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Article

Assessing the morphological identification of guard hairs from Brazilian deer

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ABSTRACT. Given the difficulty of collecting biological samples from rare and elusive species, the collection and analysis of hair is a good alternative for the identification and differentiation of mammal species. Our study aimed to test the reliability of the morphological identification of guard hairs from cervids that inhabit Brazil. We collected guard hairs from five body regions (head, neck, side of the thorax, back, and buttocks) of one male and one female of eight Brazilian cervid species, and we analyzed hair cuticular and medullar patterns. We carried out qualitative and quantitative analyses on the morphology of the medullar (total thickness and thickness of the medulla) and cuticular patterns (area and perimeter of the scales) of the guard hairs. Based on the obtained data, we found no notable morphological differences in the cuticular and medullar patterns in the guard hairs. Furthermore, our quantitative analysis demonstrated that the guard hairs are not a useful material for differentiating the Brazilian deer species.

KEYWORDS. *Blastocerus*, *Mazama*, Non-invasive sampling, *Odocoileus*, *Ozotoceros.*

Neotropical deer are difficult to capture because they occur in low density, exhibit elusive behavior, and do not tolerate human contact. Such characteristics preclude the obtainment of biological samples, such as blood, tissue, or cells in general, which are important in studies that involve wild animals.

Due to this difficulty, alternative and non-invasive techniques have been developed, such as the collection, analysis, and description of hair (Marinis & Asprea, 2006; Beja-Pereira *et al.*, 2009). Hair is a very rich biological material and is relatively easy to obtain using methods such as hair traps (*e.g.* Herr & Schley, 2009; Ebert *et al.*, 2010). Hair can provide a series of information, *e.g.*, genetics (Beja-Pereira *et al.*, 2009), ecology, based on stable isotopes (Magioli *et al.*, 2014), and morphology and taxonomy (Marinis & Asprea, 2006). The analysis of the hair microstructure has proven useful in differentiating between species; furthermore, this method enables the description of carnivore diets, one of the most frequent uses of trichology in the study of mammals (Andheria *et al.*, 2007), and it is widely applied in ecology, forensic sciences, archaeology, paleontology, and epidemiology (Quadros & Monteiro-Filho, 2006). Thus, the identification of hair is an accepted alternative for the study of rare and elusive species because the technology is non-invasive, it has a relatively low cost, and it is easy to use (Castro-Arellano *et al.*, 2008).

In regard to the Neotropical cervids *Mazama americana* (Erxleben, 1777), *Mazama gouazoubira* (Fischer, 1814), and *Mazama nana* (Hensel, 1872), the cuticular patterns on guard hair present subtle differences (Quadros & Monteiro-Filho, 2006). However, the number of analyzed individuals is small, because of the difficulty in obtaining samples from safe sources for reliable parallel; moreover, it is smaller than necessary, given the wide individual and intraspecific variation within the genus. The differences between species can be so subtle that some authors have declared that no differences exist among the medullar and cuticular patterns of *M. americana* and *M. gouazoubira* (Vázquez *et al.*, 2000). The sampling deficiency became even more evident in a study that described the hair of *M. gouazoubira*, analyzing only one animal and lacking a comparison of the results to those for other species of the same genus (Oliveira & Keller, 2011).Worsening this scenario even further is the fact that many museum pieces of the *Mazama* genus, used in studies as standards for the species, have been erroneously identified (A. M. B. Mantellatto, unpubl. data). These errors are due to a high morphological convergence among the species (Duarte *et al.*, 2008).

It should be noted that many of the cervid species that inhabit Brazil [*M. americana*, *M. nana*, *Mazama bororo* (Duarte, 1996), *Mazama nemorivaga* (Cuvier, 1817), *M. gouazoubira*, *Ozotoceros bezoarticus* (Linnaeus, 1758), *Blastocerus dichotomus* (Illiger, 1815), and *Odocoileus virginianus* (Zimmermann, 1780)] occur in sympatry in certain regions (Duarte & González, 2010), further

frustrating the identification of field samples. The scarcity of background information on all the Brazilian cervids, hinders the use of efficient tools such as trichology in biological studies of this taxon. Another limiting factor is the lack of quantitative analyses, which would otherwise diminish the subjectivity in the identification of guard hair samples.

Therefore, it is necessary to include other Brazilian cervid species in these studies, in addition to increasing the number of sample units, and always using samples with confirmed taxonomic identifications. Thus, our objective was to test the reliability of the morphological identification of guard hairs from the cervids that occur in Brazil.

MATERIALS AND METHODS

Sample collection. We sampled one male and one female of each of the Brazilian cervid species: *Mazama americana*, *M. gouazoubira*, *M. nana*, *M. bororo*, *M. nemorivaga*, *Blastocerus dichotomus*, *Ozotoceros bezoarticus*, and *Odocoileus virginianus*. The specimens were not necessarily the same age, as some species are rarely found in captivity. These animals were kept in captivity in the scientific breeding grounds of the Center for Research and Conservation of Cervids (NUPECCE, UNESP Jaboticabal), where they were all given the same type of food, were subjected to the same management regime, and spent most of their time in individual bays. The taxonomic identification of these animals was ascertained through molecular genetics and cytogenetic testing conducted when the animals were taken into captivity, these being the most reliable methods of species identification due to the morphological similarities between different deer's species.

For hair collection, the animals were chemically restrained with a combination of 1 mg/kg xylazine hydrochloride and 7 mg/kg ketamine hydrochloride (Pinho *et al.*, 2010), reducing the stress of the animals during collection and providing safety to the animal handlers.

One tuft of hair was plucked manually from the following pre-defined areas: head (frontal region, between the eyes); neck (mid-lateral region); side of the thorax (medial region of the rib cage); buttocks (posterior region, at femoral-tibial height); and back (medial region, at the height of the last thoracic vertebrae) (Fig. 1). The hair samples were stored at room temperature, in tapered plastic tubes with screw caps, until they were processed in the laboratory.

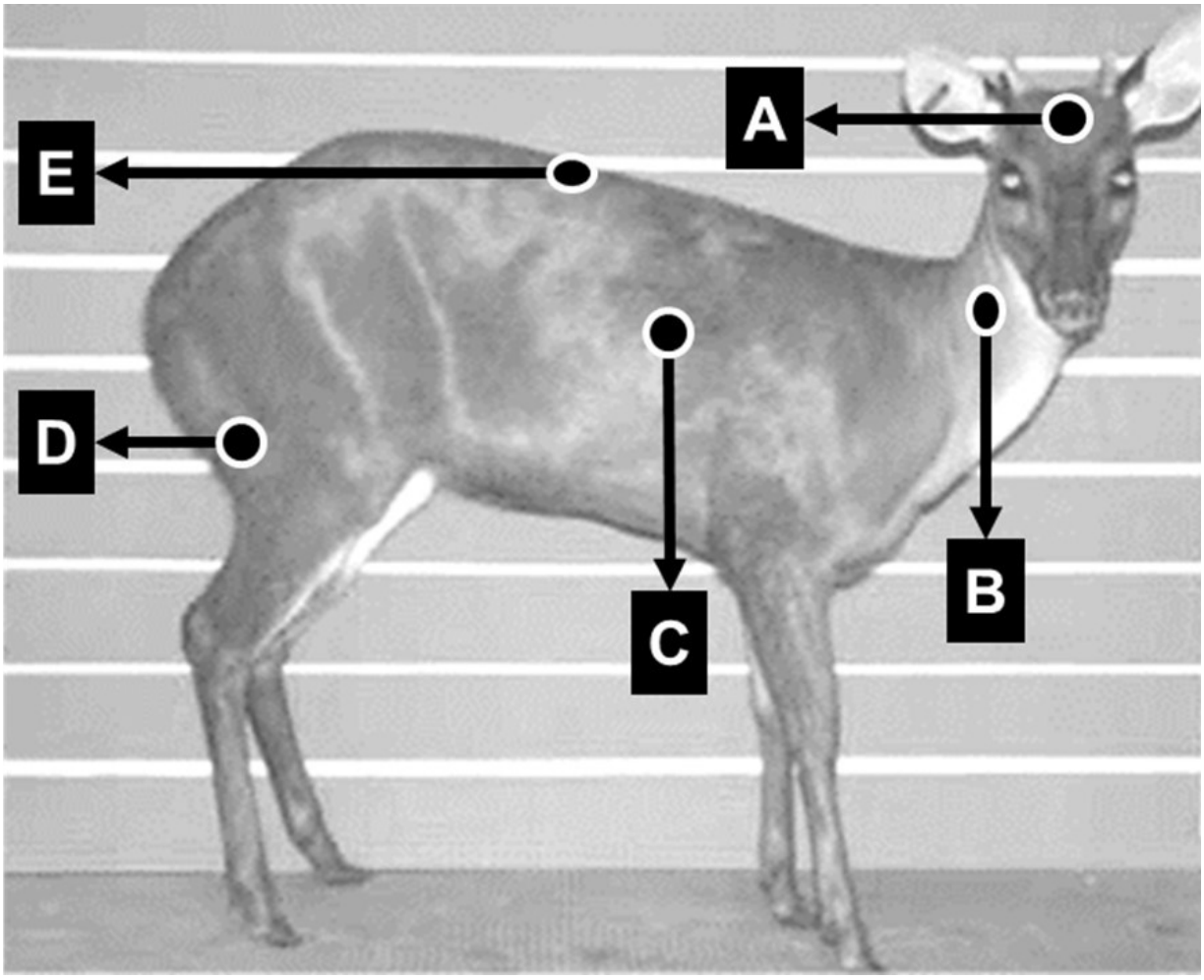


Fig. 1. *Mazama americana* (Erxleben, 1777). Body regions where samples of guard hair were collected (A, head; B, neck; C, side of the thorax; D, buttocks; E, back).

Processing and analyses of hair. Describing the morphology of the guard hairs requires an analysis of their cuticular and medullar patterns (Teerink, 1991). Thus, the collected hairs were prepared according to the methods recommended by Quadros (2002).

The guard hairs used in the analysis contained the bulb, shaft and shield. However, we used only the shield part. Three hairs from each body region were selected for processing and analysis. After the collection and selection of suitable hairs (whole and not deformed), they were cleansed in an absolute ethanol bath, and then dried with paper towel.

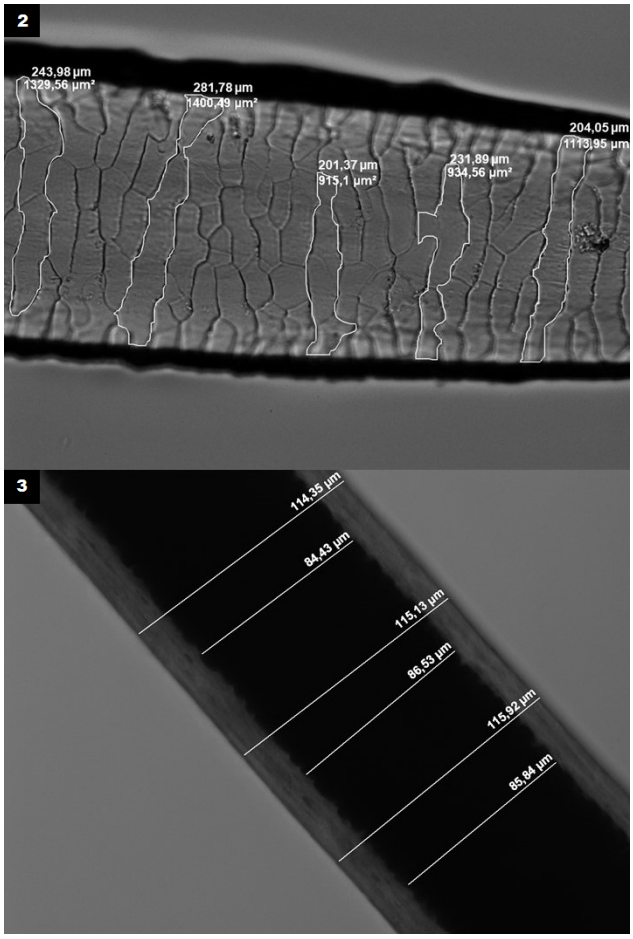
To analyze the cuticular patterns of the guard hairs, we prepared glass slides coated with a thin layer of clear nail polish, which recorded the impression of the external cuticular pattern (Quadros, 2002). The impressions on the slides were analyzed and photographed soon after their preparation, ensuring that the analyses were not impaired by the degradation of the nail polish over time.

To analyze the medullar patterns, we prepared the slides using the same hairs selected previously for the analysis of cuticular patterns. They were clarified with 30 volume hydrogen peroxide and mounted on the slides, and then analyzed and photographed under an optical microscope (Quadros, 2002). The obtained images were analyzed with the software AxionVision 4.8, which facilitated the collection of the qualitative and quantitative data.

Qualitative analyzes were based on the differentiation between the guard hairs of each body region, between the Brazilian cervid species, and between the sexes, through the search for any distinctive morphological characteristics that could be found, and their classification according to the guard hair nomenclature proposed by Quadros & Monteiro-Filho (2006).

The quantitative data measured for the cuticular patterns were the perimeter and the area of the scales. We took these measurements from five scales selected from the shield region each hair (Teerink, 1991; Fig. 2). The quantitative data on the medullar patterns were the total thickness of the hair and the thickness of the medulla. These measurements were taken from three regions along the shield of the hair (Quadros, 2002; Fig. 3).

Using the quantitative data, we ran an analysis of variance, Tukey test, and cluster analysis, to estimate the possibility of identifying the hair. These analyses were run for all of the quantitative variables individually. In the cluster analysis, we considered as sample units (input table lines) the sampled animals (n=16), and the variables were the average of the measurements (area and perimeter of the scales, total thickness, and thickness of the medulla) of the hairs from each body region (head, neck, lateral thorax, buttocks, and back), totalizing 20 variables (5 regions * 4 measurements average). Additionally, we ran discriminant function analyses to test the potential for using these characteristics to classify the samples. For this, each species was considered as a category, with 30 sample units that were analyzed pairwise. Each sample unit was defined by the average measurements of the area and perimeter of the scales, the total thickness and the thickness of the medulla of three hairs, from the five body regions of two individuals per species.



Figs. 2, 3. *Blastocerus dichotomus* (Illiger, 1815). Light micrographs of guard hairs from the lateral region of the thorax of a male individual (10x magnification): Fig. 2, measurement of the area and perimeter of the scales, from the shield of the hair; Fig. 3, measurement of the total thickness of the hair, and the thickness of its medulla, from the shield of the hair.

RESULTS

Morphology of the guard hairs. Regarding the cuticular patterns, there was little or no difference between species, body regions, or sexes. *Mazama nemorivaga*, *M. bororo*, *O. virginianus*, *B. dichotomus*, and *O. bezoarticus*, presented similar cuticular patterns among themselves, and the patterns did not differ between body regions or between sexes. Upon examination of these patterns, they were classified as transverse wave (Fig. 4). This pattern is morphologically described as having no defined angles in the shape of the scales, their contour being wavy and composing a set of smooth transitions between protrusions and recesses of varying depths; furthermore, the scales are arranged transversely in relation to the longitudinal axis of the hair (Quadros, 2002).

Finally, both females and males of *M. americana*, as well as the *M. nana* and *M. gouazoubira* females, presented differentiated cuticular patterns on the head region, which can be classified as transverse wave with ornate scale edge (Fig. 5). This pattern is similar to the previously described

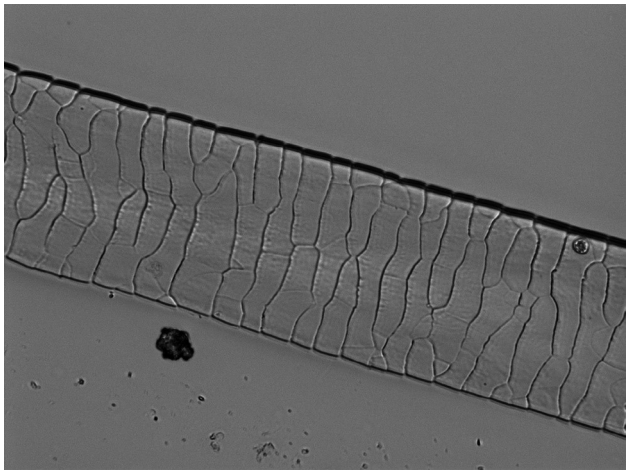


Fig. 4. *Blastocerus dichotomus* (Illiger, 1815). Light micrograph of the impression of a guard hair plucked from the lateral region of the thorax of a male individual, presenting a transverse wave cuticular pattern. This pattern is morphologically described as having no defined angles in the shape of the scales, their contour being wavy and composing a set of smooth transitions between protrusions and recesses of varying depths. Furthermore, the scales are arranged transversely in relation to the longitudinal axis of the hair (10x magnification).

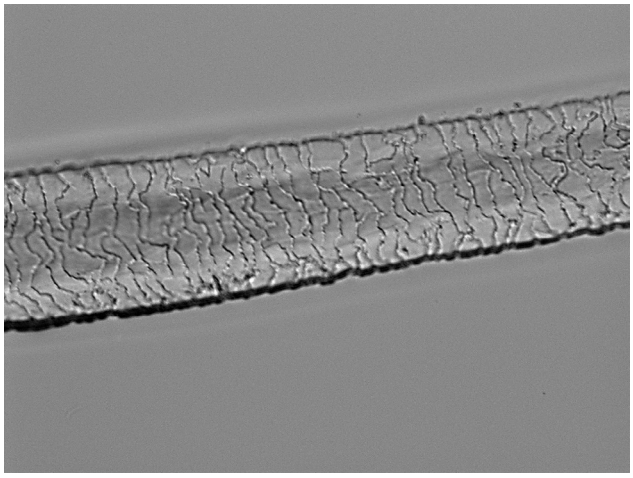


Fig. 5. *Mazama americana* (Erxleben, 1777). Light micrograph of the impression of a guard hair plucked from the lateral region of the thorax of a male individual, presenting a transverse wave with ornate scale edge cuticular pattern. This pattern is morphologically described as having no defined angles in the shape of the scales, their contour being wavy and composing a set of smooth transitions between protrusions and recesses of varying depths. Furthermore, the scales are arranged transversely in relation to the longitudinal axis of the hair and the edges may have small ridges and undulated or wavy indentations, with regular intervals or not, and with varying sizes (20x magnification).

except for the edges that may have small ridges and undulated or wavy indentations, with regular intervals or not, and with varying sizes (Quadros, 2002). The rest of the body regions, including the head region of the *M. nana* and *M. gouazoubira* males, however, were similar to those of the species *M. nemorivaga*, *M. bororo*, *O. virginianus*, *B. dichotomus*, and *O. bezoarticus*, presenting a transverse wave pattern.

The medullar patterns were similar among the different Brazilian cervid species, body regions, and sexes. According to the nomenclature proposed by Quadros & Monteiro-Filho (2006), the only medullar pattern observed was classified as reticulate (Fig. 6), which is morphologically described as a pattern that has more than one row of cells in its width and the cells anastomose with each other, circumscribing spaces of varying size and predominantly circular shape (Quadros, 2002).

Figures in the supporting information section provide images of all the cuticular and medullar patterns on guard hairs from each analyzed body region, species, and sexes.

Quantitative data analysis. The results of the cluster analyses were incongruent with the taxonomy of the species sampled so far. Therefore, the quantitative data did not prove effective in the differentiation between the min cluster analysis (Fig. 7).

The factorial analysis of variance showed that the three combined factors (species, sex, and body region) affected the averages, with a significant difference ($P<0.005$) between them. In the distinction between species, the analysis of variance without considering the factors 'sex' and 'body region' as the treatment, produced a significant result

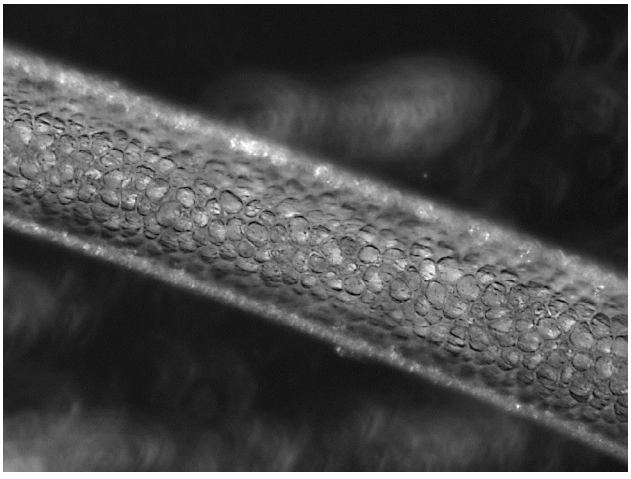


Fig. 6. *Blastocerus dichotomus* (Illiger, 1815). Light micrograph of a guard hair plucked from the lateral region of the thorax of a male individual, presenting a reticulate medullar pattern. This pattern is morphologically described asa pattern that has more than one row of cells in its width and the cells anastomose with each other circumscribing spaces of varying size and predominantly circular shape (10x magnification).

($P<0.05$), indicating that a difference exists. Subsequently, the Tukey test demonstrated a significant difference between some species (Tab. I).

Although these analyses revealed this indicative difference, the discriminant function analyses, with linear and quadratic cross-validation, showed that none of the comparisons between species obtained a correct classification percentage above 95% (Tab. II).

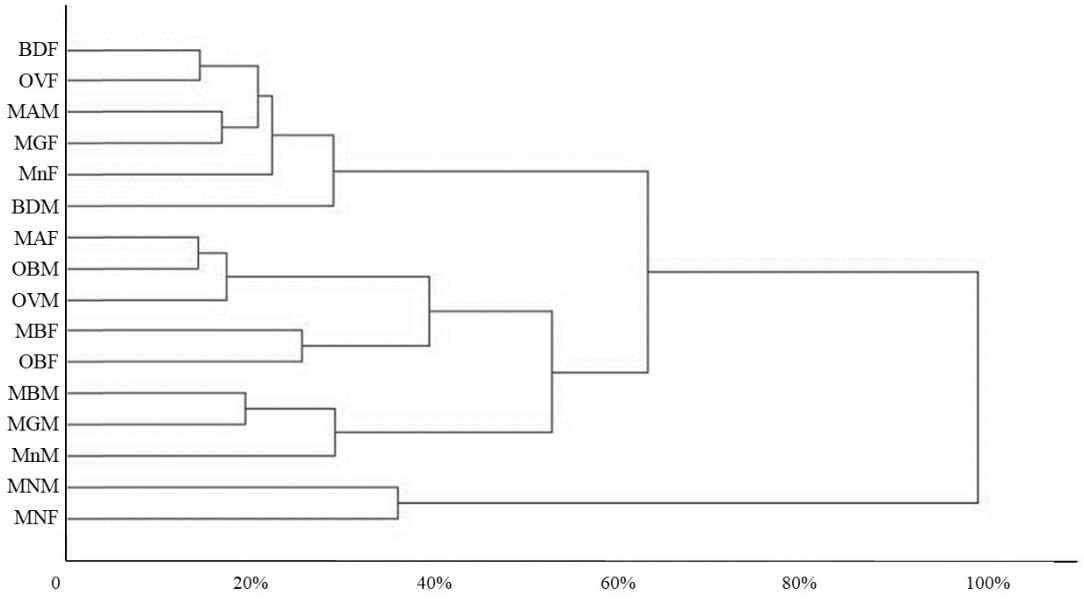


Fig. 7. Cluster analysis of cuticular and medullar patterns in guard hairs of eight species of Brazilian cervids. For each sampled animal, there were used individual characteristics of each body region (cuticular patterns: area and perimeter of the scales, and medullar patterns: total thickness of the hair and thickness of the medulla). *Blastocerus dichotomus* (Illiger, 1815) female (BDF) and male (BDM), *Odocoileus virginianus* (Zimmermann, 1780) female (OVF) and male (OVM), *Ozotoceros bezoarticus* (Linnaeus, 1758) female (OBF) and male (OBM), *Mazama nana* (Hensel, 1872) female (MnF) and male (MnM), *Mazama americana* (Erxleben, 1777) female (MAF) and male (MAM), *Mazama gouazoubira* (Fisher, 1814) female (MGF) and male (MGM), *Mazama nemorivaga* (Cuvier, 1817) female (MNF) and male (MNM), *Mazama bororo* (Duarte, 1996) female (MBF) and male (MBM).

Tab. I. Tukey test, taking into consideration the eight Brazilian cervid species (*Blastocerus dichotomus*, *Odocoileus virginianus*, *Ozotoceros bezoarticus*, *Mazama americana*, *M. bororo*, *M. nana*, *M. gouazoubira*, and *M. nemorivaga*) and all the analyzed variables (area and perimeter of the scales, thickness of the medulla and total thickness of the guard hair).

Species	Area	Perimeter	Medulla thickness	Total thickness
M. nemorivaga	A	A	A	A
M. bororo	B	B	C	C
M. americana	B	BC	AB	AB
O. virginianus	B	BC	C	BC
O. bezoarticus	B	C	BC	C
B. dichotomus	B	BC	C	C
M. gouazoubira	B	BC	C	C
M. nana	B	BC	C	C
DMS (5%)	3.121.732	438.301	438.301	256.184

Tab. II. Linear and quadratic discriminant function analysis, by pairwise comparison, of the species that presented similarities in some quantitative aspect (area or perimeter of scales, thickness of the medulla, or total thickness of the hair), indicating whether the species occur in sympatry.

Species	Sympatry	% of hits	
		Linear discriminant function	Quadratic discriminant function
M. americana X *M. gouazoubira*	YES	63.3	68.3
M. americana X *M. bororo*	YES	86.7	88.3
M. americana X *M. nana*	YES	65	63.3
M. americana X *M. nemorivaga*	YES	80	83.3
M. americana X *B. dichotomus*	YES	78.3	76.7
M. americana X *O. bezoarticus*	YES	93.3	93.3
M. americana X *O. virginianus*	YES	88.3	90
M. gouazoubira X *M. bororo*	YES	78.3	86.7

Tab. II. Cont.

Species	Sympatry	% of hits	
		Linear discriminant function	Quadratic discriminant function
M. gouazoubira X *M. nana*	YES	63.3	60
M. gouazoubira X *M. nemorivaga*	YES	75	81.7
M. gouazoubira X *O. bezoarticus*	YES	78.3	80
M. bororo X *M. nana*	YES	78.3	71.7
M. nemorivaga X *O. virginianus*	YES	78.3	76.7
B. dichotomus X *O. bezoarticus*	YES	90	91.7
M. gouazoubira X *B. dichotomus*	YES	40	56.7
M. nana X *B. dichotomus*	YES	68.3	75
M. nana X *O. bezoarticus*	YES	91.7	88.3
M. nemorivaga X *B. dichotomus*	YES	78.3	78.3
M. nemorivaga X *O. bezoarticus*	YES	88.3	86.7
M. gouazoubira X *O. virginianus*	NO	83.3	93.3
M. bororo X *M. nemorivaga*	NO	70	76.7
M. bororo X *B. dichotomus*	NO	81.7	83.3
M. bororo X *O. bezoarticus*	NO	88.3	88.3
M. bororo X *O. virginianus*	NO	78.3	81.7
M. nana X *M. nemorivaga*	NO	68.3	63.3
M. nana X *O. virginianus*	NO	73.3	85
B. dichotomus X *O. virginianus*	NO	83.3	88.3
O. bezoarticus X *O. virginianus*	NO	93.3	93.3

DISCUSSION

The morphological analysis of the guard hairs revealed little to no difference between the Brazilian cervid species. Given that the classification of these patterns, based on the nomenclature proposed by Quadros & Monteiro-Filho (2006), it was subjective, since there are no established parameters for its determination, which depends exclusively on the interpretation of the observer, the subtle differences observed may be explained by the technique.

Thus far, few authors have described and differentiated guard hairs from Brazilian cervids; furthermore, not all Brazilian species have been studied. Quadros (2002) conducted a study with some of the species, which there were also studied in the present one, and observed the following cuticular patterns: 1. irregular waved cuticle (*M. gouazoubira*), 2. smooth and distal scale edges (*M. nana*), 3. and ornate scale edges (*M. americana*). However, these results differ from those obtained in our study, despite in both it was used the same methods in the morphological analyses. These different patterns for each species may in fact exist, or they may be the result of the subjective bias inherent to the pattern interpretation proposed by Quadros & Monteiro-Filho (2006), as mentioned. The difference may even be a result of errors in the identification of the specimens used, which are frequent. Other studies do not describe the medullar patterns of the Brazilian cervids, precluding the comparison of results.

The cluster analysis of the quantitative data revealed that, due to incongruence in grouping of distinct species, the quantitative data are insufficient for the differentiation between the species, body regions, or sexes. One of the possible causes of these insufficiencies may be the immense intraspecific variation.

The analysis of variance and Tukey test showed that the measured characteristics differ between species. Thus, we ran a discriminant function analysis intending to arrive at a reliable method of identifying hair samples collected in future field studies, in cases of sympatry between two species. However, our results indicated that observed parameters were not sufficiently reliable for hair classification, since correct classification percentages never exceeded 95%. Some pairs, however, had values above 90%, indicating that if the sample size is larger in future studies there is a possibility that distinct values to be obtained.

We found low reliability in the differentiation of Brazilian cervid species through the analysis and description of guard hair microstructure, since this technique entails great subjective bias in the data analysis. This implies that previous studies, which used the same techniques performed here (*e.g.* Rocha-Mendes *et al.*, 2010; Bianchi *et al.*, 2014; Ávila-Nájera *et al.*, 2018), need to be considered with caution, since the differentiation of the analyzed cervid species, and perhaps even other animal species, may not be reliable. An alternative for the identification of hair would be the genetic analysis of the tissue in the bulb of the hair or

from other collected biological material. This technique has already proven effective for pumas (Miotto *et al.*, 2007) and it is also used to identify cervid feces (Souza *et al.*, 2013; Duarte *et al.*, 2017; Oliveira *et al.*, 2019).

It is worth emphasize that the impossibility of controlling some variables, like the age of the individuals, is a possible limitation of this study, once the hair structure could change according to this variables.

Studies that aim to validate non-invasive methods for obtaining samples, and to increase knowledge of the animal species composing the biodiversity of the planet, are essential. It is therefore imperative that such studies are rigorous and more detailed, so that the obtained data are reliable and contribute to the advancement of science and to the conservation of endangered species.

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