



# Distribution of the elusive and threatened Brazilian dwarf brocket deer refined by non-invasive genetic sampling and distribution modelling

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## Abstract

The Brazilian dwarf brocket deer (*Mazama nana*; Mammalia: Cervidae) is an elusive deer species that occupies the forests of southern Brazil, northern Argentina, and eastern Paraguay. A drastic reduction in forested areas has greatly affected the species, the least studied Neotropical deer. As do many threatened and elusive species, the Brazilian dwarf brocket deer needs a refinement of its distribution that would indicate proper sites to guide its conservation in situ. This project aimed to determine species distribution in order to establish priority areas for conservation. Given the rarity and elusiveness of the species, we proposed indirect methods to achieve this objective. We tracked and collected faecal samples in protected areas spread over southern Brazil with the help of a scat detection dog. Following species identification by PCR/RFLP and sample spatialisation, we modelled the species distribution using MaxEnt software. We found that the potential geographical distribution of the Brazilian dwarf brocket deer in Brazil is spread over the states of Paraná, Santa Catarina, northern and central Rio Grande do Sul, the extreme south of São Paulo and Mato Grosso do Sul, eastern Paraguay, and the Misiones province in Argentina. The west and centre of Paraná state and part of western Santa Catarina state were identified as high conservation priority areas.

**Keywords** Cytochrome b · Faecal DNA · *Mazama nana* · Scat detection dog · Maximum entropy modelling

## Introduction

Determining elementary parameters, such as the geographic distribution of a species, is fundamental to any ecological and conservation study; however, obtaining this information has proven difficult for some species, like *Mazama* genus and other forest elusive dwellers, precluding the sighting and capture frequently used traditional methodologies of ecological studies (Vogliotti 2003). Methods of circumventing this impasse have been developed over time, such as non-invasive genetic sampling from faecal samples (Kohn and Wayne

1997), locating faecal samples with scat detection dogs (Smith et al. 2001), and the prediction of ecological niches and geographic distribution via species distribution modelling (Duarte et al. 2017; Khadka and James 2017). These techniques can also be useful in assessing IUCN data-deficient class species that are rare or morphologically cryptic.

Modelling is particularly useful in predicting the distribution of a species for which occurrence records are scarce (Pearson 2007). One of the most commonly used algorithms for this type of modelling is MaxEnt, which involves the principle of maximum entropy to predict potential distribution (Baldwin 2009; Phillips et al. 2006) based on data regarding species occurrence (presence) and absence (not necessarily). This method is thus regarded as a fundamental component in species conservation planning, determining the potential distribution, and areas of superior suitability for the species (Pearce and Boyce 2006; Rodriguez et al. 2007). Examples of its uses for elusive species include the studies involving Geoffroy's cat (Cuyckens et al. 2016), rare birds (Marini et al. 2010), and threatened bat populations on Sardinia (Bosso et al. 2016).

Faeces collection and analysis provide important sources of information about wild animals (Kohn and Wayne 1997;

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Kohn et al. 1999). Accurately determining data, such as the species to which a sample belongs, is essential for a detailed ecological study. To this end, specific molecular markers are used to obtain information (Oliveira and Duarte 2013). González et al. (2009) developed primers that amplify a fragment of the cytochrome b gene of all species of the genus *Mazama*. The authors submitted this fragment for hydrolysis with the restriction enzymes Bstnl, Sspl, and AflII. Such enzymes induce the cleavage of the fragment into subfragments of different sizes, facilitating identification of the species through restriction patterns. A combination of occurrence data obtained by faecal DNA samples and distribution modelling using maximum entropy was satisfactorily applied to elucidate a recently described forest deer species (Duarte et al. 2017).

The Brazilian dwarf brocket deer (*Mazama nana*) is the smallest of the genus, weighing less than 15 kg and measuring only 45 cm in height, with a homogeneous reddish colouring (Duarte 1996). No information is available on species density, home ranges, use of space, or diet (Abril et al. 2010).

The species seems to be associated with Araucaria forest, semi-deciduous seasonal forest, and transitional vegetation between cerrado and ombrophilous forest (Rossi 2000). It also inhabits secondary forest areas with a high abundance of bamboo (Chébez and Varela 2001). The Brazilian dwarf brocket deer is simultaneously considered the least studied cervid in Brazil (Vogliotti 2008) and possibly the most endangered in the Neotropical region (Abril et al. 2010). In spite of this lack of information regarding its abundance, the conservation status of the Brazilian dwarf brocket deer is based on inferences concerning a predicted population decline of 30% or more, classifying it as “vulnerable” in the IUCN Red List (IUCN 2014). Over the last century, the main causes of this decline have comprised intensive wood exploitation and rapid agricultural conversion of their habitats (Vogliotti 2008). At present, the edge effect on small forest remnants, hunting, predation by domestic dogs, pesticides, and diseases acquired from domestic ungulates merits special mention (Duarte et al. 2015).

Concerning the species distribution, Cabrera (1960) reported the Brazilian dwarf brocket deer's area of occurrence as including southeastern Brazil, northeastern Argentina, and eastern Paraguay. Grubb (1990) affirmed a belief that the species can be found in Bolivia. According to Vieira (1955), the species occurs in the Brazilian states of Mato Grosso, São Paulo, and Rio Grande do Sul. This is contested by Duarte (1998), who reported the species occurrence as from the northern region of Paraná to the central region of Rio Grande do Sul, as well as areas in Paraguay and Argentina. In this same work, Duarte proposed a distribution map of species of the genus *Mazama*, with the Brazilian dwarf brocket deer in this region; however, its distribution does not extend to the eastern

regions of the three southern states of Brazil. Eisenberg and Redford (1999) described the distribution of the Brazilian dwarf brocket deer as southeastern Paraguay, the north of the Misiones province in Argentina, and the Brazilian states of Rio Grande do Sul, Santa Catarina, Paraná, and São Paulo, extending up to the southern regions of Mato Grosso do Sul and Minas Gerais. Finally, Rossi (2000) suggested the species presence in the states of Rio Grande do Sul, Santa Catarina, Paraná, and the southern region of São Paulo based on eight locations of museum collection.

Since it is an elusive species, these somehow contradictory distributions are based on very few and uncertain occurrence records, occasionally associated with the Araucaria forest, which is one of the Atlantic forest formations. As do many threatened and elusive species, the Brazilian dwarf brocket deer needs a refinement of its distribution that would indicate proper sites to guide its conservation in situ. Here, we propose an approach that combines two tools to achieve this: (i) collecting occurrence data with non-invasive genetic sampling based on faeces and (ii) modelling the occurrence data on MaxEnt in order to obtain a map of suitable areas, which will indicate its potential distribution and a scale of priority for those areas as regards its conservation.

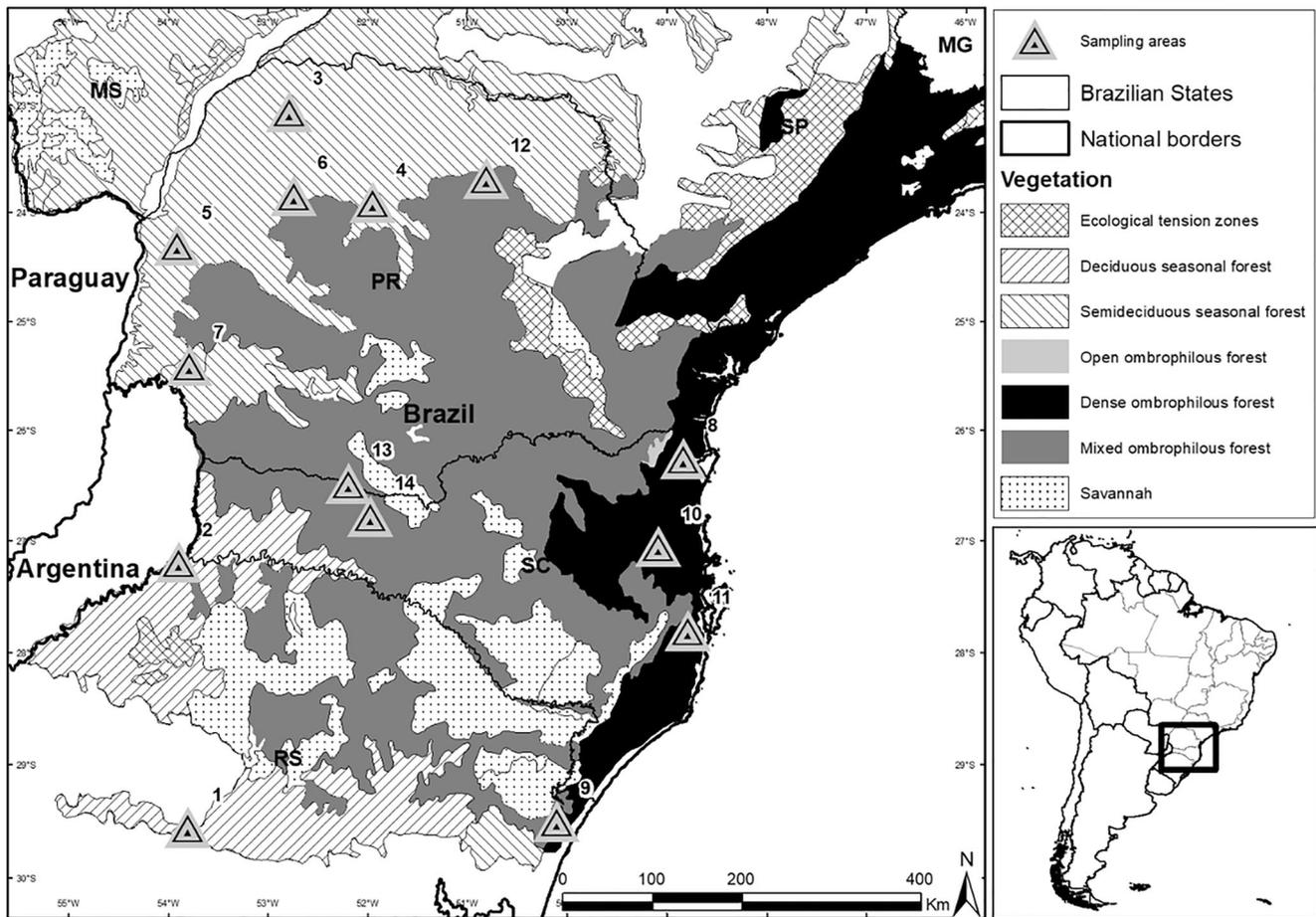
## Methods

### Study areas

We collected faecal samples from 14 protected areas in the southern region of Brazil (Fig. 1). We chose these areas for their size and the forest formations to which they belong (Table 1). Forest formations of mixed ombrophilous (Araucaria forest), dense ombrophilous, semi-deciduous seasonal, and deciduous seasonal forest were all considered. We chose areas smaller than 600 ha and greater than 5000 ha in order to detect the extremities of environmental conditions, which are potentially related to the sizes of the areas (Fleury and Galetti 2006).

### Sample collection

We collected samples in a way that would obtain data for distribution modelling, walking along transects within the protected areas. The transects were distributed so that sampling extended throughout the geographical limits of each area, avoiding the interdependence of the collection points. Based on the available time and financial resources, we sampled each protected area for a maximum of 4 days or until 20 samples were collected. Owing to logistical particularities, three areas (E. E. da Mata Preta, P. N. do Iguaçu, and P. E. Vila Rica do Espírito Santo)



**Fig. 1** Map showing the location of the protected areas sampled and the regions of the Atlantic forest according to the vegetation classification of the Instituto Brasileiro de Geografia e Estatística (IBGE 1992). The

numbers correspond to the locations shown in Table 1. *SP* = São Paulo state; *MG* = Minas Gerais state; *MS* = Mato Grosso do Sul state; *PR* = Paraná state; *SC* = Santa Catarina state; *RS* = Rio Grande do Sul state

underwent more intense sampling. The sampling period was between May 2012 and March 2015. Since the Brazilian dwarf brocket deer can occur in sympatry with other *Mazama* species, such as the red brocket deer (*M. Americana*), the small red brocket deer (*M. Bororo*), and the brown brocket deer (*M. gouazoubira*) (Duarte et al. 2017), these faecal samples were identified at the species level.

We walked the transects (total 276 km) with a scat detection dog that had previously been trained to identify deer scats. The researcher in the field always remained close to the established line of the transect, while the dog made incursions up to a maximum radius of 20 m. The geographical coordinates of each sample collection site were noted for subsequent georeferencing. We collected the faecal samples without making contact with the hand and stored them in hermetically sealed screw cap centrifuge tubes (50 mL) in order to prevent contamination of the genetic material. Approximately 40 mL of absolute ethanol was added to the tubes used to store the faecal pellets.

**Laboratory analyses**

We extracted DNA from the faecal samples using a silica-based extraction protocol (Boom et al. 1990). For identification at the species level, a 224-bp fragment of the mitochondrial cytochrome b gene was amplified by PCR, with primers designed by González et al. (2009) that amplify samples of all species of the genus *Mazama*. Following amplification confirmation in agarose gel, the products were submitted to hydrolysis with restriction enzymes SspI, AflIII, and BstN. These enzymes promote the specific cleavage of the 224-bp fragment into fragments of different sizes, facilitating species identification (González et al. 2009; Souza et al. 2013).

**Procedures for distribution modelling**

**Database of environmental variables**

In order to generate distribution models of this species, we used the database of environmental variables provided by

**Table 1** Characterisation of the study areas indicated in Fig. 1: the size in hectares, the predominant forest formation, and samples collected from each species of *Mazama*

Map location	Name of the area	Size (ha)	Sampling effort (m)	Samples per distance walked (sample/100 m)	Forest formation	Mn	Mb	Mg	Ma	No ID	Total
1	R. B. do Ibicuí Mirim	567	2009	0,29	Deciduous seasonal	0	0	4	0	2	6
2	P. E. do Turvo	16,979	1163	1,11	Deciduous seasonal	2	0	0	10	1	13
3	P. E. de Amaporã	209	1918	0	Semi-deciduous seasonal	0	0	0	0	0	0
4	P. E. Vila Rica do Espírito Santo	349	24,013	0,51	Semi-deciduous seasonal	59	0	0	0	65	124
5	P. E. de São Camilo	387	2426	0,57	Semi-deciduous seasonal	10	0	0	0	4	14
6	R. B. das Perobas	8716	1265	1,58	Semi-deciduous seasonal	0	0	0	14	6	20
7	P. N. do Iguaçú	169,696	21,256	0,96	Semi-deciduous seasonal	54	0	0	125	26	205
8	P. N. M. Mourro do Funder	48	2060	0	Dense ombrophilous	0	0	0	0	0	0
9	R. B. Mata Paludosa	117	2905	0	Dense ombrophilous	0	0	0	0	0	0
10	P. N. da Serra do Itajá	57,375	6691	0,28	Dense ombrophilous	12	4	0	0	3	19
11	P. E. da Serra do Tabuleiro	94,608	7377	0,04	Dense ombrophilous	0	2	0	0	1	3
12	P. E. do Penhasco Verde	305	1235	0	Mixed ombrophilous	0	0	0	0	0	0
13	E. E. da Mata Preta	6566	32,179	0,56	Mixed ombrophilous	5	0	6	147	24	182
14	P. N. das Araucárias	12,847	2463	0,77	Mixed ombrophilous	10	0	6	1	2	19
					Total	152	6	16	297	134	605

Species identification made using PCR/RFLP of cytochrome b 224-bp fragment. Mn—*M. nana*; Mb—*M. bororo*; Mg—*M. gouazoubira*; Ma—*M. americana*; no ID—no successful species identification: R. B. = biological reserve; P. E. = state park; P. N. = national park; P. N. M. = municipal natural park; E. E. = ecological station

the Image Processing Division (DPI) of the Brazilian National Institute of Space Research (INPE), previously interpolated for a resolution of 30 arc-seconds (approx. 1 km). From this database, we selected variables that we think would potentially affect the species distribution. We chose the following variables for a prior Pearson's correlation test: percentage of tree cover (Hansen et al. 2003), altitude (Valeriano 2008), slope, drainage network density, and distance (Ximenes 2008). We also selected the following climatic variables for this prior test (Hijmans et al. 2005): BIO15 (coefficient of variation); temperature seasonality, BIO4 (standard deviation \* 100); minimum temperature in the coldest month of the year, BIO6; mean annual temperature, BIO1; mean temperature variation, BIO2 (monthly mean, Tmax-Tmin); isothermality, BIO3, (BIO2/BIO7) (\*100); mean temperature in the coldest quarter, BIO11; and annual precipitation, BIO12. Following the test for correlation, we chose the uncorrelated variables ( $r^2 \leq 0.8$ ; Pearson's test) and removed BIO1, BIO3, BIO6, and BIO15 from the initial pool. We performed a principal component analysis (PCA) with the remaining variables, using PAST software, version 3.06 (Hammer et al. 2001) in order to exclude the variables that are least effective in explaining environmental variation in the scope of modelling analysis (Atlantic forest range). PCA revealed that drainage network density was not particularly explanatory (Supplementary material); hence, it was removed from the final group. Finally, we generated the MaxEnt model using the variables tree cover percentage, altitude, slope, drainage network distance, BIO2, BIO4, BIO11, and BIO12.

## Software application

We performed the distribution modelling with 271 georeferenced records. Some of the records were from our 152 samples identified in the laboratory as belonging to the Brazilian dwarf brocket deer, and the remainder were from additional 119 occurrence records (collected since 2003) from the Centre for Deer Research and Conservation (NUPECCE) database and from the contributions of other researchers. The algorithm automatically removed records that were in the same 1-km<sup>2</sup> environmental database pixel, leaving only one record per pixel. This resulted in 100 occurrence records to be analysed. The model was "trained" using 70% of the points and tested using the remaining 30% (Pearson 2007). The points were sampled using the bootstrap method, with ten random partitions and substitutions.

Modelling was performed by MaxEnt software version 3.3.3k (Phillips et al. 2004, 2006), which only uses data regarding the presence of the species and estimates the probability distribution of maximum entropy. Only the maximum entropy algorithm was used because it can be considered the most applicable (Baldwin 2009).

## Assessment of the model

We assessed the model using the area under the curve (AUC), calculation of the omission error, and binomial test for comparison of the two proportions (Elith et al. 2006; Fielding and Bell 1997; Phillips and Dudík 2008). We used the “minimum training presence logistic threshold” as a cutoff limit for predicting species occurrence.

## Determining priority areas for conservation

We established two categories to determine priority areas for conservation, “priority” and “high-priority,” based on the principle that a highly suitable area will be more effective for conservation once species density is higher there (VanDerWal et al. 2009). The “priority” category contained areas with environmental suitability values between 0.5 and 0.75, while the “high-priority” category contained areas with values of environmental suitability exceeding 0.75. Both categories only considered Brazilian territory and areas outside protected areas.

## Results

The scat detection dog located 591 of 605 faecal samples in the protected areas sampled. Having extracted the DNA from each, the species level identification protocol (PCR/RFLP) was applied, and 471 samples were successfully identified, an amplification success of 78% (Table 1). We found that 152 of the samples belonged to the Brazilian dwarf brocket deer, and the species was detected in several protected areas: P. E. do Turvo, P. E. Vila Rica do Espírito Santo, P. E. São Camilo, P. N. do Iguaçu, P. N. Serra do Itajaí, E. E. Mata Preta, and P. N. Araucárias (see Table 1 and Fig. 1). We additionally found records of the species in all of the Atlantic forest formations sampled: deciduous seasonal, semi-deciduous seasonal, dense ombrophilous, and mixed ombrophilous.

The average distribution model using MaxEnt software with a minimum training presence logistic threshold is presented in Fig. 2. One hundred points were used, because the MaxEnt software default only considers one point per km<sup>2</sup>, ten random partitions of the data were made, and the mean external AUC (independent test) value was  $0.943 \pm 0.009$ . The model presented a significantly different result from chance ( $p < 0.001$ ) for a one-tailed binomial proportion test, and the omission error for the average model was 0.02 with a “minimum training presence logistic threshold” of 0.0219. The environmental variables that contributed most to the model were the percentage of tree cover (33.7%), temperature seasonality (BIO4, 28.5%), and mean daily temperature variation (BIO2, 21.9%).

According to environmental suitability data, the species might occupy a large part of the southern region of Brazil (except for the fields of southern Rio Grande do Sul), eastward to the Misiones province in Argentina, and eastern Paraguay and include the southernmost areas of Mato Grosso do Sul and São Paulo. Four large regions with high levels of environmental suitability occur within this distribution (Fig. 2). These are the Serras of northeastern Rio Grande do Sul, the Serra do Mar between southern São Paulo and northeastern Santa Catarina, the central and northeastern regions of Paraná (encompassing Telêmaco Borba municipality), and a large region that includes the Iguaçu National Park, the Misiones province to the east, and central Santa Catarina.

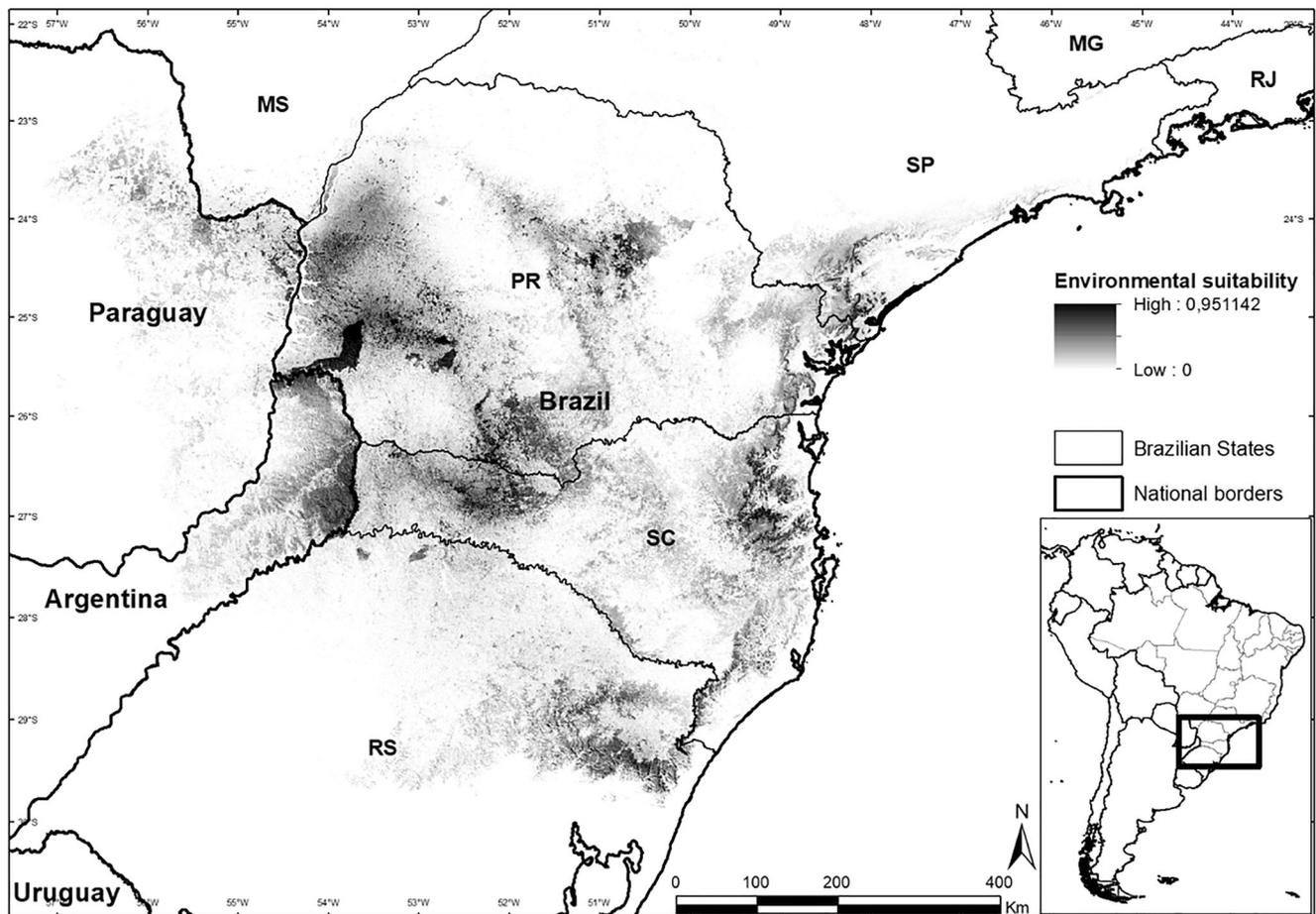
Figure 3 shows the priority areas for species conservation based on the environmental suitability pixels extracted from the distribution model. These are sites outside protected areas and are divided into “priority areas” and “high-priority areas.” Four major pixel clusters represent these areas. One is at the northeast of Rio Grande do Sul state where the Serra Gaúcha is found; a second is at the northeast of Santa Catarina state, featuring the edge of a mountain range at the coastal plain; a third is in central Parana state where the Ponta Grossa plateau is found; and the fourth is a continuum of pixels from western Paraná state to western Santa Catarina state that cross the Iguaçu region and the Santa Catarina sierra and western plateau.

## Discussion

This study is a significant advance in our knowledge of the distribution of the threatened Brazilian dwarf brocket deer. Such new information, which builds on mere incipient knowledge in previous literature, was obtained via the use of non-invasive methodologies with high levels of detectability when applied to elusive species.

The geographic distribution determined by the maximum entropy model largely coincides with that predicted in the literature (Cabrera 1960; Duarte 1996; Eisenberg and Redford 1999; Grubb 1990; Rossi 2000; Vieira 1955). The species distribution does not extend to Minas Gerais (Eisenberg and Redford 1999) or indeed to most of the southeastern region of Brazil (Cabrera 1960). Contrary to Duarte’s (1998) predictions, there are strong indications involving occurrence records and the high environmental suitability foreseen in the model that the species occupies the eastern regions of Paraná and Santa Catarina, principally the Serra do Itajaí. It may also be present in the southernmost region of Mato Grosso do Sul, as mentioned by Vieira (1955) but disputed by Duarte (1998).

The distribution model for Brazilian dwarf brocket deer is a starting point from which new field studies can confirm information regarding the expected presence and absence of the



**Fig. 2** Distribution modelling (maximum entropy model,  $AUC = 0.943 \pm 0.009$ ,  $p < 0.001$ ) for the Brazilian dwarf brocket deer (*Mazama nana*). SP = São Paulo state; MG = Minas Gerais state; MS = Mato Grosso do Sul state; PR = Paraná state; SC = Santa Catarina state; RS = Rio Grande do Sul state

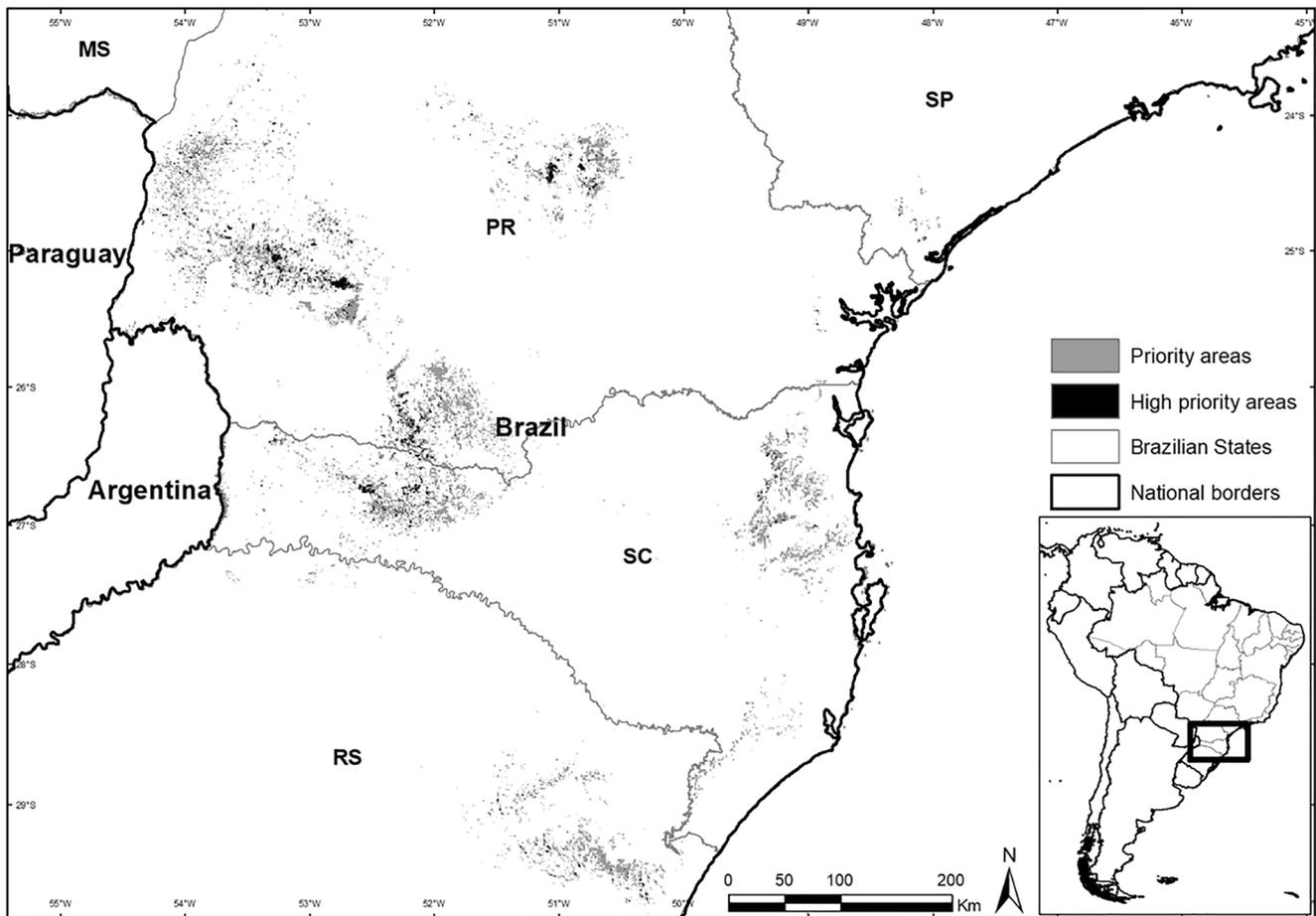
species. Moreover, it is important to understand the ecological interaction of the *Mazama* species, given that the Brazilian dwarf brocket deer has been detected in sympatry with the red brocket deer, the small red brocket deer, and the brown brocket deer (Duarte et al. 2017). Evidence for this interaction was proposed by Vogliotti (2008), who suggested that the Brazilian dwarf brocket deer occurs in areas of higher density vegetation in the lower strata, while the red brocket deer occurs in forest areas with more open lower strata. There are doubts as to how this habitat partition occurs between different species of *Mazama* and whether the current distribution is influenced by eventual competition. One example is the southernmost part of São Paulo, where the model indicates the potential distribution of the Brazilian dwarf brocket deer, but to date, only small red brocket deer, brown brocket deer, and red brocket deer have been recorded (Duarte et al. 2017).

The variables that contributed most to the model (percentage of tree cover, temperature seasonality, and mean daily temperature variation) indicate the association of this species with forested areas, cooler winters and hot summers, and high daily temperature amplitude: characteristics that are common

in southern Brazil. This type of information may be key to explaining the interaction with other sympatric species of the genus *Mazama*, and future studies could verify whether these same variables (and at what levels) determine such occurrences. The association between the Brazilian dwarf brocket deer and the mixed ombrophilous forest appears to be weak, as several occurrence records have been identified in other forest formations, such as dense ombrophilous forest and the deciduous and semi-deciduous seasonal forest (Table 1).

The occurrence of Brazilian dwarf brocket deer in some protected areas (Table 1) should be considered in management plans, formal documents that guide local public policies. A second threatened species detected, the small red brocket deer, should receive the same attention. It is also important that management plans for protected areas take into account the main threats to these species, which include hunting, predation by domestic dogs, pesticides, and diseases acquired from domestic ungulates.

A detailed diagnosis of priority areas for the Brazilian dwarf brocket deer conservation is required, including mapping forest remnants, monitoring population tendencies, and detecting



**Fig. 3** Priority areas for the conservation of the Brazilian dwarf brocket deer (*Mazama nana*) in Brazil outside of the protected areas. Priority areas have environmental suitability values (maximum entropy model)

above 0.5 and high-priority areas above 0.75. *SP* = São Paulo state; *MG* = Minas Gerais state; *MS* = Mato Grosso do Sul state; *PR* = Paraná state; *SC* = Santa Catarina state; *RS* = Rio Grande do Sul state

potential threats. In general, more studies are required to qualify the information regarding these locations and to enable the creation of protected areas and adoption of precautionary measures in the licencing of enterprises. It is also necessary to test the hypothesis that density is positively correlated with habitat suitability (VanDerWal et al. 2009), once there is not a consensus (Yañez-Arenas et al. 2012), and it would affect conservation actions based only on habitat suitability.

Prospecting populations and occurrence data by collecting faeces with detection dogs, identifying these samples with molecular markers, and modelling its occurrences in order to refine the geographical distribution of an elusive and/or morphologically cryptic species is an approach that has proved to be effective. This set of tools can help in the status assessment and conservation planning of several data-deficient species that might be at risk of extirpation. Such approaches could be applied to elucidate the distribution issues of muntjac, such as the Gongshan muntjac (*Muntiacus gongshanensis*), the Roosevelt’s muntjac (*M. rooseveltorum*), the Fea’s muntjac (*M. feae*), and the Annamite muntjac (*M. truongsongensis*), following a taxonomic review (Timmins and Duckworth 2016a,

b, c; Timmins et al. 2016). It is also applicable to some forest deer like the red brocket deer and the Central America red brocket deer (*Mazama temama*), which need to have their taxonomic status resolved and then their distributions delimited (Duarte and Vogliotti 2016; Bello et al. 2016). Another group of animals that would benefit from this approach is the Asian Chevrotains (*Tragulus javanicus* and *Tragulus williamsoni*), whose populations should be mapped and their habitat requirements identified (Duckworth et al. 2015; Timmins et al. 2015).

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