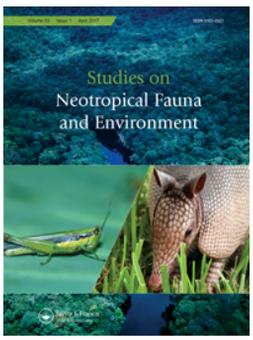

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ORIGINAL ARTICLE

Low accuracy of identifying Neotropical deer species by scat morphology

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ABSTRACT

Morphometric feces data are used to identify ungulates, but their effectiveness is questioned by numerous authors. Herein, we evaluated the efficiency of this tool in discriminating scat samples from Neotropical deer with sympatric distributions. We performed discriminant analysis of previously identified scat samples (n = 204). The accuracy of discriminant analysis (56–92%) was lower than the confidence limit established in this study in all sympatric combinations expected in these biomes. These results demonstrate serious limitations regarding the use of scat morphometry for species identification of Neotropical deer and reinforce the need to use non-invasive genetic techniques.

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Fecal samples; *Mazama*; scat morphology; fecal DNA; Cervidae

Introduction

For numerous animal species with elusive or nocturnal habits, or those that are rare or aggressive, there is a serious lack of ecological studies that involve large samples based on direct observation and the capture of individuals. Since many of these species are subject to some degree of the threat of extinction, this scarcity of research data limits important and urgent actions for conservation management.

One way to circumvent the difficulties involved in the capture and observation of individuals belonging to rare and elusive species is the use of indirect evidence of the presence of these species, including tracks (Wilson & Delahay 2001) and feces (Putman 1984). Scats are products that provide a wealth of information about individual animals, such that it is possible to conduct ecological studies of habitat use (Rivero et al. 2005), diet (Stewart et al. 2003; Nájera-Hillman & Mandujano 2013) and density (Periago & Leynaud 2009), as well as genetic (Fagundes 2005) and endocrinological studies (Pereira et al. 2006).

Deer species that occur in Brazil (*Blastocerus dichotomus, Ozotoceros bezoarticus, Odocoileus virginianus, Mazama gouazoubira, Mazama nemorivaga, Mazama americana, Mazama nana* and *Mazama bororo*) are no exceptions to the rule, and studies on these species are rare. This scenario is aggravated when considering species of the genus *Mazama*, which are nocturnal, shy, elusive and inhabit dense forests (Barrette 1987; Vogliotti 2003). Another aggravating factor, given the context of lack of studies, is that *M. nana, M. bororo, B. dichotomus*, and *O. bezoarticus* appear on both national (Duarte et al. 2012a, 2012b, 2012c, 2012d) and international lists (IUCN 2014) of endangered fauna and are classified as vulnerable.

Historically, fecal analysis has been used to circumvent this issue with ungulates (Rost & Bailey 1979; Acevedo et al. 2010), in general, and certain species of the genus *Mazama* in particular (Rivero et al. 2005; Periago & Leynaud 2009). However, identification at the species level is frequently based only on pellet morphometry, which has been widely criticized in the literature (Seton 1925; Chame 2003; Vogliotti 2008). The problem is even more complex when the geographical distributions of these species exhibit extensive overlap, as is the case of Brazilian deer species, where sympatry in several areas has been documented (Weber & Gonzalez 2003; Duarte & González 2010). Important examples include the Atlantic Forest, whose forest formations harbor *M. americana, M. gouazoubira, M. nana*, and *M. bororo*, and the Cerrado and Pantanal, where *B. dichotomus, O. bezoarticus, M. americana*, and *M. gouzoubira* co-occur.

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Similar to scat identification, methodologies involving the recording of tracks were widely used in the past, though there are now serious reservations as to their reliability (Angeli et al. 2014).

Therefore, in this paper, we compared the morphology of fecal samples of six species of Neotropical deer, and samples from wild and captive deer of one species, in order to evaluate the accuracy of identifying deer species based on scat morphometry.

Materials and methods

To incorporate more samples and all *Mazama* species in our database, we conducted two initial experiments. To validate the inclusion of captive deer samples and ensure that the captive condition, particularly diet, does not influence pellet morphology, we tested the differences between fecal samples from wild and captive deer and the effect of preserving samples in absolute ethanol on fecal morphology. Finally, discriminant analysis was performed to determine the potential of the technique to accurately identify deer species based on their scat samples.

The values for the length and width of each fecal sample consisted of the mean of the measurements of each pellet (n = 15–30 per sample). These measurements were made with the aid of a precision caliper 0.05 mm. Samples from wild deer were collected with the assistance of scat detection dogs and identification was determined by DNA (Vogliotti 2008; Oliveira et al. 2012; Oliveira & Duarte 2013). The DNA was extracted and a fragment of the mitochondrial cytochrome b gene was amplified and cut with specific restriction enzymes (González et al. 2009; Souza et al. 2013). We were unable to determine individuals' age in this sample pool.

Comparisons between samples of wild and captive deer were performed by testing the difference between the measurements (length and width) of 30 scat samples of *M. americana* collected in the wild and 30 scat samples from 18 individuals of *M. americana* maintained in captivity. The fecal samples of captive deer were collected at the Deer Research and Conservation Center (NUPECCE) of the Faculty of Agricultural and Veterinary Sciences (FCAV) of São Paulo State University (UNESP), Jaboticabal campus. These individuals are all adults and maintained in individual stalls and are fed twice a day, when their water is changed. They receive commercial horse feed in the morning and a variety of fresh forage in the afternoon. The wild deer scat samples were collected previously in Iguaçu National Park (Vogliotti 2008). The unpaired t-test was used to perform these comparisons, with BioEstat 5.3 software (Ayres et al. 2007), at a 5% level of significance.

The effect of preserving scat samples in absolute ethanol was evaluated using 10 fresh samples (30 pellets per sample) collected equally from individuals of the species *M. americana, M. gouazoubira, M. nana, M. nemorivaga*, and *M. bororo* maintained in captivity. The samples were collected and measured before and after being preserved for 24 h in ethanol. This time span is considered to be sufficient to simulate storing conditions, in which samples remained stored for weeks or years, since ethanol quickly penetrates samples and changes their color in about 10 min. The paired t-test was used to perform these comparisons, with BioEstat 5.3 software (Ayres et al. 2007), at a 5% level of significance.

To determine whether fecal morphology is effective as an identification tool at the species level, 204 samples collected in the wild from the following species were used: *M. americana* (n = 56), *M. gouazoubira* (n = 30), *M. nana* (n = 28), *M. bororo* (n = 30), *O. bezoarticus* (n = 30), and *B. dichotomus* (n = 30). All the sample measurements were plotted on a chart showing length as a function of width, to produce a broad view of the data. Discriminant function analysis was performed between all the species that can occur in sympatry in the Atlantic Forest, the Cerrado and the Pantanal. This analysis was also performed between pairs of species that, in some areas, can occur in sympatry in isolation from the others, such as Iguaçu National Park, where *M. americana* and *M. nana* co-occur. Discriminant function analysis was performed using Statistica 7.0 software (Statsoft Inc. 2007), and was considered adequate only for functions correctly classified in more than 95% of the samples. In this analysis, in addition to length and width values, the product values and ratios of these measurements were incorporated.

Results

Fecal samples from wild and captive deer showed no significant differences in either measurement, length (t = −0.311; p = 0.756) or width (t = 0.343; p = 0.732).

No effect was observed due to preservation in absolute ethanol on either measurement, length (t = 0.090; p = 0.928) or width (t = −0.050; p = 0.960), when comparing scat samples before and after preservation.

Analysis of the variation in length and width of the fecal samples showed extensive overlapping among the values of five of the deer species analyzed, while only the values of *B. dichotomus* scat samples showed some separation (Figure 1). Even so, numerous samples from

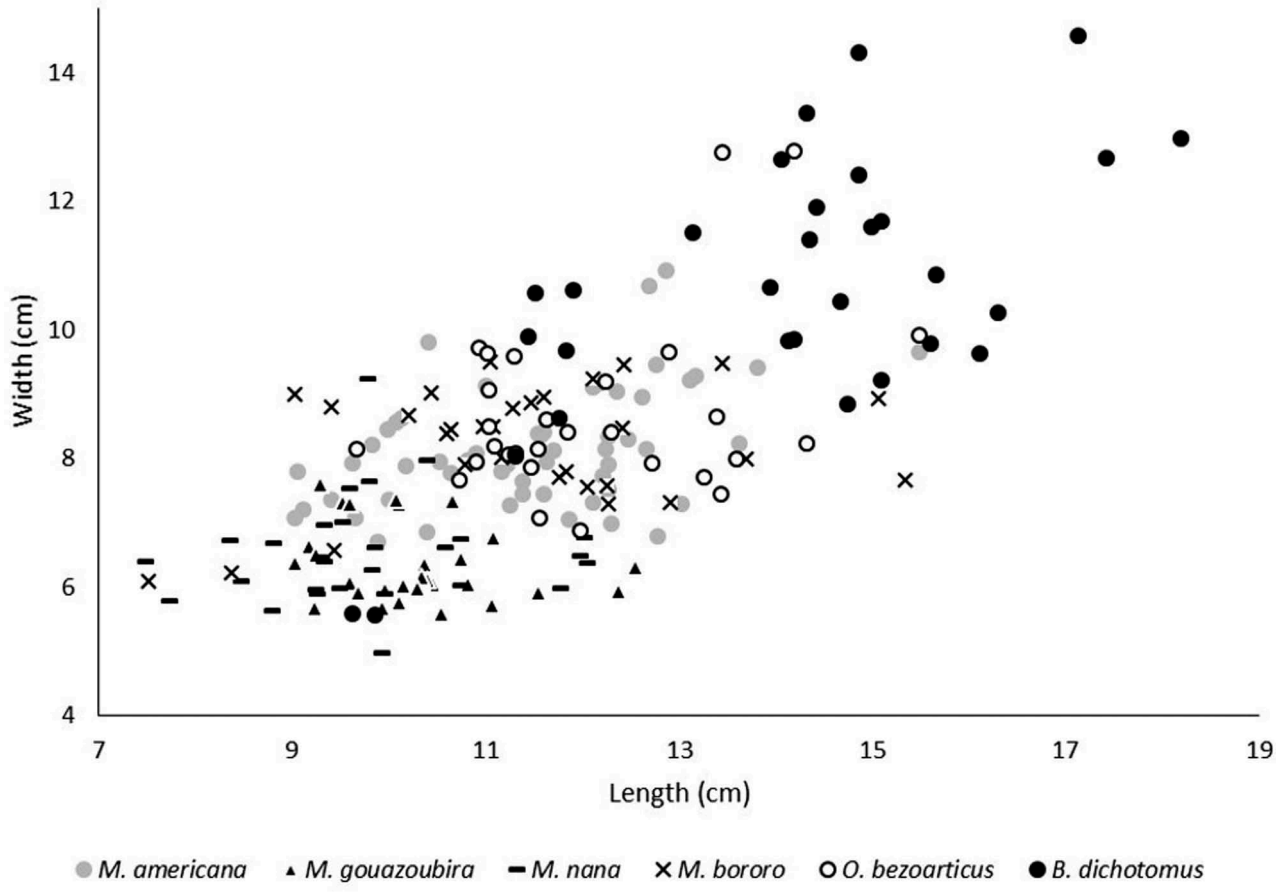

Figure 1. Length of the pellets as a function of width of the pellets for different fecal samples of Neotropical deer.

this species showed values that overlapped with the other species.

Discriminant analysis identified classification functions among species occurring in sympatry in the Atlantic Forest and between species occurring in sympatry in the Cerrado and the Pantanal (Table 1). None of the functions achieved an adequate percentage of correct classification of the samples, with only 55.6% of correct classifications in the Atlantic Forest and 59.0% of correct classifications in the Cerrado and the Pantanal. Even when species were paired for this analysis, the functions did not achieve an adequate level of correct classifications, with errors above 7.8% (Table 2).

Discussion

Samples from captivity and storage conditions

Analysis of the results indicated no difference between the scat samples from wild or captive deer, nor between samples that were fresh or preserved in absolute ethanol. This finding allowed for the inclusion of a sample

Table 1. Classification functions for groups of species of the genus *Mazama*, *Ozotoceros* and *Blastocerus* that can occur in sympatry in the Atlantic Forest, the Pantanal and the Cerrado.

Species	Discriminant function	Correct classification
Sympatric species in the Atlantic Forest		
M. americana	DF = (24.81 × lgth) + (155.71 × wdth) + (305.40 × ratio) + (−8.15 × prod) + (−610.30)	55.6%
M. gouazoubira	DF = (18.38 × lgth) + (155.15 × wdth) + (333.31 × ratio) + (−7.81 × prod) + (−606.66)	
M. nana	DF = (19.07 × lgth) + (154.08 × wdth) + (326.74 × ratio) + (−7.80 × prod) + (−596.80)	
M. bororo	DF = (23.88 × lgth) + (156.38 × wdth) + (310.23 × ratio) + (−8.12 × prod) + (−615.23)	
Sympatric species in the Cerrado and the Pantanal		
M. americana	DF = (10.71 × lgth) + (120.32 × wdth) + (305.96 × ratio) + (−5.20 × prod) + (−524.49)	59.0%
M. gouazoubira	DF = (4.84 × lgth) + (118.97 × wdth) + (330.37 × ratio) + (−4.87 × prod) + (−515.47)	
O. bezoarticus	DF = (11.05 × lgth) + (121.19 × wdth) + (306.74 × ratio) + (−5.25 × prod) + (−533.45)	
B. dichotomus	DF = (8.34 × lgth) + (120.22 × wdth) + (314.43 × ratio) + (−5.02 × prod) + (−529.02)	

Functions abbreviations: *Lgth*, length of the pellet; *wdth*, width of the pellet; *ratio*, lgth/wdth; *prod*, lgth × wdth.

Table 2. Classification functions for species of the genera *Mazama*, *Ozotoceros* and *Blastocerus* that can show sympatry between two species in the Atlantic Forest, Cerrado and Pantanal.

Species	Discriminant function	Correct classification
Sympatric species in the Atlantic Forest		
M. americana	DF = (2.30 × lgth) + (7.79 × wdth) + (−45.29)	87.0%
M. gouazoubira	DF = (2.21 × lgth) + (5.87 × wdth) + (−31.16)	
M. americana	DF = (2.14 × lgth) + (7.07 × wdth) + (−41.44)	85.8%
M. nana	DF = (1.94 × lgth) + (5.52 × wdth) + (−28.80)	
M. americana	DF = (1.69 × lgth) + (5.91 × wdth) + (−34.21)	66.4%
M. bororo	DF = (1.63 × lgth) + (6.02 × wdth) + (−34.98)	
M. gouazoubira	DF = (3.59 × lgth) + (10.08 × wdth) + (−51.15)	58.3%
M. nana	DF = (3.46 × lgth) + (10.36 × wdth) + (−51.57)	
M. gouazoubira	DF = (2.06 × lgth) + (6.07 × wdth) + (−30.60)	83.8%
M. bororo	DF = (2.15 × lgth) + (8.02 × wdth) + (−45.68)	
M. nana	DF = (1.77 × lgth) + (5.41 × wdth) + (−27.15)	84.8%
M. bororo	DF = (1.92 × lgth) + (6.96 × wdth) + (−40.05)	
Sympatric species in the Cerrado and the Pantanal		
M. americana	DF = (2.15 × lgth) + (4.87 × wdth) + (−32.64)	63.1%
O. bezoarticus	DF = (2.24 × lgth) + (5.17 × wdth) + (−36.79)	
M. americana	DF = (1.63 × lgth) + (2.48 × wdth) + (−19.82)	83.5%
B. dichotomus	DF = (1.84 × lgth) + (3.29 × wdth) + (−30.78)	
M. gouazoubira	DF = (2.79 × lgth) + (4.32 × wdth) + (−28.85)	92.1%
O. bezoarticus	DF = (3.16 × lgth) + (6.04 × wdth) + (−45.63)	
M. gouazoubira	DF = (1.77 × lgth) + (1.22 × wdth) + (−13.61)	92.2%
B. dichotomus	DF = (2.06 × lgth) + (2.48 × wdth) + (−27.64)	
O. bezoarticus	DF = (1.78 × lgth) + (1.62 × wdth) + (−18.29)	76.8%
B. dichotomus	DF = (1.96 × lgth) + (2.04 × wdth) + (−24.84)	

Functions abbreviations: *Lgth*, length of the pellet; *wdth*, width of the pellet.

bank of scats preserved in ethanol in our comparative study between species. Even though samples collected in the wild are more interesting because they are probably composed of more than one individual and represent the population variation to a greater degree, their collection is a limiting factor for many groups of animals. Thus, despite all the sources of variation inherent to the captive condition in comparison with free-living, using samples from animals in captivity expands the possibilities of analyzing feces reliability for species identification of other taxa, even on a preliminary basis. Regarding samples preserved in ethanol, the main means of storing fecal samples for all types of genetic studies, this finding is of interest because it allows researchers to combine species identification by fecal morphometry rather than using a specific molecular marker to achieve the same, saving time normally spent following laboratory protocols (Beja-Pereira et al. 2009). Furthermore, the possibility of using samples preserved in ethanol allows us to analyze the relationships between the fecal morphometry of species not evaluated here, even those from other biomes.

Our results contradict the idea that diet has a strong influence on scat shape and size (Chame 2003) and the observation by Hibert et al. (2008) that captive samples show important differences from wild African ungulates. However, these characteristics have only now been tested directly on species of Neotropical ungulates, so drawing definitive conclusions about how much influence diet may have is premature.

Tool reliability and implications

Although the shape and size of scat samples allows for the differentiation of large groups of mammals (e.g. orders and certain families), identification on a finer scale is debatable (Chame 2003). Although some differences were observed in this study, the levels of accuracy obtained in the discriminant analyses were extremely low when comparing within each biome. Even paired comparisons of possible sympatries, where discrimination is easier, did not meet the pre-established confidence limit and indicated that the overlap in size could lead to important errors in the application of fecal analysis to identify fecal samples of Neotropical deer species. This finding is in agreement with that observed for ungulates from the African savanna (Hibert et al. 2008), and more specifically for duikers (Bowkett et al. 2009), African forest bovids that present converging morphological and ecological characteristics with deer of the genus *Mazama* (Barrette 1987).

This finding elicits reflections directly linked to ecological studies and the conservation management of these species, since records have detected the presence of deer species solely through visual identification of their feces (Gaspar 2005; Lyra-Jorge 2007). In

addition, studies on density estimation using pellet-group counts are very common among cervids (Mandujano 2014), including South American populations (Rivero et al. 2004; Periago & Leynaud 2009). In Neotropical species, Chame (2003) has already highlighted the impossibility of differentiating between *M. gouazoubira* and *M. americana* by measuring scat samples. However, Rivero et al. (2004) obtained different results with the same species and affirmed that differentiation was possible, reporting density estimates and habitat use for both species (Rivero et al. 2004, 2005). Our analysis for these same two species showed accuracy of only 86%, indicating a large overlap between them, a fact not observed by Rivero et al. (2004), who focused on the average values of the measurements, rather than where they overlapped. Bowkett et al. (2009) also emphasized that the mean values for these species were different, but that overlapping values prevented accurate discrimination. Besides this methodological problem, differences in the size of individuals, such as the presence of fawns in the population, leads to higher variability in scat sizes, which further complicates discrimination among species due to the larger overlap in scat sizes. Significant differences have been described, for example, in the size of fecal deposits between different age groups of caribou (*Rangifer tarandus*) (Ball 2010).

The alleged relationship between body size and the size of fecal pellets suggests potential differentiation between animals of different sizes, such as the marsh deer (approximately 100 kg) and the brocket deer (approximately 20 kg). However, observations show that even among such different species, the discriminant functions showed accuracy of 92%, below the pre-established ideal of 95%.

Conclusion

Given these findings, it is evident that the use of fecal analysis to identify the feces of non-Amazon Brazilian deer species, in any situation that sympatry could occur between species, should not be implemented. Our findings also suggest extreme caution in using this tool with other Neotropical deer species. On the other hand, the use of noninvasive genetic techniques has already established itself as an effective tool in ecological studies (Beja-Pereira et al. 2009; Souza et al. 2013) and the current costs and accessibility (approximately US$10–15 per sample) no longer represent limitations to their application. Thus, the identification of fecal samples from deer at the species level by genetic methods is the most reliable option available.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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