Tools and Technology

Noninvasive Techniques Provide Novel Insights for the Elusive Bush Dog (*Speothos venaticus*)

KAREN E. DeMatteo, *University of Missouri, Division of Biological Sciences, 226 Tucker Hall, Columbia, MO 65211, USA; and WildCare Institute, Saint Louis Zoo, One Government Drive, St. Louis, MO 63110, USA*

MIGUEL A. RINAS, *Ministerio de Ecología RNRyT, Leandro N Alem 4907, 3300, Posadas, Misiones, Argentina*

CARINA F. ARGÜELLES, *Instituto de Biología Subtropical—nodo Posadas, Universidad Nacional de Misiones, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina and Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Dpto. de Genética, Félix de Azara 1552, CPA 3300LQH, Posadas, Misiones, Argentina*

JUAN PABLO ZURANO, *Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Dpto. de Genética, Félix de Azara 1552, CPA 3300LQH, Posadas, Misiones, Argentina*

NICOLE SELLESKI, *Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Dpto. de Genética, Félix de Azara 1552, CPA 3300LQH, Posadas, Misiones, Argentina*

MARIO S. DI BITETTI, *Instituto de Biología Subtropical—nodo Puerto Iguazú, Universidad Nacional de Misiones, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina and Asociación Civil Centro de Investigaciones del Bosque Atlántico (CeIBA), Bertoni 85 (3370), Puerto Iguazú, Misiones, Argentina*

LORI S. EGGERT, *University of Missouri, Division of Biological Sciences, 226 Tucker Hall, Columbia, MO 65211, USA*

ABSTRACT The bush dog (*Speothos venaticus*), a small and rarely seen canid from Central and South America, has proven extremely challenging to locate and study in the wild, making the development of species-specific and comprehensive carnivore conservation strategies difficult. From May to August 2011, a detection-dog–handler team located 34 scats from bush dogs in the northern ($n = 26$) and central ($n = 8$) zones of Misiones, Argentina. We identified 22 unique genotypes (14 northern and 8 central) and assigned sex to 100% of the genotyped scats. Only half of the scats were located inside 7 protected areas (4 northern and 3 central); the remaining half were located in 4 sites outside of protected areas (3 northern and 1 central). Results suggest low but significant differentiation between zones for bush dogs ($F_{ST} = 0.049$, $P = 0.010$). Bush dogs demonstrated high habitat-use flexibility and a close association with altered habitat; however, altered habitat may not be optimal for the species because of the potential for lower prey densities and risk of exposure to life threatening diseases by domestic dogs. The effectiveness of noninvasive techniques (detection dogs, genetic analyses of scat, Geographic Information System technology) in studying the ecology of bush dogs not only opens the door for additional studies of a species that has proven difficult to study with standard survey techniques, but also provides an alternative approach that conservationists can use independent of habitat type and presence of humans. © 2014 The Wildlife Society.

KEY WORDS conservation, detection dogs, genetics, habitat use, noninvasive techniques, *Speothos venaticus*.

As intensive urbanization and agricultural development continue to negatively affect landscapes, maximizing the protection of existing biodiversity is a priority. Many conservation strategies are developed using the broad ecological requirements of apex or top-level predators, which promotes direct protection of large areas and indirect protection of numerous species and multiple ecosystems (Wikramanayake et al. 1998, Sanderson et al. 2002, Sergio et al. 2008). Historically within the Neotropics, only 2 species have been described as top-level predators or species that have large home ranges, highly carnivorous diets, and

the ability to inhabit a variety of habitats: the large-bodied jaguar (*Panthera onca;* 50–100 kg) and puma (*Puma concolor;* 30–70 kg). However, preliminary field data and opportunistic observations in the wild suggest that the ecological requirements of the bush dog (*Speothos venaticus*), which typically exists in family groups with young from one or more litters (DeMatteo 2008, Michalski 2010, Lima et al. 2012), are greater than its relatively small body size (5–6 kg) suggests (Silveira et al. 1998, DeMatteo 2008, DeMatteo and Loiselle 2008, Michalski 2010, Lima et al. 2012). That is, the bush dog more closely resembles the jaguar and puma and contrasts with other Neotropical felids and canids that have small home ranges, have omnivorous–carnivorous diets, and are habitat specialists (Eisenberg and Redford 1999). The bush dog has a large home range (140–150 km^2; Beisiegel 1999, Lima et al. 2012), is a habitat generalist over

its broad Neotropical distribution, and is highly carnivorous (Beisiegel and Zuercher 2005, DeMatteo et al. 2011); these are all characteristics that are consistent with it being defined as an apex predator, similar to the jaguar and puma, both of which require large ranges to survive. Based on these preliminary data for the bush dog, it is evident that Neotropical conservation strategies should take into account the ecological requirements of this small canid. However, the Near Threatened bush dog has proven difficult to study because standard field techniques (such as camera-traps, transect surveys, and radiocollars) have had limited success with the species (Beisiegel 2009; DeMatteo et al. 2009b, 2011; Michalski 2010; Lima et al. 2012).

In Argentina, the province of Misiones contains the largest remaining tract of Upper Paraná Atlantic Forest ecoregion (Fig. 1); however, the region has a heterogeneous landscape where protected areas are located in a matrix of altered habitat with forest patches that vary in size and connectivity. Uncontrolled vehicle velocity on numerous paved and dirt roads puts animals at risk and scattered human settlements
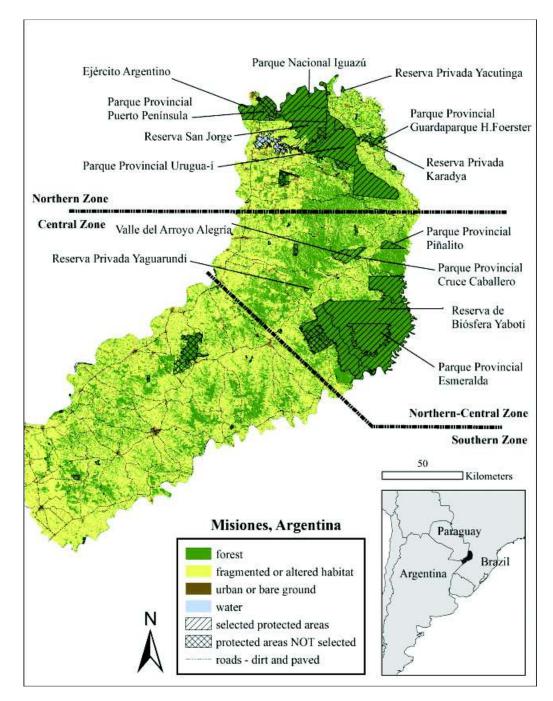

Figure 1. Location of the province of Misiones, Argentina, in South America (inset). Map of Misiones showing both the protected areas (8 northern and 6 central) included in the 2011 survey and the protected areas not included in the survey. These areas are shown in relation to the land-use pattern existing in Misiones in 2009: forest, fragmented or altered areas, and urban or bare ground (Izquierdo et al. (2008) land cover map updated by A. Izquierdo for 2009 personal communication).

increase the potential of human–wildlife conflict (De Angelo et al. 2013). Despite these factors, the region still has a largely intact species assemblage (Galindo-Leal and de Gusmão Câmara 2003); however, carnivore densities are often lower than at other Neotropical sites (Di Bitetti et al. 2008*a*, De Angelo et al. 2011). Wide-ranging carnivores confirmed in the northern and central portions of Misiones include bush dogs (DeMatteo 2008, DeMatteo and Loiselle 2008, DeMatteo et al. 2009*b*, Gil and Lobo 2012), jaguars (Paviolo et al. 2008), pumas (Kelly et al. 2008, Paviolo et al. 2009, De Angelo et al. 2011), and ocelots (*Leopardus pardalis;* Di Bitetti et al. 2006, 2008*a*).

Managing for the long-term survival of the apex carnivores in this ecoregion requires understanding the degree of overlap or separation among the species-specific ecological niches and whether protection, habitat fragmentation, and land-use patterns affect these relationships (Di Bitetti et al. 2010). To date, information on the bush dog's ecological requirements has been missing. The objective of this study was to use noninvasive techniques to detect the presence of bush dogs so we could begin to gain insight into the vulnerability of this small, carnivorous, social canid and its role in intact, and fragmented Neotropical ecosystems. Detection dogs, which have proven to be effective at surveying wildlife species in a diverse array of habitat types (Smith et al. 2003; Wasser et al. 2004; Cablk and Heaton 2006; Long et al. 2007*a*, *b*; Vynne et al. 2011*b*), including the rough terrain and vegetation in Misiones (DeMatteo et al. 2009*b*), were used to locate scats. DNA extracted from the scats was used to confirm species and identify individuals. Genetic data were paired with Geographic Information System analyses to examine habitat preferences and estimate connectivity between protected areas.

STUDY AREA

The Misiones province of Argentina was bordered by Brazil and Paraguay (Fig. 1). Misiones was primarily composed of forest (45.2%), followed by agricultural areas (19.4%) and large plantations (10%); however, roughly 40.3% of forest was located in protected areas (*n* = 71). Although 90–95% of the province was historically covered with Upper Paraná Atlantic Forest, differences in the history of human colonization, land-use change, and protection efforts in Misiones have resulted in land-use differences between the northern-central and southern portions of the province (Izquierdo et al. 2008,2011; Fig. 1). The majority of forest (66.1%) was located in the north-central portion of the province. Half of native forest in Misiones was unprotected (52.5% or 483,171.75 ha) and located in a mosaic of monoculture stands of pine (*Pinus* sp.), *Eucalyptus*, and native *Araucaria angustifolia* (192,096.45 ha), small-scale agriculture (perennial crops of tea and yerba mate; 159,511.32 ha), areas of subsistence agriculture (typically a mixture of highly fragmented degraded or secondary forest, small-scale agriculture and pasture; 89,835.84 ha), pastures (typically dominated by African grasses; 44,418.33 ha), bare ground (28,094.85 ha), or urban areas (15,482.7 ha; Fig. 1).

Even though a relatively large portion of the native forest was under protection, the level of effective protection against poaching or illegal extractive activities was highly variable among protected areas (Paviolo et al. 2008) and this, in turn, differentially affected the populations of medium and large mammals. Most unprotected forests in Misiones acted as population sinks for several large mammals (e.g., jaguars, De Angelo et al. 2013; red brocket deer [*Mazama americana*], Di Bitetti et al. 2008*b*), but other species can thrive in forests under relatively high-anthropic pressure (e.g., dwarf brocket deer [*M. nana*], Di Bitetti et al. 2008*b*). Despite the large number of protected areas in the northern-central portion of Misiones (*n* = 49), there was a large tract of unprotected forest south of Parque Provincial (P. P.) Urugua-í and north of Valle del Arroyo Alegría–P. P. Piñalito that essentially divided the protected areas into 2 zones—northern (NZ) and central (CZ). The degree of connectivity between these 2 zones was unknown, so this study separated the survey areas into the NZ and the CZ and used genetic analyses to try to gain insight into this question.

The province was characterized by humid, subtropical climate with no distinct dry season (Crespo 1982). Average monthly rainfall typically exceeded 100 mm; however, in October and November rainfall was >200 mm. The hot season (late Sep to mid-Apr) was characterized by warm days (30–35° C) and moderate nights (18–24° C). In contrast, the cool season had moderate days (23–27° C) and cool nights (9–12° C).

METHODS

We collected scats from May to August 2011 (late autumn to early spring) when lower daily temperatures were optimal for the detection dog to locate scats. In the NZ of Misiones, we surveyed 8 protected areas (Table 1), selected areas around each, and one dirt or paved road (Fig. 1). Although a portion of the surveyed road borders the eastern edge of P. P. Urugua-í and the southwestern edge of the Biological Corridor (area in between P. P. Urugua-í and P. P. Guardaparque H. Foerster), the majority is immersed in areas with subsistence farming, small villages, livestock, and free-ranging domestic animals. We counted Ejército Argentino (a military area where 93.9% of the managed land is continuous forest that was logged until the 1990s [all remaining forests in Misiones have been logged to varying degrees] but remains in relatively good condition) as a protected area because the military presence in the area combined with the provincial–national park guard patrols in adjacent areas affords a high level of protection to local wildlife. In the CZ, we surveyed 6 protected areas (Table 1), selected areas around each, and a parcel of private land (200 ha; Fig. 1). We treated two of these protected areas (P. P. Esmeralda and Reserva de Biósfera de Yabotí) as a single area because the provincial park is completely contained within the Biosphere Reserve (Fig. 1). We counted Valle del Arroyo Alegría (a large parcel on the northern edge of P. P. Cruce Caballero where 87.76% of the land is continuous forest) as a protected area because of its recent designation by Alto Paraná as an area of 'High

Table 1. Summary of protected areas for the northern and central zones and of the 56 unique survey routes walked during the 2011 field survey for bush dog scat, as conducted in Misiones, Argentina.

Area	Northern zone	Central zone
All protected areas		
Total no.	21	28
Total area (ha)	211,858.71	306,070.49
Range (ha)	10.38–84,000	19.15–236,313
Mean area (ha) $\pm$ SD	7,566 $\pm$ 18,151.79	14,574.79 $\pm$ 50,016.40
Surveyed protected areas		
Total no.	8	6
Total area (ha)	178,322	280,600
Range (ha)	90–84,000	400–236,313
Survey routes		
No. unique routes	39	17
Total length (km)	333.41	113.85
Range (km)	0.44–23.39	0.49–11.8
Mean distance (km) $\pm$ SD	8.55 $\pm$ 4.94	6.70 $\pm$ 2.92
Total survey routes—protected areas		
No. unique routes	39	
Total length (km)	299.83	
Range (km)	0.49–19.11	
Mean distance (km) $\pm$ SD	7.69 $\pm$ 3.90	
Total survey routes—outside protected areas		
No. unique routes	6	
Total length (km)	48.31	
Range (km)	0.44–11.61	
Mean distance (km) $\pm$ SD	8.05 $\pm$ 4.09	
Total survey routes—spanning borders of protected or unprotect areas		
No. unique routes	11	
Total length (km)	109.74	
Range (km)	3.67–23.39	
Mean distance (km) $\pm$ SD	9.98 $\pm$ 6.04	

Conservation Value' where preventing illegal activities such as poaching was a high priority (Fig. 1).

Survey routes ($n = 56$) consisted of 2-lane paved roads, 1- to 2-lane dirt roads, established trails through forested areas, and existing machete-cut trails through forest (Table 1). The majority of routes (69.6%) were completely within protected areas, 10.7% were completely outside protected areas, and 19.7% were partially inside or outside protected areas (Table 1). The higher number and total length of trails walked in the NZ compared with the CZ (Table 1) is a reflection of several factors: 1) although P. P. Esmeralda – Reserva de Biósfera Yabotí represents the largest combined protected area in the northern-central zone, the provincial park has few existing trails, which makes the majority of the park inaccessible; and the Biósfera has ongoing logging activity, which makes access to some areas impossible; 2) there are overall fewer protected areas in the CZ compared with the NZ (Table 1); and 3) the area of those in the CZ is smaller compared with those in the NZ (Table 1). Daily distance covered and time to cover this distance was dependent on sample processing time and how extreme the working conditions were for the dog (temp, precipitation, sun exposure).

Detection-Dog–Handler Training

The detection dog had worked in the region previously and had been trained using scat samples from both wild and captive animals, for which species identification had been genetically confirmed. DeMatteo et al. (2009b) demonstrat-

ed that using scats from captive animals in training did not impede the dog's ability to recognize target species in the field. The dog was trained to indicate on 5 target species (jaguar, puma, ocelot, oncilla [*Leopardus tigrinus*], and bush dog) and to ignore several non-target species (maned wolf [*Chrysocyon brachyurus*], margay [*Leopardus wiedii*], jagua-rondi [*Puma yagouaroundi*], tayra [*Eira barbara*], raccoon [*Procyon lotor*], coyote [*Canis latrans*], bobcat [*Lynx rufus*], red fox [*Vulpes vulpes*], and various venomous snakes, including *Porthidium* sp. and *Bothriechis* sp.). Although some non-target species were not present in the area, they helped fine-tune the dog's search image.

Sample Collection

We collected all scats that the dog indicated on as long as mould was not evident, which meant scat condition ranged from fresh with moist mucus layer to hard and dry. We georeferenced each scat location using a Global Positioning System unit (Garmin eTrex®; Garmin, Schaffhausen, Switzerland) and recorded condition and approximate age (Prugh et al. 2005, Smith et al. 2006, Michalski et al. 2011), macroscopic evaluation of dietary components, and location relative to trail or road. Environmental factors such as rain, sun, and insects, can cause an inaccurate assessment of scat condition. Despite these factors, we made a best guess when classifying each scat as fresh ($\leq$24 hr), moderately old (between 24 hr and 3 days), or old (>3 days).

We swabbed the surface of the scat with a cotton-tipped applicator soaked in phosphate buffered saline solution in order to collect cells sloughed from the digestive tract of the animal (Ball et al. 2007, Rutledge et al. 2009). Each scat was swabbed in triplicate (2 for DNA extraction in United States and 1 for storage in Argentina). In order to maximize the quantity of DNA collected with each swab, when possible, we swabbed different locations on the scat with each replicate. We stored each swab dry in a 1.7 mL polypropylene tube, labeled, and secured it with parafilm. We collected each scat and stored it in a labeled, 18-oz Whirlpak® bag (Nasco, Fort Atkinson, WI) as a backup for DNA extraction and for future diet analyses. At the end of each field day, we preserved samples at −20°C.

Genetic Analyses

We extracted DNA from 2 independent swabs using a QIAGEN (Venlo, Netherlands) DNeasy™ DNA extraction kit following the protocol described in Vynne et al. (2011a). Extractions were carried out in a room separate from the lab in which polymerase chain reaction (PCR) amplifications were performed using dedicated equipment to prevent contamination. Negative controls (no scat material added to the extraction) accompanied each set of extractions and were used in species identification PCRs to test for contamination.

To identify species, we amplified a 110-base-pair (bp; 171-bp with primers) carnivore-specific region of mitochondrial cytochrome *b* gene (Farrell et al. 2000; 5′-AAACTG-CAGCCCCTCAGAATGATATTTGTCCTCA-3′; 5′-TATTCTTTATCTGCCTATACATRCACG-3′) with a modified version of the protocols and reagents of Farrell et al.

(2000) and Miotto et al. (2007). Amplifications were performed on a MyCycler Thermal Cycler System (Bio-Rad, Hercules, CA) in 25-μL volumes containing 2 μL of DNA extract, 1× PCR Gold buffer (Applied Biosystems, Foster City, CA), 0.3 μM each forward and reverse primer, 200 μM each dNTP, 5 mm $MgCl_2$, 150 μg/mL BSA (Ambion® – Life Technologies, Grand Island, NY), and 1U AmpliTaq Gold DNA polymerase (Applied Biosystems, Grand Island, NY). To minimize the potential for contamination in all reactions (including microsatellites and sexing), we performed PCR set up in an Ultraviolet PCR Chamber and included negative controls (no DNA added). The PCR profile consisted of 10 min denaturation at 95° C, followed by 40 cycles at 95° C for 30 s, at 49° C for 45 s, at 72° C for 45 s, and a final 30 min extension at 72° C. We sequenced purified PCR products using the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems) and analyzed them in an ABI 3100 Genetic Analyzer (Applied Biosystems). We edited and aligned sequences using Lasergene Seqman 8.1 (DNASTAR, Madison, WI) and compared them with entries in GenBank using the Basic Local Alignment Search Tool (BLAST) to identify sequences from Neotropical species that were identical with sample sequences. In addition to the single cytochrome *b* sequence available for bush dogs in GenBank (AF028155), we used blood and scat samples from captive animals to establish an additional sequence for comparative analyses (St. Louis Zoo; Table 2). We extracted DNA from captive scat using the same technique as study samples. We extracted DNA from blood samples using standard phenol – chloroform procedure (Sambrook et al. 1989), followed by dialysis against 1× TNE2 (10 mm Tris-HCl, 10 mm NaCl,

2 mm EDTA) and an ethanol precipitation. We obtained species identifications using the same methods as we used with study samples.

Scat samples identified as bush dog were subsequently genotyped to distinguish individuals. We used 8 dinucleotide microsatellite loci that were previously optimized for use with noninvasive samples (allele size < 200 bp) from bush dogs (DeMatteo et al. 2009a). We used an annealing temperature of 60° C with all reactions. Loci were amplified in 2 multiplex reactions: Group 1, 6FAM-SVE1, VIC-SVE5, NED-SVE8, PET-SVE4; Group 2, 6FAM-SVE3, VIC-SVE7, NED-SVE6, PET-SVE2. We performed all amplifications in 10-μL volumes using a QIAGEN Multiplex PCR Kit and manufacturer's instructions. A positive control or reference sample was included in all reactions to standardize allele calling. We determined fragment sizes in an ABI 3100 Genetic Analyzer and scored them against a GS600LIZ molecular size standard (Applied Biosystems) using Gene-Mapper® 4.01 (Applied Biosystems).

For each locus, a genotype was confirmed in either 4 identical homozygous profiles (confirmed homozygote) or 2 heterozygous profiles (confirmed heterozygote; Hedmark et al. 2004). We estimated the probability that 2 siblings or related individuals (parent–offspring) would have the same genotype (probability of identity for siblings, $P_{ID (sib)}$; Waits et al. 2001) in GenAlEx 6.4 (Peakall and Smouse 2006). We assigned individual identities using GenAlEx 6.4 and confirmed them by eye based on unique genotypes at a minimum of 6 microsatellite loci ($P_{ID (sib)} = 0.0045$, which is less than the threshold of < 0.01; Waits et al. 2001).

For all species, we performed tests for deviations from Hardy–Weinberg equilibrium at each locus, linkage

Table 2. The 4 mitochondrial (mtDNA) haplotypes (110 bp) found among the 22 individual bush dogs in Misiones, Argentina, from scat surveys conducted in 2011, as compared with the 2 haplotypes used as a reference in species identifications: the mtDNA sequence generated from captive bush dog blood and scat (St. Louis Zoo) and GenBank reference sequence AF028155.

ID	mtDNA sequence																													
St. Louis Zoo	T	A	G	G	A	C	G	A	G	G	C	T	T	A	T	A	C	T	A	C	G	G	G	T	C	C	T	A	T	A
Haplotype 1												C											A							
Haplotype 2												C											A							
Haplotype 3												C											A							
Haplotype 4												T											A							
AF028155												T											G							
St. Louis Zoo	T	A	T	T	C	A	T	A	G	A	G	A	C	A	T	G	A	A	A	T	A	T	T	G	G	A	A	T	C	G
Haplotype 1											A										T								C	G
Haplotype 2											A										T								C	G
Haplotype 3											A										C								C	G
Haplotype 4											A										T								T	A
AF028155											A										T								C	G
St. Louis Zoo	T	A	T	T	A	T	T	A	C	T	T	T	C	A	A	C	C	A	T	A	G	C	T	A	C	A	G	C	A	T
Haplotype 1		A	T			T			T	G												C								
Haplotype 2		A	T			T			C	G												C								
Haplotype 3		A	T			C			C	G												C								
Haplotype 4		G	C			T			T	G												T								
AF028155		A	T			T			T	T												T								
St. Louis Zoo	T	C	A	T	A	G	G	T	T	A	T	G	T	A	C	T	A	C	C	A										
Haplotype 1								C																						
Haplotype 2								C																						
Haplotype 3								C																						
Haplotype 4								T																						
AF028155								T																						

disequilibrium, and genic differentiation of alleles with an alpha of 0.05 (with Bonferroni correction; Rice 1989) using GenePop 4.0.10 (Raymond and Rousset 1995). We performed tests for presence of null alleles using Micro-Checker 2.2.3 (Van Oosterhout et al. 2004). We calculated allelic richness with and without correction for unequal samples sizes using rarefaction (HP-RARE v. J-6-2006; Kalinowski 2005). We estimated differentiation between subpopulations (NZ and CZ) with F_{ST} (nuclear DNA) and Φ_{PT} (nuclear and mitochondrial DNA) values in GenAlEx 6.4 (Peakall and Smouse 2006). Negative PhiPT values (which are analogous to F_{ST} values) indicate great differences between 2 random individuals from the same population, rather than between 2 random individuals from different populations (Arnason and Palsson 1996). We estimated inbreeding between subpopulations (NZ and CZ) with F_{IS} in GenAlEx 6.4.

We sexed scat samples genotyped to the individual level using a multiplexed amplification of DBY7 (118 bp; Seddon 2005) and modified DBX6 fragments (155 bp: DBX6-F: TACGCTGGGTCTTAGTTTCTTGA and DBX6-R: TGGTAGATGAGTTTAACTGCCCTATT). Although the original DBX6 fragment (249 bp; Seddon 2005) was previously successfully used to sex bush dog blood samples (DeMatteo et al. 2009a), it did not reliably amplify with the current scat samples, likely because of its large size (>200 bp). Using known blood samples from bush dogs, we selected a shorter DBX6 fragment and then tested it with a minimum of 6 blood samples and 3 scat samples of both sexes. We performed the amplification using the protocol outlined by Seddon (2005) with the exception of a lower annealing temperature (58° C). After amplification, 5 uL of PCR product was electrophoresed with a 100 bp ladder on a 4% agarose gel and visualized by GelStar (Lonza Group, Basel, Switzerland) staining. We used a minimum of 2 replicates to identify males and 3 replicates to identify females. We included known male and female scat samples in all reactions for comparison. We used a binomial test to test for a skew in the proportions of males versus females.

Assessment of Genotyping Errors
If a genotype that was determined to be heterozygous had one or more replicates in which only 1 of the 2 expected alleles was represented (homozygous), we interpreted these replicates to have allelic dropout. We calculated the rate of allelic dropout (no. of heterozygous replicates with allelic dropout/total no. of heterozygous replicates [with and without allelic dropout]) for each locus and each individual sample. If a homozygous profile had an allele in a single replicate that could not be reproduced in 4 additional replicates, we interpreted this as a false allele or an allele-like artifact generated by PCR. The percentage of amplifications with false alleles was calculated for each species by dividing the number of false alleles by the total number of replicates.

Habitat Use and Connectivity Assessment
For analyses examining habitat preference and connectivity, we used 30-m × 30-m land-cover raster grids of Misiones, Argentina, described in Izquierdo et al. (2008) but updated

for 2009 by A. Izquierdo (CONICET and Center for Atlantic Forest Investigation [CeIBA], Argentina, personal communication). To gain insight into species habitat or landscape use in the heterogeneous matrix of intact and modified habitat, we used ArcGIS 10.1 to create buffers centered on georeferenced point locations for the 34 scats genetically identified to species level to simulate daily (total area = 10 km^2) and long-term (total area = 100 km^2) habitat-use patterns. Although habitat preference may affect directional movements across a landscape and a circular buffer pattern may not always reflect reality, this analysis attempted to determine how locations may be related to habitat preference (e.g., core protected areas) and habitat avoidance (e.g., altered habitat). We used a binomial test to test for the significance of sample location relative to habitat type and degree of protection. We based the null hypothesis (i.e., the expected proportion of scats in each habitat type) on the sampling effort (distance walked) in each of them.

Although we surveyed fewer areas outside of protected areas, by examining the location of the sample relative to the edge of the protected area and the habitat that surrounds that specific location, we gained insight into habitat use and preference. We used a straight-line distance between each scat and the nearest protected area to examine habitat-use flexibility (i.e., is a species always found deep in the core of protected areas or is it found in a variety of habitats including core protected areas and edges?). We used an F-test to test for significant differences between scats located inside versus outside of protected areas. For this analysis, we considered each protected area independent and not connected to adjacent protected areas because the majority of adjoining areas fall under different management authorities (private, provincial, national) and likely differ in level of protection and monitoring (Paviolo et al. 2008).

We examined the degree of connectivity between protected areas with ArcGIS 10.1 using a buffer along the edge (1 km) versus a buffer in the immediate vicinity (10 km). Although these distances are relatively small compared with the distances between some protected areas, this connectivity analysis helped determine the degree of isolation for each protected area and to identify areas of concern. In this connectivity analysis, in contrast to the individual species points, we considered adjacent protected areas a single unit to eliminate duplication or over-representation of habitat along shared borders.

RESULTS

Assessment of Genotyping Errors
For the 8 microsatellite loci, the overall allelic dropout rate was 25.3%. With the exception of 1 locus (SVE4) that had no allelic dropout, the average rate of allelic dropout for the 7 remaining loci was consistent (range = 19.7–35.3%; $\bar{x} \pm$ SD = 28.9 ± 5.6). Two-thirds (66.5%) of the total allelic dropout across loci could be attributed to a subset of bush dog samples that we classified as moderately old ($n = 8$) or old ($n = 15$). The percentage of amplifications with false alleles for bush dogs was low (range = 0–4.5%). Three loci

(SVE4, SVE5, SVE6) had no false alleles, 4 loci (SVE2, SVE3, SVE7, SVE8) had 2 amplifications (range = 1.4–2.0%) with false alleles, and 1 locus (SVE1) had 7 amplifications (4.5% total) with false alleles.

Genetic Analyses

We located 329 scats—241 in the NZ and 88 in the CZ. Of these, 289 could be identified to species-level, with 34 (11.8%, NZ = 26, CZ = 8) confirmed as bush dog and 255 (88.2%, NZ = 190, CZ = 65) confirmed as 1 of the 4 felid targets (31 jaguar, 27 puma, 66 ocelot, 131 oncilla). About half of the remaining scat either failed to amplify (n = 2) or amplified only DNA from prey items contained in the scat (n = 16). Visual examination of the latter scats confirmed the presence of hair from one of several genetically confirmed prey (South American field mouse [*Akodon montensis*], black-footed pygmy rice rat [*Oligoryzomys nigripes*], collared peccary [*Pecari tajacu*]). The remaining 22 scats were found to contain DNA from multiple species, including at least one of the target species. This result may represent contamination of the scat by urine of scent-marking animals (scent-marking being a common behavior in carnivores), and all were excluded from further analyses.

Of the 34 bush dog scats, 26 (76.5%, NZ = 18, CZ = 8) successfully amplified at both microsatellite and DBY7-DBX6 loci, allowing us to identify 22 individuals (NZ = 14 and CZ = 8) and their respective sexes (NZ = 8 M and 6 F, CZ = 6 M and 2 F; Table 3). Of the 14 individuals identified in the NZ, 4 of them (3 M and 1 F) were found 2 times each with a mean distance (±SD) between scats of 1.11 ± 1.41 km (range = 0.00–3.00 km; Table 4) and sexing results consistent across samples. Unlike the NZ, the 8 samples in the CZ represent unique individuals, with no individual found more than once. The proportion of males versus female scats located (17 M and 9 F) was not significantly different than expected (1:1 M:F, binomial test, $P = 0.169$).

Four mitochondrial DNA (mtDNA) haplotypes (Table 2) were found in the 22 individuals identified using micro-satellites—3 in the 14 individuals of the NZ and 2 in 8 individuals of the CZ. Haplotype 1 (Hp1) was the most common, with an 85.7% frequency in the NZ and 87.5% in

the CZ. Haplotype 2 (Hp2, 7.2%) and 3 (Hp3, 7.2%) were unique to the NZ, while haplotype 4 (Hp4, 12.5%) was unique in the CZ.

Despite the bush dog's broad distribution, they have always been reported as rare, independent of human disturbance (DeMatteo 2008). Although this study represents a large sample size for bush dogs, the number of individuals in each population is smaller than the recommended minimum of 25–30 individuals needed to accurately estimate allele frequencies, expected heterozygosity, and F_{ST} (Hale et al. 2012). These values were calculated for the 2 populations (NZ and CZ) because of the potential importance of the findings, but should be interpreted with caution. The number of alleles per locus varied from 4 to 8 in the NZ, 4 to 6 in the CZ, and 5 to 11 when the 2 zones were combined (Table 5). When rarefaction was applied to account for the large difference in samples size between the 2 zones, the average allelic richness across loci was similar between the 2 zones (NZ = 5.16 and CZ = 5.87; Table 5). No evidence of genotypic linkage disequilibrium was found. In the CZ, all loci conformed to expectations under Hardy–Weinberg equilibrium (HWE), but 3 loci (SVE2, SVE4, SVE8) in the NZ deviated significantly from HWE expectations (Table 5). Two of these loci (SVE2 and SVE4) plus SVE6 deviated significantly when the 2 zones were combined (Table 5). Although the 3 loci in the NZ had moderate amplification success ($\bar{x} = 68.5\%$; range = 61.1–77.8%), the percentages were lower than the overall average across all loci ($\bar{x} = 81.3\%$; range = 61.1–100%). Analysis in Microchecker (http://www.microchecker.hull.ac.uk/) suggested the presence of one or more null alleles for SVE2, but not for SVE4, SVE6, or SVE8. An alternative explanation for these significant deviations from HWE is that the homozygote excess is due to significantly more inbreeding than expected within the subpopulations ($F_{IS} = 0.274$, $P = 0.010$).

The finding that a greater number of individual bush dogs were identified in the NZ (n = 14) than in the CZ (n = 8; Table 3) may reflect real differences in populations or maybe be due to differences in sampling effort between the areas (NZ = 333.41 km and 241 scats and CZ = 113.85 km and 88

Table 3. Summary of 34 bush dog scats and 26 genotyped or genotyped/sexed scat found in protected and unprotected areas in the northern and central zones of Misiones, Argentina, during 2011 field surveys (no. of hectares in parentheses). Sexes or gender for the 22 bush dog individuals (male–female) are reported. Four individuals (3 M and 1 F) were found 2 times. In the table, an * indicates that 1 individual of that sex (male or female) was found 2 times, while ** indicates that 2 individuals of that sex (male) were found 2 times.

Area	No. scats species ID	No. scats genotyped	No. genders identified (M–F)
Northern Misiones	26	18	8–6
Ejército Argentino (6,951 ha)	2	2	1*–0
P. P. Puerto Península (6,900 ha)	5	4	2–1*
P. P. Guardaparque H. Foerster (4,309 ha)	1	0	—
Reserva San Jorge (21,163 ha)	1	0	—
Outside protected area	17	12	5**–5
Central Misiones	8	8	6–2
P. P. Esmeralda (31,569 ha) and Reserva de Biósfera Yabotí (236,313 ha)	3	3	3–0
Valle del Arroyo Alegría (8,000 ha)	1	1	0–1
P. P. Piñalito (3,796 ha)	3	3	2–1
Outside protected area	1	1	1–0

Table 4. Summary of the 4 individual bush dogs identified twice with sex and location where the scats were found during 2011 field surveys in northern and central zones of Misiones, Argentina. For each individual, the mean distance between scat (km), calculated using ArcGIS 10.1, is noted (the SD is not reported because no individual was recorded > 2 times). If scat were found adjacent to each other the distance is equal to zero.

Sex	Zone	Location	No. scats	Mean distance (km)
M	North	Ejército Argentino	2	1.36
F	North	P. P. Puerto Península	2	3.00
M	North	Outside protected area (Route 101)	2	0.00
M	North	Outside protected area (P. P. Guardaparque H. Foerster)	2	0.06

scats) due to restricted sampling opportunities, the smaller number of protected areas, and the smaller average size of protected areas in the CZ. Based on nuclear microsatellite loci, acknowledging the low power to estimate F_{ST}, our results indicated low but significant differentiation between the NZ and CZ for bush dogs ($F_{ST} = 0.049$, $P = 0.010$). When PhiPT is calculated with nuclear microsatellite loci, our results suggest moderate and significant differentiation between the NZ and CZ for bush dogs ($\Phi_{PT} = 0.066$, $P = 0.010$). In contrast, results using maternally inherited mtDNA indicated a lack of genetic differentiation between populations ($\Phi_{PT} = -0.029$, $P = 0.500$).

Habitat Use and Connectivity Assessment

Almost half ($n = 16$) of bush dog scats were located inside protected areas, the remainder ($n = 18$) were located outside (Table 3). Based on the distance surveyed inside versus outside protected areas (3.5:1 inside:outside), the fraction of samples located outside was significantly higher than expected (binomial test, $P < 0.001$). Scats were located in all surveyed protected areas with the exception of P. P. Urugua-í; however, scats were located along the main road that borders P. P. Urugua-í. Scats located outside protected areas were in 3 distinct areas in the NZ (around P. P. Foerster, W–SW of P. P. Urugua-í, along surveyed road) and one area in the CZ (E of P. P. Cruce Caballero – Valle del Arroyo Alegría).

For bush dogs, the distance to edge values for scats located inside ($n = 16$; range = 0.00–4.79 km; $\bar{x} \pm SD = 1.37 \pm 1.43$ km) versus outside protected areas ($n = 18$; range = 0.03–3.25 km; $\bar{x} \pm SD = 0.83 \pm 1.10$ km) were not significantly different (F-test, $F_{1,32} = 1.710$, $P = 0.144$).

Bush dog scats were primarily associated with forest (82.4%) versus altered habitats (plantations = 5.9%, agricultural areas = 8.8%, mixed use = 3.0%, pasture = 0%); however, as the buffer area was increased (from 0 km^2 to a max. of 100 km^2), there was a large decline in the quantity of forest (−16.2%) and an increase in the quantity of fragmented or altered habitat (plantations [+0.6%], mixed use [+4.7%], pasture [+2.6%], and other minor land-use types [+1.9%], which includes bare soil and urban areas). Based on the habitat surveyed (10:1 forest:altered), the proportion of samples located in forest versus altered habitat was not significantly different than expected (binomial test, $P = 0.123$).

DISCUSSION

By using 2 noninvasive techniques—a detection dog and genetic analyses of scats—we confirmed the presence of bush dogs of both sexes in the northern and central zones of Misiones, Argentina. This is the first time such a large-scale survey for the bush dog has been carried out using the combination of these 2 techniques. This small canid is notoriously difficult to study with standard survey techniques such as camera-traps having poor results and population estimates being based on tracks or opportunistic sightings (DeMatteo 2008, DeMatteo and Loiselle 2008, DeMatteo et al. 2011). Physically trapping the species has also proven

Table 5. Genetic diversity levels for bush dogs ($n = 34$ scat or 22 individuals) found during 2011 surveys in the north and central zones of Misiones, Argentina. § = locus did not amplify, N_S = number of scats, N_I = number of individuals detected, A = number of alleles (without rarefaction), A_R = allelic richness with rarefaction (14 genes), A_u = number of unique alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, P_{ID} = probability of identity, $P_{ID(sibs)}$ = probability of identity for siblings. Significant departures from Hardy–Weinberg equilibrium after Bonferroni correction are indicated ($^*P < 0.050$ and $^{**}P < 0.010$).

Zone	N_S	N_I		SVE1	SVE2	SVE3	SVE4	SVE5	SVE6	SVE7	SVE8
North	26	14	A	6	8	4	7	7	4	7	8
			A_R	4.372	6.638	2.933	6.524	5.381	3.053	5.557	6.844
			A_u	2	4	2	7	1	1	4	5
			H_o	0.500	0.500*	0.600	0.833**	0.867	0.267	0.833	0.786*
			H_e	0.546	0.844	0.572	0.877	0.777	0.356	0.819	0.881
Central	8	8	A	6	4	4	§	6	4	4	6
			A_R	5.608	4	3.750	§	5.850	3.742	4	6
			A_u	2	0	2	§	0	1	1	3
			H_o	0.750	0.286	0.250	§	0.875	0.125	0.571	0.714
			H_e	0.683	0.648	0.517	§	0.825	0.442	0.714	0.846
North and central	34	22	A	8	8	6	7	7	5	8	11
			A_R	4.619	5.720	3.216	6.524	5.401	3.200	5.430	7.548
			H_o	0.577	0.421**	0.478	0.833**	0.870	0.217*	0.760	0.762
			H_e	0.581	0.802	0.559	0.877	0.790	0.380	0.805	0.883
			P_{ID}	0.210	0.083	0.033	0.046	0.280	0.075	0.410	0.078
			$P_{ID\,(sib)}$	0.520	0.380	0.330	0.340	0.550	0.370	0.670	0.380

difficult, which explains the limited direct field studies (DeMatteo 2008, Lima et al. 2012). Genetic analyses have been previously done with wild bush dog scat ($n = 11$; Zuercher et al. 2003); however, these were limited to species identification in a single protected area in Paraguay.

The present study demonstrates that not only is it possible to identify scat to the individual level but that it is also possible to evaluate habitat-use variability among individuals. Four individuals were located twice both inside and outside of protected areas. Although the number of individuals or the distance between scats is limited, our study demonstrates that repeated sampling of individuals is possible. Future surveys with bush dogs using these techniques could be modified to maximize sampling in an area in order to determine the number of groups present, minimum area used per group, and relatedness between groups. To date, radiocollar studies have investigated area use by a single group (Lima et al. 2012); however, the questions of whether adjacent groups overlap, degree of relatedness between adjacent groups, and how adjacent groups may or may not vary in home-range size have been impossible to address.

Despite the relatively small sample size (Leberg 2002), the average allelic richness and overall heterozygosity found among bush dogs in Misiones was relatively high and similar to levels found among another carnivores, including a population of dholes (*Cuon alpinus;* also a social canid) in southern India (Iyengar et al. 2005). Similar to our results with bush dogs, Iyengar et al. (2005) also found one predominant haplotype across the population and suggested that this may be the result of dholes undergoing periods of 'booms and busts' for unknown reasons. This type of cycling can result in a loss or skew of genetic diversity, and stabilizing this variability depends on gene flow among populations. This type of skew or loss may be what is reflected in the northern zone of Misiones, where both homozygote excess and significantly higher levels of inbreeding were detected. These 'busts' in the northern zone may result from a variety of causes, including declines due to exposure to lethal domestic dog diseases (DeMatteo 2008, DeMatteo et al. 2011). Although a relatively high level of allelic diversity remains in both the northern and central zones, nuclear microsatellite loci revealed that little or moderate genetic differentiation exists between the 2 zones. The fact that our results indicated greater differentiation at nuclear microsatellite loci than that observed at mtDNA could be interpreted as evidence of female dispersal, which has been suggested from captive bush dog studies (Macdonald 1996) but not confirmed in the wild. However, it could also reflect the fact that microsatellite loci are more informative than mtDNA over short evolutionary timescales. Fragmentation in Misiones is relatively recent, so this disparity in differentiation between nuclear and mitochondrial DNA could be evidence that it is affecting dispersal between formerly contiguous populations.

Bush dogs were extremely flexible in their habitat use; we located scats in areas surrounded by forest, plantations, agriculture, mixed-use areas, and pastures. Other studies have confirmed the presence of bush dogs in fragmented or altered habitat (DeMatteo and Loiselle 2008, Oliveira 2009, Michalski 2010, Gil and Lobo 2012, Lima et al. 2012). In our study, the proportion of scat found in fragmented or altered habitat was similar to observations of bush dogs occurring in these modified habitat types (20% of historical bush dog locations; DeMatteo and Loiselle 2008). We also found that bush dogs used the fragmented habitat more than was expected. Although this might be interpreted as a positive result, preliminary field data from the Brazilian Cerrado ecoregion suggest that use of fragmented or altered habitat does not come without a cost. Specifically, data suggest that increasing levels of fragmentation may alter the species' ecological requirements, resulting in an increase in the minimum area needed to support a group (E. S. Lima, Universidade do Estado de Mato Grosso, Brazil, personal communication per DeMatteo et al. 2011). Although this information is from a single habitat type (the Cerrado), it is suspected to be true across the variety of habitats that bush dogs inhabit and is a reflection of a negative relationship between prey density and habitat fragmentation (DeMatteo et al. 2011). This potential increase in the minimum area needed to support a group of bush dogs with increased levels of habitat fragmentation will inevitably push bush dogs closer to human populations and increase the risk of either direct or indirect exposure to lethal domestic dog diseases (DeMatteo 2008). Exposure to diseases has been identified as a severe threat to the long-term survivability of the species (DeMatteo et al. 2011), with strong field evidence demonstrating that diseases from domestic dogs can have devastating effects on bush dogs, mainly because the species lives in groups (Mann et al. 1980; Steinel et al. 2001; Leite Pitman et al. 2003; Jorge et al. 2007*a*, *b*, 2008; Lima et al. 2012).

Our results in the Upper Paraná Atlantic Forest ecoregion further emphasize the need to understand the effect of fragmentation and altered habitat on the bush dog's ecological requirements across its broad distribution. Despite the bush dog's flexibility in habitat use, our measures of gene flow, genetic differentiation, and inbreeding suggest that movement between the northern and central zones may be hindered for bush dogs and that there are limits to the degree of habitat alteration that the species can tolerate.

Threats to landscape connectivity occur throughout the bush dog's distribution, including the northern and central zones of Misiones. In this region, protected areas, especially those along the eastern boundary between the northern and central zones, are at risk of becoming isolated islands because the area is still undergoing changes in land use, particularly from forests to grasslands for cattle grazing (Izquierdo et al. 2008). Our finding that the quantity of forest declines with increasing distance from protected areas supports this risk and suggests that long-term landscape connectivity in this area could be in jeopardy. Within protected areas there are increased human impacts that may negatively affect populations of bush dogs and other wildlife (Chebez and Hilgert 2003, Giraudo et al. 2003, Holz and Placci 2003). On one hand, a large proportion of the protected areas of

Misiones is not under strict protection but is under multiple-use categories where timber logging and other land uses are allowed, which differentially affects some mammal species (Di Bitetti et al. 2008a, 2010; Paviolo et al. 2008; De Angelo et al. 2013). It is important to assess the potential impact of these activities on bush dog populations. On the other hand, there is increasing pressure by private entrepreneurs and the current Misiones government to build paved roads within protected areas. Most protected areas would be crisscrossed by multiple roads with the inevitable increase in transit and road-kills (Paviolo et al. 2009, De Angelo et al. 2013). This development will have definite negative impacts on carnivores because of their sensitivity to road kills (Haines et al. 2005,2006), and this may also pose another threat to bush dogs.

MANAGEMENT IMPLICATIONS

The ability to revise and develop conservation strategies at both the species level and ecosystem level requires knowledge of a species' basic ecological requirements. The failure to detect the presence of a species with standard field techniques may result in the incorrect conclusion that a species does not occur there, when in reality the species may be actively avoiding the altered habitat, artificial structures, or animal trails typically associated with these methods. This study demonstrated that switching the focus from attracting the target species to a specific location to using detection dogs to locate evidence associated with the species' natural behavior and movement patterns is very effective with bush dogs. The ability to extend the use of detection dogs to locate noninvasive samples from other species, especially in human-occupied areas, allows for comparative studies on how niche overlap or separation differ in intact versus altered habitat so that comprehensive conservation strategies can be developed not only for apex predators but across the broader ecological community.

ACKNOWLEDGMENTS

LITERATURE CITED

Arnason, E., and S. Palsson. 1996. Mitochondrial cytochrome *b* DNA sequence variation of Atlantic cod, *Gadus morhua*, from Norway. Molecular Ecology 5:715–724.

Ball, M. C., R. Pither, M. Manseau, J. Clark, S. D. Peterson, S. Kingston, N. Morrill, and P. Wilson. 2007. Characterization of *target* nuclear DNA from faeces reduces technical issues associated with assumptions of low-quality and quantity template. Conservation Genetics 8:577–586.

Beisiegel, B. M. 1999. Contribuição ao estudo da história natural do cachorro do mato, *Cerdocyon thous*, e de cachorro vinagre, *Speothos venaticus*. Dissertation, Instituto de Psicologia, Universidade de São Paulo, Brazil. [In Spanish.].

Beisiegel, B. M. 2009. First camera-trap record of bush dogs in the state of São Paulo, Brazil. Canid News 12.5 [online]. http://www.canids.org/canidnews/12/Bush_dogs_in_Sao_Paulo.pdf.

Beisiegel, B. M., and G. L. Zuercher. 2005. *Speothos venaticus*. Mammalian Species 783:1–6.

Cablk, M. E., and J. S. Heaton. 2006. Accuracy and reliability of dogs in surveying for desert tortoise (*Gopherus agassizii*). Ecological Applications 16:1926–1935.

Chebez, J. C., and N. Hilgert. 2003. Brief history of conservation in the Paraná forest. Pages 141–159 *in* C. Galindo Leal, and I. de Gusmão Câmara, editors. The Atlantic Forest of South America. Center for Applied Biodiversity Science. Island Press, Washington, D.C., USA.

Crespo, J. A. 1982. Ecología de la comunidad de mamíferos del Parque Nacional Iguazú, Misiones. Revista del Museo Argentinao Cienicas Naturales. Ecologia 3:45–162 [In Spanish].

De Angelo, C., A. Paviolo, and M. S. Di Bitetti. 2011. Differential impact of landscape transformation on pumas (*Puma concolor*) and jaguars (*Panthera onca*) in the Upper Paraná Atlantic Forest. Diversity and Distributions 17:422–436.

De Angelo, C., A. Paviolo, T. Wiegand, R. Kanagaraj, and M. S. Di Bitetti. 2013. Understanding species persistence for defining conservation actions: a management landscape for jaguars in the Atlantic Forest. Biological Conservation 159:422–433.

DeMatteo, K. E. 2008. Using a survey of carnivore conservationists to gain new insight into the ecology and conservation status of the bush dog. Canid News 11.3 [online]. www.canids.org/canidnews/11/Bush_dog_-data_survey.pdf.

DeMatteo, K. E., and B. A. Loiselle. 2008. New data on the status and distribution of the bush dog (*Speothos venaticus*): evaluating its quality of protection and directing research efforts. Biological Conservation 141:2494–2505.

DeMatteo, K., F. Michalski, and M. R. P. Leite-Pitman. 2011. *Speothos venaticus*. IUCN red list of threatened species. Version 2011.2. www.iucnredlist.org. Accessed 21 Oct 2012.

DeMatteo, K. E., P. G. Parker, and L. S. Eggert. 2009a. Isolation and characterization of dinucleotide microsatellite loci in the bush dog (*Speothos venaticus*). Molecular Ecology Resources 9:1219–1220.

DeMatteo, K. E., M. A. Rinas, M. M. Sede, B. Davenport, C. F. Argüelles, K. Lovett, and P. G. Parker. 2009b. Detection dogs: an effective technique for bush dog surveys. Journal of Wildlife Management 73:1436–1440.

Di Bitetti, M. S., C. D. De Angelo, Y. E. Di Blanco, and A. Paviolo. 2010. Niche partitioning and species coexistence in a Neotropical felid assemblage. Acta Oecologica 36:403–412.

Di Bitetti, M. S., A. Paviolo, and C. De Angelo. 2006. Density, habitat use and activity patterns of ocelots (*Leopardus pardalis*) in the Atlantic Forest of Misiones. Argentina. Journal of Zoology 270:153–163.

Di Bitetti, M. S., A. Paviolo, C. D. De Angelo, and Y. E. Di Blanco. 2008a. Local and continental correlates of the abundance of a Neotropical cat, the ocelot (*Leopardus pardalis*). Journal of Tropical Ecology 24:189–200.

Di Bitetti, M. S., A. Paviolo, C. A. Ferrari, C. De Angelo, and Y. Di Blanco. 2008b. Differential responses to hunting in two sympatric species of brocket deer (*Mazama Americana* and *M. nana*). Biotropica 40:636–645.

Eisenberg, J. F., and K. H. Redford 1999. Mammals of the Neotropics. Volume 3. The central Neotropics: Ecuador, Peru, Bolivia, Brazil. University of Chicago Press, Chicago, Illinois, USA.

Farrell, L. E., J. Roman, and M. E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9:1583–1590.

Galindo-Leal, C., and I. de Gusmão Câmara. 2003. Atlantic Forest hotspot status: an overview. Pages 3–11 *in* C. Galindo Leal and I. de Gusmão

Câmara, editors. The Atlantic Forest of South America. Center for Applied Biodiversity Science. Island Press, Washington, D.C., USA.

Gil, G. E., and J. M. Lobo. 2012. Situación del zorro vinagre (*Speothos venaticus*) en el extreme sur de su distribución (Argentina). Interciencia 37:21–28. [In Spanish].

Giraudo, A. R., E. Krauczuk, V. Arzamendia, and H. Povedano. 2003. Critical analysis of the protected áreas in the Atlantic Forest of Argentina. Pages 245–261 in C. Galindo Leal, and I. de Gusmão Câmara, editors. The Atlantic Forest of South America. Center for Applied Biodiversity Science, Island Press, Washington, D.C., USA.

Haines, A. M., M. E. Tewes, and L. L. Laack. 2005. Survival and sources of mortality in ocelots. Journal of Wildlife Management 69:255–263.

Haines, A. M., M. E. Tewes, L. L. Laack, J. S. Horne, and J. H. Young. 2006. A habitat-based population viability analysis for ocelots (*Leopardus pardalis*) in the United States. Biological Conservation 132:424–436.

Hale, M. L., T. M. Burg, and T. E. Steeves. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS ONE 7:e45170. doi: 10.137/journal.pone.0045170

Hedmark, E., Ø. Flagstad, P. Segerström, J. Persson, A. Landa, and H. Ellegren. 2004. DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. Conservation Genetics 5:405–410.

Holz, S., and G. Placci. 2003. Socioeconomic roots of biodiversity loss in Misiones. Pages 207–226 in C. Galindo Leal, and I. de Gusmão Câmara, editors. The Atlantic Forest of South America. Center for Applied Biodiversity Science. Island Press, Washington, D.C., USA.

Iyengar, A., V. N. Babu, S. Hedges, A. B. Venkataraman, N. Maclean, and P. A. Morin. 2005. Phylogeography, genetic structure, and diversity in the dhole (*Cuon alpinus*). Molecular Ecology 14:2281–2297.

Izquierdo, A. E., C. D. De Angelo, and T. M. Aide. 2008. Thirty years of human demography and land-use change in the Atlantic Forest of Misiones, Argentina: an evaluation of the forest transition model. Ecology and Society 13:3. http://www.ecologyandsociety.org/vol13/iss2/art3/

Izquierdo, A. E., H. R. Grau, and T. M. Aide. 2011. Implications of rural–urban migration for conservation of the Atlantic Forest and urban growth in Misiones, Argentina (1970–2030). AMBIO 40:298–309.

Jorge, R. S. P., E. S. Lima, and L. E. B. Lucarts. 2008. Sarna sarcóptica ameaçando cachorros- vinagres (*Speothos venaticus*) de vida livre em Nova Xavantina – MT in XXXIII Congresso Anual de Sociedade de Zoológicos do Brasil, Sorocaba. Brasil. [In Portuguese.] http://www.szb.org.br/

Jorge, R. S. P., R. G. Morato, M. Pereira, E. S. Lima, K. Scheffer, P. Carnieli, Jr., I. Kotait, and F. Ferreira. 2007a. Rabies antibodies in a bush dog captured in the Pantanal, Brazil. Page 94 in Proceedings of the 56th annual Wildlife Disease Association conference. Wildlife Disease Association, Estes Park, Colorado, USA.

Jorge, R. S. P., C. M. Nunes, C. M. Trinconi, H. B. Paula, A. K. K. Dias, and F. Ferreira. 2007b. Leishmania spp. in wild carnivores captured in the Pantanal, Brazil. Page 42 in Proceedings of the 56th annual Wildlife Disease Association conference. Wildlife Disease Association, Estes Park, Colorado, USA.

Kalinowski, S. T. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5:187–189.

Kelly, M. J., A. J. Noss, M. S. Di Bitetti, L. Maffei, R. L. Arispe, A. Paviolo, C. D. De Angelo, and Y. E. Di Blanco. 2008. Estimating puma densities from camera trapping across three study sites: Bolivia, Argentina, and Belize. Journal of Mammalogy 89:408–418.

Leberg, P. L. 2002. Estimating allelic richness: effects of sample size and bottlenecks. Molecular Ecology 11:2445–2449.

Leite Pitman, M. R. P., F. V. Nieto, and L. Davenport. 2003. Amenaza de enfermedades epidémicas a las conservación de carnívoros silvestres en la Amazonía peruana. Pages 227–231 in M. R. P. Leite Pitman, N. C. A. Pitman, and P. C. Alvarez, editors. Alto Purús: Biodiversidad, Conservación y Manejo. Center for Tropical Conservation and INRENA, Perú. [In Spanish.].

Lima, E. S., K. E. DeMatteo, R. S. P. Jorge, M. L. S. P. Jorge, J. C. Dalponte, H. S. Lima, and S. Klorfine. 2012. First telemetry study of *Speothos venaticus*: providing information on home range, activity, and habitat selection. Wildlife Research 39:512–519.

Long, R. A., T. M. Donovan, P. Mackay, W. J. Zielinski, and J. S. Buzas. 2007a. Effectiveness of scat detection dogs for detecting forest carnivores. Journal of Wildlife Management 71:2007–2017.

Long, R. A., T. M. Donovan, P. Mackay, W. J. Zielinski, and J. S. Buzas. 2007b. Comparing scat detection, dogs, cameras, and hair snares for surveying carnivores. Journal of Wildlife Management 71:2018–2035.

Macdonald, D. W. 1996. Social behaviour of captive bush dogs (*Speothos venaticus*). Journal of Zoology 239:525–543.

Mann, P. C., M. Bush, M. J. G. Appel, B. A. Beehler, and R. J. Montali. 1980. Canine parvovirus in South American canids. Journal of American Veterinary Medical Association 177:779–783.

Michalski, F. 2010. The bush dog *Speothos venaticus* and short-eared dog *Atelocynus microtis* in a fragmented landscape in southern Amazonia. Oryx 44:300–303.

Michalski, F., F. P. Valdez, D. Norris, C. Sieminski, C. K. Kashivakura, C. S. Trinca, H. B. Smith, C. Vynne, S. K. Wasser, J. P. Metzger, and E. Eizirik. 2011. Successful carnivore identification with faecal DNA across a fragmented Amazonian landscape. Molecular Ecology Resources 11:862–871.

Miotto, R. A., F. P. Rodrigues, G. Ciocheti, and P. M. Galetti Jr. 2007. Determination of the minimum population size of pumas (*Puma concolor*) through fecal DNA analysis in two protected Cerrado areas in the Brazilian southeast. Biotropica 39:647–654.

Oliveira, T. A. 2009. Distribution, habitat utilization and conservation of the vulnerable bush dog *Speothos venaticus* in northern Brazil. Oryx 43:247–253.

Paviolo, A., C. D. De Angelo, Y. E. Di Blanco, and M. S. Di Bitetti. 2008. Jaguar *Panthera onca* population decline in the Upper Paraná Atlantic Forest of Argentina and Brazil. Oryx 42:554–561.

Paviolo, A., Y. E. Di Blanco, C. D. De Angelo, and M. S. Di Bitetti. 2009. Protection affects puma abundance and activity patterns in the Atlantic Forest. Journal of Mammalogy 90:926–934.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Prugh, L. R., C. E. Ritland, S. M. Arthur, and C. J. Krebs. 2005. Monitoring coyote population dynamics by genotyping faeces. Molecular Ecology 14:1585–1596.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rutledge, L. Y., J. J. Holloway, B. R. Patterson, and B. N. White. 2009. An improved field method to obtain DNA for individual identification from wolf scat. Journal of Wildlife Management 73:1430–1435.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sanderson, E. W., K. H. Redford, C. B. Chetkiewicz, R. A. Medellin, A. R. Rabinowitz, J. G. Robinson, and A. B. Taber. 2002. Planning to save a species: the jaguar as a model. Conservation Biology 16:58–72.

Seddon, J. M. 2005. Canid-specific primers for molecular sexing using tissue or non-invasive samples. Conservation Genetics 6:147–149.

Sergio, F., T. Caro, D. Brown, B. Clucas, J. Hunter, J. Ketchum, K. McHugh, and F. Hiraldo. 2008. Top predators as conservation tools: ecological rationale, assumptions, and efficacy. Annual Review of Ecology, Evolution, and Systematics 39:1–19.

Silveira, L. A., A. T. A. Jacomo, F. H. G. Rodrigues, and J. A. F. Diniz-Filho. 1998. Bush dogs (*Speothos venaticus*) in Emas National Park, central Brazil. Mammalia 62:446–449.

Smith, D. A., K. Ralls, A. Hurt, B. Adams, M. Parker, B. Davenport, M. C. Smith, and J. E. Maldonado. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6:339–346.

Smith, D. A., K. Ralls, A. Hurt, B. Adams, M. Parker, and J. E. Maldonado. 2006. Assessing reliability of microsatellite genotypes from kit fox faecal samples using genetic and GIS analyses. Molecular Ecology 15:387–406.

Steinel, A., C. R. Parrish, M. E. Bloom, and U. Truyen. 2001. Parvovirus infections in wild carnivores. Journal of Wildlife Disease 37:594–607.

Van Oosterhout, D., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Vynne, C., M. R. Baker, Z. K. Breuer, and S. K. Wasser. 2011a. Factors influencing degradation of DNA and hormones in maned wolf scat. Animal Conservation 15:184–194.

Vynne, C., J. R. Skalski, R. B. Machado, M. J. Groom, A. T. A. Jácomo, J. Marinho-Filho, M. B. Ramos Neto, C. Pomilla, L. Silveira, H. Smith, and S. K. Wasser. 2011*b*. Effectiveness of scat-detection dogs in determining species presence in a tropical savanna landscape. Conservation Biology 25:154–162.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249–256.

Wasser, S. K., B. Davenport, E. R. Ramage, K. E. Hunt, M. Parker, C. Clarke, and G. Stenhouse. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Canadian Journal of Zoology 82:475–492.

Wikramanayake, E. D., E. Dinerstein, J. G. Robinson, U. Karanth, A. Raninowitz, D. Olson, T. Mathew, P. Hedao, M. Conner, G. Hemley, and D. Bolze. 1998. Large mammal conservation: the tiger as a case study. Conservation Biology 12:865–878.

Zuercher, G. L., P. S. Gipson, and G. C. Stewart. 2003. Identification of carnivore feces by local peoples and molecular analyses. Wildlife Society Bulletin 31:961–970.

Associate Editor: Blanchong.