 2018).

Here, we pooled available published data on craniometry of non-Amazon *Mazama* and *Subulo* species with a new dataset of individuals identified primarily through genetic analysis and distinct skin traits. We tested whether linear discriminant functions can effectively classify species based on craniometrics and whether skull size and shape vary among species. Additionally, we examined whether a latitudinal evolutionary trend (Tamagnini et al., 2021) could be observed for a widely distributed species, *S. gouazoubira*. This broader understanding of intraspecific skull variation is important for improving the accuracy of species classification at a regional level and may provide insights into the evolutionary history of brocket deer.

Materials and methods

Data source

We gathered craniometric data from a total of 80 skulls representing five brocket deer species from six distinctive sources, including published studies and original datasets (Tab. 1). Literature sources included Sandoval et al. (2023), Bernegossi et al. (2023), Peres et al. (2021a), González et al. (2018), and Borges (2017). Species identifications from González et al. (2018) were updated according to Mantellatto et al. (2020). Additionally, original data were gathered from specimens housed at the Natural History Museum of Curitiba (MHNCI) in Paraná State, Brazil, based on molecular identifications provided by Mantellatto et al. (2020) (n=26), supplemented by additional identifications based on morphological traits (n=11). We excluded infants and young juveniles and included subadults as well as adults in the sample data, based on the presence of the third molar and/or antler development. The inclusion of subadults aimed to capture the intraspecific variability present in natural museum collections and to increase the effective sample size.

Craniometrics

We measured 35 skull dimensions using a digital calliper (accuracy: 0.1mm), following the criteria outlined by Von den Driesch (1976). The measured traits included total length (LT), condylobasal length (CBL), basal length (BL), short skull length (SSL), premolar 1 – prosthion (PREPRO), basicranial axis (BACR), basifacial axis (BAF), median frontal length (MFL), lambda-nasion (LN), lambda-rhinion (LR), lambda-prosthion (LP), akrokranion (ACI), greatest length of the nasals (GLN), median palatal length (MPL), oral palatal length (OPL), lateral length of the premaxilla (LLPRMAX), length of the cheektooth row (LCHEE), length of the molar row (LMR), length of the premolar row (LPREM), greatest inner length of the orbit (GLOR), greatest inner height of the orbit (GHOR), greatest mastoid breadth (GMBOO), greatest breadth of the occipital condyles (GBOC), greatest breadth at the bases of the paraoccipital processes (GBPP), greatest breadth of the foramen magnum (GBFM), height of the foramen magnum (HFM), greatest neurocranium breadth (GBBC), least frontal breadth (LFBO), greatest breadth across the orbits (GBAO), least breadth between the orbits (LBBO), zygomatic breadth (ZYB), greatest breadth across the nasals (GBN), greatest breadth across the premaxillae (GBPM), and basion (defined as the highest point of the superior nuchal crest – BNUCR).

Statistical analyses

All statistical analyses were conducted using R version 4.4.1 (R Core Team, 2024). Missing data, accounting for 8.8% of the entire dataset, were handled through imputation rather than the exclusion of observations or variables, following the methodology outlined by Mera-Gaona et al. (2021). Data imputation was performed using the multivariate imputation by chained equations (MICE) package, employing the predictive mean matching method (Van Buuren and Groothuis-Oudshoorn, 2011), with a fixed seed value of 1.

We included *M. rufa* ($n = 4$) within *M. americana* (referred to as the *americana* group) in the analyses due to both the low sample size and the historical uncertainty surrounding the classification of *M. rufa* specimens as *M. americana* before its recent taxonomic revision (Peres et al., 2021a). Age was treated as a binary variable, distinguishing between the subadult (0) and adult (1) classes. We were unable to include sex classes in the analysis due to sample size limitation.

Prior to analysis, multivariate normality of the data was assessed using the Henze-Zirkler and Mardia tests, implemented in the "MVN" package (Korkmaz et al., 2014). Data were log-transformed, and multivariate normality was confirmed through both Henze-Zirkler (HZ = 0.99, p > 0.05) and Mardia tests (Skewness = 7907.56, p > 0.05, Kurtosis = -0.95, p > 0.05; Suppl. Fig. 1). Multicollinearity was evaluated by pairwise correlations tests using the function "cor" from the default

Table 1 – Craniometric data source and sample size for five brocket deer species included in this study.

Source	Species				
	M. americana	*M. jucunda*	*M. nana*	*M. rufa*	*S. gouazoubira*
Borges (2017), Bernegossi et al. (2023)	–	–	–	–	1
González et al. (2018)	–	1	–	–	8
MHNCI (Mantellatto et al., 2020)	2	8	7	-	8
MHNCI	5	2	1	–	3
Peres et al. (2021a)	11	4	4	4	–
Sandoval et al. (2023)	1	–	–	–	10
Total	19	15	12	4	30

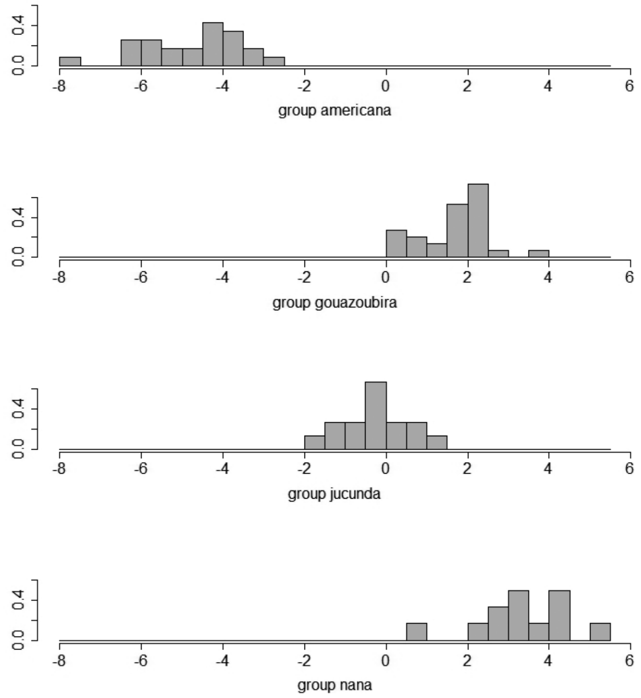

Figure 1 – Distribution of LDA projections onto the first discriminant axis for four sympatric brocket deer species.

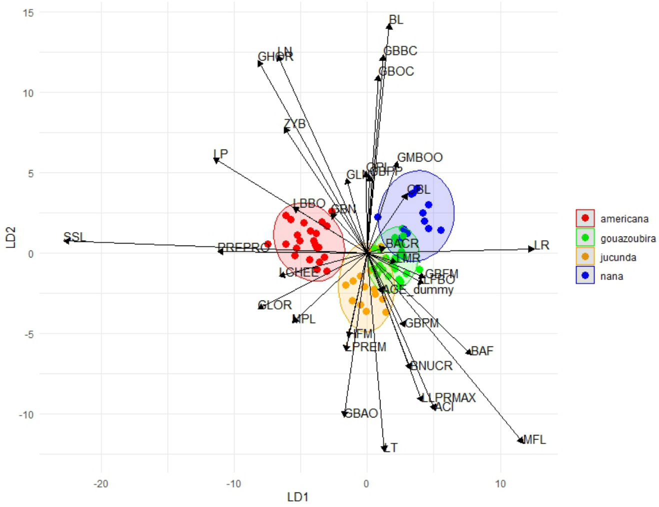

Figure 2 – Two-dimensional LDA scatterplot showing projections onto the first two axes (explaining 91.7% of total variance). Arrows indicate variable directions and effect intensities for the classification of four sympatric brocket deer species.

package "stats", and by estimating variance inflation factor (VIF), using the function "vif" from the "car" package (Fox and Weisberg, 2019).

Linear discriminant function analysis (LDA) was performed using the "lda" function from the "MASS" package (Venables and Ripley, 2002) to assess the potential for discriminating among groups based on craniometric variables. To evaluate the effects of multicollinearity on classification results, we compared the accuracy of the confusion matrix from the LDA with that of a correlation-adjusted Shrinkage Discriminant Analysis (SDA), using the "sda" package (Ahdesmaki et al., 2021). We used Pillai, Wilks, Hotelling-Lawley, and Roy's tests, implemented via the "manova" function, to evaluate differences among group scores. The percentage of explained variance was obtained for each discriminant function and individual variable. Grouping patterns were visually analyzed using observation scores, depicted in up to three-dimensional plots generated using the "plotly" package (Sievert, 2020).

Model validation was conducted through cross-validation techniques (James et al., 2013), including leave-one-out (LOOCV) and K-fold cross-validation (with fold sizes of K=5, 10, and 15), allowing comparisons of model performance. Average success rate and F-score were employed to assess model performance. F-score provides a balanced measure of the model's accuracy by combining precision and recall into a single value. We set recall and precision as evenly weighted – the F1-score (see Li et al., 2016).

Two approaches were used for evaluating the full model reduction. First, we reran the analysis using only the ten most important variables for each axis and compared the results with the full model. Second, we applied a stepwise forward variable selection method based on Wilk's Lambda criterion using the 'greedy.wilks' function from the 'klaR' package (Weihs et al., 2005). Variables were included at a significance level of p=0.05. The selected variables were then used to run discriminant analysis on a simplified dataset for comparison with the full model. Additionally, pairwise t-tests were conducted to explore differences among groups in univariate analysis.

To investigate the influence of latitude on the craniometrics of *S. gouazoubira*, linear regression was performed using the most important skull variables as dependent variables, with latitude as the continuous independent variable. The most important variables were determined based on their contribution to the full model, stepwise selection, and univariate analysis, specifically those variables that significantly distinguished *S. gouazoubira* from the other species.

Results

Linear discriminant analysis

Correlation exceeded 0.5 in 68 % of the pairwise comparisons between variables, and VIF values were above 10 for 18 (∼50 %) of the variables (LT, CBL, BL, SSL, PREPRO, BACR, BAF, MFL, LN, LR, LP, ACI, GLN, OPL, LCHEE, GBOC, GBAO, ZYB). To assess the impact of multicollinearity on classification, we compared the accuracy between LDA and SDA. The LDA of the full model correctly classified 97.5 % of brocket deer species observations (Tab. 2), while the classification accuracy applying SDA was 96.3 %. Therefore, we proceeded with LDA results for further analysis. MANOVA tests indicated significant differences for the overall LDA (Pillai=2.09, $p<0.05$; Wilks=0.01, $p<0.05$; Hotelling-Lawley =11.96, $p<0.05$; Roy = 8.79, $p<0.05$) as well as for each component (LD1: F=228.35, $p<0.05$; LD2: F=66.15, $p<0.05$; LD3: F=24.97, $p<0.05$).

The first discriminant axis (LD1) had the highest eigenvalue (180.27), explaining 76.3 % of the total variance. LD2 had an eigenvalue of 38.44 (15.4 % of the variance), and LD3 had an eigenvalue of 27.6 (8.3 % of the variance). Coefficient scores for each variable and discriminant function are shown in Suppl. Table 1. The distribution of individual scores for LD1 showed significant group segregation, with some overlap in boundary areas (Fig. 1). The ten most important morphological traits for classification along LD1 accounted for 85 % of its variance, with 32 % solely explained by SSL. For LD2 and LD3, BL contributed 11 % to LD2, while LR accounted for 26 % of LD3. Nine variables ranked among the top ten contributors to two axes: BAF, BL, GBBC, GHOR, LCHEE, LN, LP, LR, and MFL (Tab/ 3).

Plotting the first and second axes, which together explained 91.7 % of the variance, highlighted the distinct influences of each variable on species classification (Fig. 2). SSL and LP were key for distinguishing

Table 2 – Confusion matrix of observations vs. full model predictions.

Group/species	*americana*	*gouazoubira*	*jucunda*	*nana*
americana	23	0	0	0
gouazoubira	0	29	1	0
jucunda	0	0	14	0
nana	0	1	0	12

Table 3 – The ten most important morphological traits of brocket deer skull for group classification by linear discriminant functions based on the explained variance of each variable.

LD1		LD2		LD3	
Trait	Variance (%)	Trait	Variance (%)	Trait	Variance (%)
SSL	32	BL	11	LR	26
LR	10	LT	8	ZYB	11
MFL	8	GBBC	8	LCHEE	9
LP	8	LN	8	BAF	8
PREPRO	8	GHOR	8	BL	6
GHOR	4	MFL	8	GBBC	5
GLOR	4	GBOC	7	LBBO	4
BAF	4	GBAO	6	LP	4
LN	3	ACI	5	GBFM	4
LCHEE	3	LLPRMAX	5	LFBO	3

species of the *americana* group, while LR, LT, and MFL differentiated *M. jucunda* and *S. gouazoubira*. BL, LR, and GBBC were the most influential traits in separating *M. nana* from the others. A 3D scatterplot of individual coefficients across all discriminant functions showed clear separation among the four groups, with minimal overlap at the boundaries (Fig. 3; see Suppl. Mat. 2 for an interactive plot).

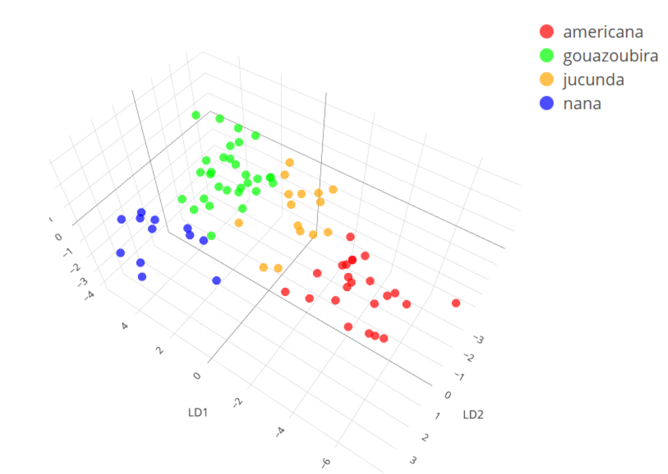

Figure 3 – Three-dimensional LDA scatterplot of four sympatric brocket deer species. An interactive version of this plot is available in the Supplementary Files.

Cross-validation of full model

Cross-validation using the LOOCV method correctly classified 96.1 % of the observations among the four groups/species. The average F1-score for the three configurations of the K-fold method ranged between 0.87–0.90, while the total variance from tests among all K-fold combinations ranged from 60–100 % of correct classifications (Fig. 4).

Model simplification

1) LDA-basis simplified model

The discriminant analysis with the 20 variables representing the 10 most relatively important for each axis in the full model (Tab. 3) plus AGE_dummy resulted in differences among groups (Pillai=1.87, $p<0.05$; Wilks = 0.03, $p<0.05$; Hotelling-Lawley=9.71, $p<0.05$, Roy=7.75, $p<0.05$). The first axis explained 81 % of the total variance, with SSL remaining the most important trait (Suppl. Table 2), while the subsequent axes explained 10 % and 9 %, respectively. The overall F1-score for the classifications by group was 0.94, with 100 % of correct classifications for the *americana* group, 90.3 % for *M. jucunda*, 93.3 % for *S. gouazoubira*, and 86.9 % for *M. nana*. Cross-validation by LOOCV resulted in greater F1-score (0.90) than by K-fold (0.80-0.83).

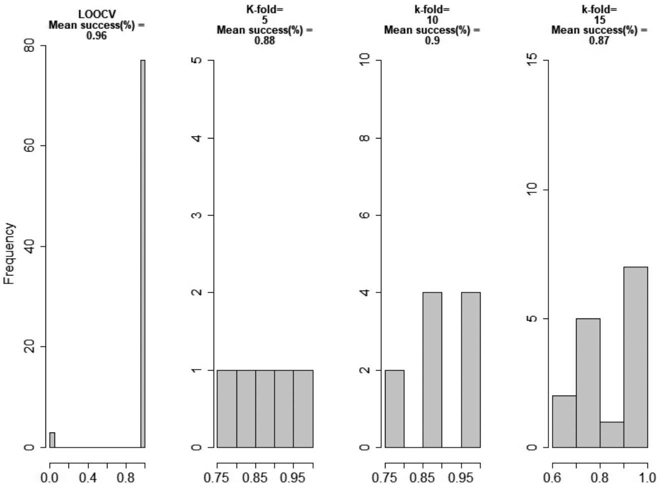

Figure 4 – Distribution and mean classification success across four cross-validation procedures: LOOCV and K-fold (K = 5, 10, and 15).

2) Stepwise selection-basis simplified model

Stepwise forward selection indicated nine morphological traits as important variables, with LP as the most important trait (Suppl. Table 3). The discriminant analysis with these variables resulted in differences among groups (Pillai = 1.73, $p < 0.05$; Wilks = 0.04, $p < 0.05$; Hotelling-Lawley = 7.12, $p < 0.05$, Roy = 5.61, $p < 0.05$). The first axis explained 81.4% of the total variance, with ZYB as the most important trait (Suppl. Table 4), while the subsequent axes explained 11.3% and 7.2%, respectively. The F1-score for the classifications by group was 0.94, with 100% for the *americana* group, 89.6% for *M. jucunda*, 93.5% for *S. gouazoubira*, and 86.9% for *M. nana*. Cross-validation by LOOCV resulted in greater F1-score (0.91) than by K-fold (0.78-0.81).

Univariate analysis

Results of univariate pairwise t-tests with the log-transformed data were significant in 79 % of 210 paired tests. Simultaneously distinguishing the four groups occurred with eight variables (LT, LP, CBL, BL, BAF, LLPRMAX, LCHEE, MFL). Tests with significant results distinguishing at least three groups occurred as follows: 13 variables distinguished the groups *americana* and *M. jucunda* from the others (ZYB, OPL, SSL, LR, ACI, GLOR, GBPP, GBAO, GLN, GBBC, LMR, LFBO, LN), three variables distinguished *M. americana* and *M. nana* from the others (BNUCR, PREPRO, MPL), while only one distinguished *S. gouazoubira* and *M. nana* simultaneously (LPREM) (Suppl. Table 5).

Latitudinal variance in S. gouazoubira

A total of 12 variables among those considered the most important were tested as dependent variables of latitude in the linear regression mod-

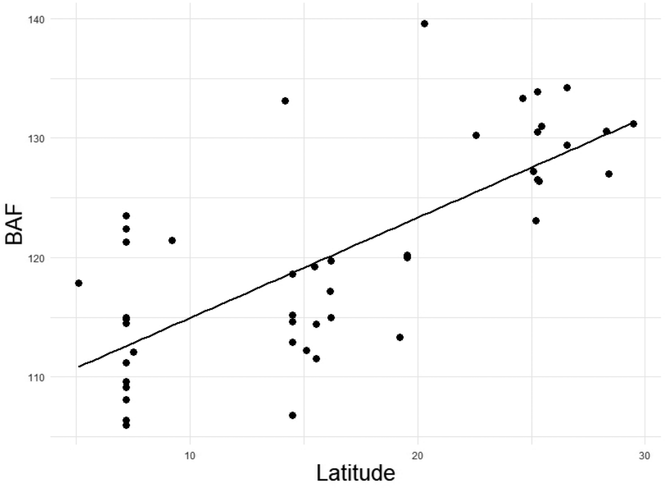

Figure 5 – Linear regression showing the relationship between basifacial axis (BAF) length and latitude of origin for the Gray brocket deer *(Subulo gouazoubira)* in South America (R2=0.53).

els. Nine of them had significant results, with BAF having the greatest influence (R^2=0.53, Tab. 4, Fig. 5).

Table 4 – Sample size, coefficient of determination and significance of linear regressions tests for selected skull variables of the four sympatric groups/species of brocket deer.

Variable	n	R^2	p	Signif.
BAF	47	0,53	<0.001	***
LR	50	0,26	<0.001	***
LT	58	0,22	<0.01	**
LP	49	0,19	<0.01	**
CBL	57	0,16	<0.01	**
BL	56	0,15	<0.01	**
LLPRMAX	59	0,12	<0.01	**
MFL	53	0,12	<0.05	*
ZYB	60	0,07	<0.05	*
LCHEE	62	0,04	>0.05	-
LPREM	62	0,03	>0.05	-
SSL	59	0	>0.05	-

Discussion

The application of linear discriminant function analysis to assign brocket deer species based on their craniometrics yielded a significant success rate, particularly when considering the full model including the standard 35 skull variables (along with age). Although the use of reduced models resulted in a slight decrease in the success rate of classifications, the outcomes remained relatively similar. This suggests that further strengthening of model simplification could be achieved with a larger sample size per group (Maas and Hox, 2005). Thus, our study demonstrates that using observations of confirmed identified individuals leads to high accuracy in classification modelling by linear discrimination functions (Thier et al., 2020). Therefore, there is substantial potential for applying linear discriminant functions to successfully classify unidentified specimens of mammals (Suchentrunk et al., 2007), including brocket deer (González et al., 2018). Furthermore, our findings highlight the importance of predictive multivariate analysis with craniometrics as an additional tool for supporting taxonomic distinctiveness among brocket deer species.

In overview, *Mazama americana* can be distinguished from other species due to their greater size in most skull traits, while the opposite is true for *M. nana* (Peres et al., 2021a; Abril et al., 2010). The inclusion of *M. jucunda* in the comparisons results in three well-distinct size classes, even when considering only the first discriminant dimension. However, the addition of *S. gouazoubira* in the comparisons introduces some overlap in the distribution of observations of skull traits (Fig. 1). These patterns place the latter species between *M. jucunda* and *M. nana*, which become clearly evident only when considering the three dimensions of discriminant analysis (Fig. 2 and fig:figure3). Although some variables were able to distinguish the four species classes in the univariate approach, overlapping confidence intervals were frequent due to the small scale of the variables, making univariate comparisons unreliable for species identification (Suppl. Table 5). However, when significant, univariate tests consistently showed the same body size order that we found in the multivariate analysis, suggesting an overall allometric differentiation trend among the species. These results support previous study that did not find differences in skull shape patterns in three-dimensional comparisons among some *Mazama* and *S. gouazoubira*, which all have similar short-nosed skulls compared to larger species (Merino et al., 2005).

Relative Influence of Skull Traits

Nearly one-third of the skull variables were highlighted by the discriminant functions, with almost half of the total variance in the full model explained by SSL and LR sizes, which are highly correlated (r=0.79) and dominated the first and third axes. SSL represents the size between the first pair of premolars and the basion of the cranium, while LR adds the distance between premolars and the rhinion to the measurement (Von den Driesch, 1976). However, among other important variables, width-related ones such as ZYB, GBBC, and GBOC, along with variables representing specific traits like the orbital area (GHOR and GLOR), also showed significant relative influence. Thus, the most important skull variables were related to general three-dimensional allometric differences, not solely the length of the skull. Although length-related variables were key for explaining the observed variance in multivariate analysis and distinguishing the four groups/species, their scale and amplitude are greater compared to other types, which may explain their greater relative influence. Given this, there is no suggestive evidence for substantial variation in the general shape of the skull among the species. This pattern is consistent with the strong conservatism in skull traits observed among small species of Old-World deer, which is thought to be caused by eco-physiological constraints (Croitor, 2024).

It is important to highlight that the sample size of individuals with confirmed sex identification was not large enough for comparative analysis, making sex a confounding factor in our results, as males tend to be larger than females (González et al., 2018; Merino et al., 2005). Even though uncertainty regarding sex classes is included in our results, it did not seem to significantly affect the overall outcome of four well-distinct groups/species projected by the linear discriminant functions. A focused analysis with only sexed individuals could potentially increase predictive power for classifying brocket deer.

On the other hand, addressing concerns about multicollinearity, our attempts to reduce its effects, including the application of Shrinkage Discriminant Analysis (SDA), did not lead to significant improvements in classification rates. The accuracy of the SDA was comparable to that of the full LDA model. As an additional approach, we explored a three-dimensional plot of the first three principal components (PCA) to visualize potential effects of multicollinearity. However, the PCA failed to correctly group the species as effectively as the LDA (Suppl. Fig. 2). These findings suggest that collinearity, while present, did not substantially undermine classification accuracy. This aligns with the understanding that concerns around variance inflation are more critical for regression models than for classification purposes.

Furthermore, reducing multicollinearity in craniometric studies is challenging due to the inherent relationships among numerous skull measurements (see Von den Driesch, 1976). In contexts with limited sample sizes, as in our study, removing correlated variables could actually reduce classification accuracy. The inclusion of all variables in the full model helped to mitigate bias, potentially minimized issues like missing data or measurement errors, and ultimately improved the reproducibility of the results. Thus, the primary goal of maximizing classification accuracy justified the inclusion of all variables, even in the presence of collinearity.

Finally, although our approach could increase the risk of overfitting, two different model simplification methods were tested, yielding consistent overall results across different variable selections. Additionally, despite using <9 % imputed data, analyses with different seeds and randomizations produced consistent classification rates, reinforcing the robustness of the findings. While the classification rate was generally high, the influence of individual skull traits on the results must be interpreted with caution, given the intrinsic correlation among variables.

Evolutionary pathways for inter- and intraspecific craniometric variation

The multidimensional differences in skull size among brocket deer species align with variations in their average body size (Azevedo et al., 2021). However, whether and to what extent the evolutionary process of speciation of brocket deer has been directly influenced by variation in body size and the latitudinally variable environmental conditions remains unclear.

From a broad-scale and historical perspective, the latitudinal effect appears to be another significant factor in body and skull size, especially for wide-ranging deer species. The Bergmann's rule (increase of body size related to increase in latitude) is commonly observed and proposed as an adaptive process for many mammals and other endothermic species at intra- and interspecific levels, as a response towards the optimization of the trade-off between the body surface (area/volume) and its temperature regulation (Pincheira-Donoso, 2010; Diniz-Filho et al., 2007; Gilbert et al., 2006; Ashton, 2004; Mayr, 1956). While a strong correlation between size and latitude was found in examinations of a few cervid species (Clauss et al., 2013), results at the family level may not corroborate Bergmann's or Allen's rules (Gohli and Voje, 2016).

We demonstrated that the skull size of *S. gouazoubira* is partially determined by latitudinal position, which could influence between 5–53 % of the variance in some of the skull measurements. This intraspecific variation may represent up to 30 % of the actual size (e.g., BAF – Fig 5) and supports predictions of Bergmann's rule (Gilbert et al., 2006; Ashton et al., 2000; Mayr, 1956). Moreover, such a significant allometric pattern emphasizes that skull size, as a proxy for full body size, is an important morphological trait under natural selection among brocket deer (Smith et al., 1986).

Refined hypotheses for explaining the natural causes of latitudinal variation in body size among closely related species consider the optimal combination of resource availability and seasonal environmental constraints determining optimal body size, regulated by energy costs in each region (Mariño et al., 2023; Rubalcaba et al., 2022). Indeed, at the interspecific level, recent studies have also provided supplementary controversial information in this context. For instance, the largest of the gray brocket deer species, which was recently validated (Sandoval et al., 2024), inhabits tropical areas at the lowest latitudes of South America. On the other hand, the smallest species among South American cervids are distributed in temperate to subtropical latitudes (i.e., *Pudu* spp., *Pudella carlae*, and *M. nana* – see Barrio et al., 2024; Peres et al., 2021a). While Bergmann's rule and other hypotheses on optimization are originally related to interspecific comparisons, we found that the smallest individuals of *S. gouazoubira* were at the lowest latitudes, which could be explained by thermoregulation needs (He et al., 2023). Thus, it appears that the latitudinal influence on deer evolutionary processes must be primarily taken into consideration as a species-specific process.

In this context, the clearly distinct multidimensional size classes among brocket deer craniometrics led us to suggest that their speciation might have been largely driven by adaptive processes related to body size variation, a common process among mammals (Baker et al., 2015; Cooper and Purvis, 2010). While body size is an important trait as a secondary sexual characteristic favouring larger individuals, we hypothesize that body size may have favoured disruptive selection rather than a unidirectional process. Although larger male individuals are more likely to successfully breed in deer polygamous mating systems (Newbolt et al., 2017), smaller individuals benefit from lower energy requirements and enhanced mobility in dense forest environments, the primary habitat structure of these species, thus optimizing their potential niche (Gilbert et al., 2006). As a result, while selection pressures might also favour smaller individuals due to these ecological advantages, a trend towards uniformity in cranial patterns may reflect similar eco-physiological constraints, as observed among Old-World deer (Croitor, 2024).

The generalist herbivory of brocket deer denotes strong niche conservatism, which may also explain the absence of adaptive differences in skull and body shape among species, while competition for food may likely exerted the main selective pressure, especially during unfavourable climatic conditions (Olalla-Tárraga et al., 2017). Typical climatic seasonality and instability in high latitude and elevation regions may facilitate the speciation of distinct size-classes of closely related species due to more intense adaptive processes (Morales-Barbero et al., 2021; Diniz-Filho et al., 2007), which may occur independently of phylogeny (Diniz-Filho et al., 2009). Thus, the speciation process among brocket deer might have been mainly driven by disruptive selection of body size, likely also due to food competition and as a result of the occupation of similar forest environments (Duarte et al., 2008). Such conditions, combined with the occurrence of chromosomal polymorphism, would favour speciation due to the unlikely potential fertility among distinct size-class and polymorphic populations of brocket deer (Galindo et al., 2021).

Additionally, the combination of slightly overlapped distributions in skull traits of *S. gouazoubira* with *Mazama* spp. suggests that this species might have undergone an early niche displacement due to interspecific competition with sympatric *Mazama* species before size-class disruption occurred (Ferreguetti et al., 2015). This hypothesis is supported by the fact that *S. gouazoubira* is the most habitat generalist among brocket deer species, and therefore was likely displaced from tropical and subtropical forests to forest edges, riparian and dry forests, savannahs, and even grassland-like habitats (González et al., 2020; Gallina-Tessaro et al., 2019). There is also known evidence of differences in daytime activity: the Gray brocket is mainly diurnal, while *Mazama* species are nocturnal (Srbek-Araujo et al., 2019). Temporal differentiation was also correlated with differences in habitat use and occupancy probability between *M. americana* and *S. gouazoubira* (Grotta-Neto, 2020; Ferreguetti et al., 2015; Rivero et al., 2005), which suggests daytime partitioning as another component of their ecological niche that would favour species coexistence (Grotta-Neto, 2020; Lucherini et al., 2009; Kronfeld-Schor et al., 2001).

Such differences in the realised niche and our results on latitudinal variation in skull size suggest that the relative influence of body size on the adaptive process of the Gray brocket may have eased while intraspecific selection increased as the species spatially expanded throughout its potential adaptive niche throughout the speciation process. This hypothesis aligns with the consistent estimates of earlier phylogenetic differentiation of the species from *Mazama* among brocket deer, which are not a monophyletic group (Barrio et al., 2024; Sandoval et al., 2024; Duarte et al., 2008; Gilbert et al., 2006).

Conclusion

We have assembled the most comprehensive dataset on the craniometrics of sympatric brocket deer species, combining both published and original data. This foundational dataset is crucial for future research aimed at refining classification models for brocket deer based on skull morphology. While the phylogenetic relationships within these groups remain under investigation, our study supports the use of linear discriminant functions applied to craniometric data as a statistical tool for validating the taxonomic classification of brocket deer. We observed that species classification is primarily driven by overall skull size rather than specific sub-part variation, although factors such as sex and latitude may introduce some bias. Our findings suggest that the distinct multidimensional variation in skulls among brocket deer species has likely arisen from disruptive selection on body size. Furthermore, we provide additional evidence to refine hypotheses regarding the evolutionary history of brocket deer species. Our results also highlight the importance of maintaining and leveraging biological collections and exploring cost-effective methods (Cook and Light, 2019; Trail, 2021; Ferguson, 2020).

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Supplementary Material 1 - Table S1 The linear coefficients of each log-transformed morphological skull trait for each linear discriminant function in the full model.
Supplementary Material 1 - Table S2 Linear discriminant coefficients from the 21-variables model.
Supplementary Material 1 - Table S3 F statistics and p value obtained during stepwise forward variable selection process.
Supplementary Material 1 - Table S4 Linear discriminant coefficients from the stepwise selection-basis model.
Supplementary Material 1 - Table S5 Results from pairwise t tests among most important variables.
Supplementary Material 1 - Figure S1 Quantile-Quantile plot after data log-transformation.
Supplementary Material 1 - Figure S2 Three-dimensional plot of the three main principal components considering four sympatric groups/species of brocket deer.
Supplementary Material 2 Interactive 3D plot.
Supplementary Material 3 Dataset.
Supplementary Material 4 Autorotating 3D plot.