



Prioritizing conservation areas to mitigate connectivity loss and local extinction risk of a small carnivore (*Leopardus guttulus*) in South America

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Abstract

Effective conservation management depends on the maintenance of key areas that allow population connectivity across the landscape. However, the lack of knowledge of how habitat conversion affects species movement hinders the identification of these areas. Here, we analyzed the impact of habitat fragmentation on landscape connectivity for *Leopardus guttulus*, a small Neotropical felid threatened by the high habitat fragmentation across the Atlantic Forest, and mapped and ranked the most important core areas and corridors for conservation actions. We also estimated genetic diversity indices and predicted the viability of the current core areas in the future. Our analyses suggest that *L. guttulus* populations are fragmented, and connectivity links between populations are few and weak. We predict that due to their size, estimated density and low connectivity, some current core areas may not maintain viable populations in the long-term. Also, ongoing land-use changes may further isolate remaining populations, leading to progressive reductions in the populations they support. In this study, we spatially prioritize the most critical areas for *L. guttulus* conservation and highlighted the urge that exists in the adoption of management measures for its conservation.

Keywords CDPOP · Connectivity modelling · Fragmentation · Habitat loss · Spatial conservation prioritization · UNICOR

Introduction

Human-induced habitat loss and fragmentation are considered the greatest global threats to biodiversity conservation (Fahrig 2003; IUCN Species Survival Commission 2015). The loss of natural habitat decreases the capacity of a landscape to support populations and reduces population connectivity by subdividing them and enhancing their isolation

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(Cushman 2006; Cushman et al. 2013; Haddad et al. 2015, 2017). Small and isolated populations are more prone to the effects of inbreeding and genetic drift, leading to genetic diversity loss and increasing population extinction probability (Gibbs 2001; Traill et al. 2010). Although these negative effects of habitat loss and fragmentation might be mitigated by the maintenance of regional connectivity among populations and the protection of suitable areas (Beier et al. 2008), the longterm persistence of a metapopulation system requires the implementation of a network of protected and connected areas (D'Aloia et al. 2019). In this context, spatial identification of the most essential areas for population connectivity is crucial for the implementation of effective conservation actions (Cushman et al. 2018; Hearn et al. 2018; Kaszta et al. 2019; Wasserman et al. 2013).

The Atlantic Forest is extremely threatened. It had a wide distribution of forest cover in the past, extending along almost the entire Brazilian coast and expanding inland into north-eastern Argentina and eastern Paraguay, covering more than 1.7 million km² (da Fonseca 1985). Currently, the biome's forest cover encompasses less than 30% of its original extent, most of it in small and increasingly isolated fragments (ca. 97% of the remaining forest is in fragments smaller than 0.5 km²) (Vancine et al. 2023). Due to its high biodiversity and habitat loss, this biome is considered a hotspot for biodiversity conservation (Myers et al. 2000). The ongoing severe habit loss, fragmentation, and degradation of this ecosystem poses great threats for long-term survival of the species inhabiting it.

The southern tigrina (*Leopardus guttulus*) was recently recognized as a separate species from the northern tigrina (*Leopardus tigrinus*) (Trigo et al. 2013). It is one of the smallest felids from South America (Sunquist and Sunquist 2002), with a geographic distribution extending from central to southern Brazil, eastern Paraguay, and northeastern Argentina (de Oliveira et al. 2016). Although the exact limits of this species' geographic range are not well understood (Trigo et al. 2013), most of its known distribution is within Brazil, where it seems to present a coextensive occurrence with the Atlantic Forest (Sartor et al. 2021) but may also enter the Cerrado biome (Central Brazil) to some extent (Trigo et al. 2013; Sartor et al. 2021).

As a recently recognized species, the specific habitat requirements of *L. guttulus* are still under investigation, with recent studies providing important contributions to their understanding (ex. Cruz et al. 2019; Sartor et al. 2021). *Leopardus guttulus* occurrence seems to be limited by the extent of forested habitats, although it may tolerate some degree of habitat alteration, inhabiting mosaics of forest and small-scale agriculture as well as forest-dominated landscapes (Cruz et al. 2019; de Oliveira et al. 2016; Sartor et al. 2021). This species is found mainly outside protected areas, probably due to the high abundance of *Leopardus pardalis*, a superior competitor and predator, within most protected areas (de Oliveira et al. 2016). Despite its small size, the species present wide home ranges (5–18 km²; Kasper et al. 2016) and lower densities (1–5/100 km²; de Oliveira et al. 2016) than typically found for other small felid species (e.g., *Leopardus guigna*, Sanderson et al. 2002; *Leopardus geoffroyi*, Caruso et al. 2012; and *Prionailurus bengalensis*, Ghimirey et al. 2023). According to the IUCN Red List, *L. guttulus* is considered vulnerable to extinction. Some estimates suggest that there may be as few as 6047 mature individuals in the wild, distributed in small and likely fragmented populations (de Oliveira et al. 2016). Furthermore, some populations have experienced large reductions in their numbers during recent years, and a decline of ca. 10–30% is expected for the next three generations (i.e., less than 20 years) due to habitat loss (de Oliveira et al. 2016).

Given the high fragmentation of the Atlantic Forest, most forest fragments may not be large enough to support long-term viable populations of *L. guttulus*. If this is the case, the maintenance of connectivity between forest patches will be critical for the conservation of the

species in the long term. Therefore, in this study, we used empirically parameterized connectivity modeling analyses to identify and prioritize key areas and corridors to determine spatial actions for species conservation (e.g., Cushman et al. 2018; Kaszta et al. 2019, 2020). In addition, we estimated current genetic diversity, population structure and effective population size using microsatellite data. Finally, we simulated demogenetic processes with an individual-based, spatially explicit landscape genetic model (CDPOP, Landguth and Cushman 2010) to assess the viability of the identified core areas in the future.

Materials and methods

Resistance surface and the study area

We utilized a landscape resistance map optimized for the entire distribution of *L. guttulus* by Sartor et al. (2022) with an empirical landscape genetics method. Briefly, Sartor et al. (2022) used a restricted multivariate optimization approach (Shirk et al. 2010; Castillo et al. 2014) to verify the relationship between landscape variables and the genetic data of 135 individuals. They compared a large pool of candidate resistance models based on their AIC score using linear mixed effects regression, which showed that gene flow in this cat species is mainly influenced by landcover variables, with low resistance within forested habitats and high resistance in cropland areas and primary roads (Supplementary Information, Table S1). However, as the species is mainly found within the Atlantic Forest, and its occurrence in the Cerrado is not well known, with only a few scattered genetically confirmed records in this biome (Trigo et al. 2013; Sartor et al. 2021), in this study, we decided to limit our analyses to the Atlantic Forest area located within the species' range recognized by the IUCN (de Oliveira et al. 2016) (Fig. 1).

Effective population size and genetic diversity

We analyzed the current genetic diversity of the species using a microsatellite dataset from Sartor et al. (2021). This dataset comprised information of 13 microsatellite loci for 135 individuals of *L. guttulus* distributed mainly within the Atlantic Forest. All individuals were sampled from 2000 to 2017 and were either road-kills or captive/museum individuals with known origin. We assessed the number of alleles per locus, allelic richness, observed and expected heterozygosity and the inbreeding coefficient (F_{IS}) with the programs FSTAT 2.9.3.2 (Goudet 2002) and Arlequin 3.5.1.2 (Excoffier et al. 2005). We analyzed the existence of population structure with the software Structure v.2.3.4. (Pritchard et al. 2000) under the admixed and correlated allele frequency model. We conducted ten independent runs for each value of K (1–4) using 1,000,000 MCMC iterations following a burn-in period of 500,000 steps, without putative population information. We also estimated current effective population size with the programs LDNe (Waples and Do 2008) and NeEstimator (Do et al. 2014), assuming a random mating model and critical values (P_{crit}) of 0.05, 0.02 and 0.01.

Source points for connectivity modeling and simulations

Connectivity modelling analysis requires source locations to reflect species density and distributions across the study area (Compton et al. 2007; Cushman et al. 2009).

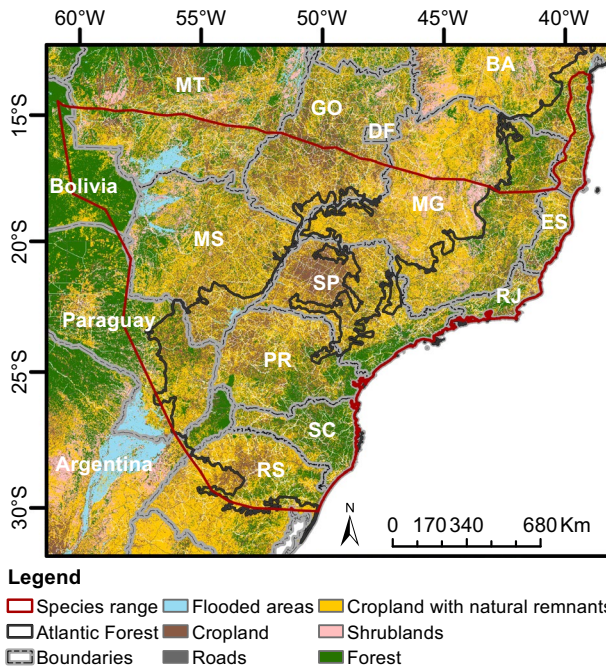


Fig. 1 Map of the study area, showing landcover in 2009 (ESA GlobCover 2009 Project). The *L. guttulus* range according to the IUCN (de Oliveira et al. 2016) is delimited by a red line, while the boundaries of the Atlantic Forest biome are outlined in black. Brazilian states are identified as follows: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Espírito Santo (ES), Bahia (BA), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS) and Federal District (DF). (For color figures, please refer to the online version)

Therefore, we used a previously developed predicted habitat suitability map (Sartor et al. 2021) to generate individual location points in the landscape proportionally to the suitability values. This technique has been widely used to create source points to model corridors and core areas for species conservation (e.g., Macdonald et al. 2018; Wan et al. 2018, 2019; Kaszta et al. 2020) and assumes that areas with high habitat suitability prediction tend to have a higher density of individuals. Thus, using the Create Spatially Balanced Points Tool in ArcGIS, we randomly generated 15,000 points within a 50 km buffer around the Atlantic Forest area, respecting the species' range. This distribution was proportional to the habitat suitability model, meaning that pixels with higher suitability values are more likely to receive a source point (see Fig. S1 of the Supplementary Information for an example of the location of the source points). Of these points, we randomly subsampled a set of them to represent pixels where an individual is present, while the remaining points represented vacant sites. To evaluate the impact of the initial population size in the subsequent analyses, we considered two estimates of population sizes to determine source points where an individual is present: 6047 individuals estimated by de Oliveira et al. (2016) using the species expected maximum area of occupancy and population density; and the lowest effective population size estimated with the genetic data (see Results).

Connectivity modeling

We used UNICOR (Landguth et al. 2012) to model connectivity with two approaches—resistance kernel (Compton et al. 2007) and factorial least cost path (Cushman et al. 2009)—using only the occupied source points of the two datasets as initial point locations. Both the resistant kernel and factorial least cost path approaches predict movement across a resistance surface based on the density, distribution and dispersal ability of individuals (Cushman et al. 2013, 2018). The resistance kernel approach calculates the cost of moving from each occupied source cell, sequentially, to every other cell on the landscape within a cost distance threshold. The result is a cumulative surface created by summing the kernels of all source points and represent the expected movement of individuals in each pixel (Compton et al. 2007). The factorial least cost path analysis calculates the least cost path between all pairs of points and then sums them to create a map of path density representing the strength of linkage among areas (Cushman et al. 2009, 2010).

As the dispersal ability of the species is not known, the dispersal threshold was estimated based on a plausible conservative evaluation of a mean dispersal rate of 3–8 km in medium–low quality areas (i.e., shrublands, flooded areas and cropland with natural vegetation remnants within it), a value similar to the mean maximum moving distance within the home range for this species (Oliveira-Santos et al. 2012). We specified three dispersal thresholds of 150,000, 200,000 and 250,000 cost units for the resistant kernel analysis and 600,000, 800,000 and 1,000,000 cost units for the factorial least cost path analysis. We know this dispersal rates may be low, but due to the great difference in the resistance values, a higher number could lead to an overestimation on connectivity. With this approach, in areas with low resistance, the species will be able to disperse great distances, and in areas with high resistance, its dispersal capacity will be reduced.

Furthermore, in some areas of the resistance layer, roads had lower resistance values than the surrounding landscape (e.g., cropland; Supplementary Information Table S1). This could induce the factorial least cost path analysis to create paths along the roads, instead of through the roads, which would not be realistic for a species that is known to not select roads for habitat features. Still, the landscape surrounding the roads probably influence the density of individuals that will cross the road and likely additively increase the effective resistance of road segments depending on their surrounding context. Therefore, we added the primary and secondary road resistance values to the landcover resistance layer [see Sartor et al. (2022) for a description on the layers] such that roads would always have higher resistance than the surrounding landscape. For example, roads crossing forested areas would present relatively lower resistance, while roads crossing cropland areas would present higher resistance due to this additive context effect.

Identifying main core areas and corridors

We evaluated and ranked the importance of the core areas and corridors generated by the connectivity analyses to prioritize conservation actions. We defined core areas as those patches with cumulative resistance kernel values higher than the 10th percentile of the kernel surface (Macdonald et al. 2018). Similar to Cushman et al. (2018), we identified three main characteristics to determine core area importance: (1) the size of the patch, as larger patches sustain more individuals; (2) the sum of the kernel, reflecting the density of individuals moving through this area; and (3) the number of patches to which each core area

was connected, as isolated populations are more susceptible to extinction. We ranked the patches according to each measure and then calculated the average of the three ranks, creating a composite score (Supplementary Information, Fig. S2A). In case of a tie, the core area with a higher sum of the kernel value per km² was given precedence, as it reflects higher movement density per unit area.

To evaluate the importance of the corridors, we considered the following measures: (1) the standardized maximum value of the factorial least cost path surface for each corridor, because it reflects the number of predicted linkages between source points in each corridor (corridor strength); (2) the standardized sum of the kernel values of all patches that the corridor connects with, since it demonstrates the density of individuals that it connects; (3) the number of patches connected by the corridor, as a measure of connectivity; and (4) the standardized sum of the kernel values of the patches indirectly connected to the patches that this corridor connects, reflecting the connectivity of the network (Cushman et al. 2018). We multiplied the values of each measure and then ranked the corridors (Supplementary Information, Fig. S2B). Corridors that are strong, connected to important patches and that are linked to more areas were ranked higher, while those with lower strength and fewer connections, linked to less significant patches, were considered to have lower importance to conservation.

Predicting population size and genetic diversity in the future

We used CDPOP (Landguth and Cushman 2010) to simulate demogenetic processes on the current landscape, aiming to evaluate the genetic diversity and species occurrence in the future. CDPOP is a landscape genetics program that simulates birth, death, mating and dispersal processes as functions of movement cost between locations. The CDPOP model enables spatially explicit estimation of population dynamics, density and genetic diversity, enabling it to estimate possible local extinctions and changes in genetic diversity based on present connectivity conditions of the landscape and current population genetic information (e.g., Kaszta et al. 2019, 2020).

We used the complete source point datasets described above as locations of *L. guttulus* individuals in the landscape and vacant areas, and considered the genetic data generated by Sartor et al. (2021) as initial genetic information. We used a linear mating and dispersal probability function and, for each source point dataset, we tested two maximum mating and dispersal cost-weighted distances: 200,000 and 300,000 cost units. Mating was random, and fecundity was parameterized on a Poisson probability drawn with means of 2.1, 2.2, and 2.3 offspring per female. This fecundity estimate represents how many mature individuals a female leaves by the end of its life and, for a stable population (i.e., for the offspring to replace the parents) the mean value should be 2 (Macdonald et al. 2018; Kaszta et al. 2019). Therefore, we considered the above cited mean fecundity values because it is close to 2 and using any value below 2.1 led to population extinction, and above 2.3 to indefinite population growth. In total, we generated 12 scenarios, encompassing all possible combinations of the parameters above mentioned (i.e., initial population size, mating and dispersal distance and mean fecundity).

For each scenario, we simulated gene flow for 200 non-overlapping generations, as it has been shown to be sufficient time for individual-based spatially-explicit genetic simulations to reach spatial genetic equilibrium (Landguth et al. 2010; Cushman and Landguth 2010). To account for stochastic variability, we ran 50 Monte Carlo runs. We extracted the number of extant individuals, total number of alleles in the population, observed (H_o) and

expected heterozygosity (H_e) at the 200th generation of each simulation run and calculated the mean among all the runs. A workflow diagram summarizing the methodological processes of the connectivity and simulations for the future can be found in Fig. S3 of the Supplementary Information.

Results

Current effective population size and genetic diversity

The analysis of the microsatellite data demonstrated that the species can be considered a single genetic population with moderate to high levels of genetic variability and low inbreeding (Supplementary Information, Table S2). Structure software identified $K=1$ based on the log-likelihood of each value of K ($\ln \Pr(X/K)=-5600$) (Supplementary Information, Figs. S4 and S5), indicating limited genetic sub-structure across the species' distribution.

The results of the effective population size analysis showed a great variation of estimates depending on the rare allele exclusion criteria applied and included “infinite” in the confidence interval (Supplementary Information, Table S3). As the programs were not able to estimate effective population size based on the rare allele exclusion criteria of 0.05, we only considered the results from exclusion criteria of 0.02 and 0.01, whose values ranged from 895 to 1519 individuals, respectively. We considered the effective population size to be one-tenth of the census size (Frankham 1995). Therefore, according to our analyses, the *L. guttulus* population size should range from 8950 to 15,190 individuals. Together with the estimate of 6704 individuals from Oliveira et al. (2016), the estimate of 8950 was the second value used to determine initial population sizes and occupied source points for the connectivity and demogenetic simulation analyses.

Location and importance of core areas and corridors

The resistance kernel analysis identified a large number of core areas. We discuss and prioritize core areas larger than 1,000 km² (smaller core areas can be seen in Fig. S6 of the Supplementary Information) as we believe these are the most important areas able to sustain viable populations in the long term, along with the paths that connect them. We identified 14–20 patches as such core areas, depending on the initial population size and dispersal threshold considered, with great difference in size and predicted strength between them (Supplementary Information, Tables S4, S5 and S6). However, regardless of the initial population size and dispersal threshold, the main core areas identified were highly consistent across all analyses (Fig. 2A, B; Supplementary Information, Fig. S7). Thus, we choose to highlight the five highest ranked areas for each analysis.

Core area 1 extends from Rio Grande do Sul to Rio de Janeiro state, along the Brazilian coast, with predicted internal connectivity higher in the central areas, in Santa Catarina, Paraná and São Paulo states. Core area 2 covers almost the entire Argentine province of Misiones, in addition to small surrounding areas from Brazil, and had the highest predicted movement density proportionally to its area. Core areas 3 and 4 are located in the Brazilian states of Minas Gerais and Espírito Santo, respectively. These core areas are much smaller than Core area 1 and present significantly lower sums of the kernel per km² than the first two. Finally, Core area 5 varied when using distinct initial population sizes and dispersal

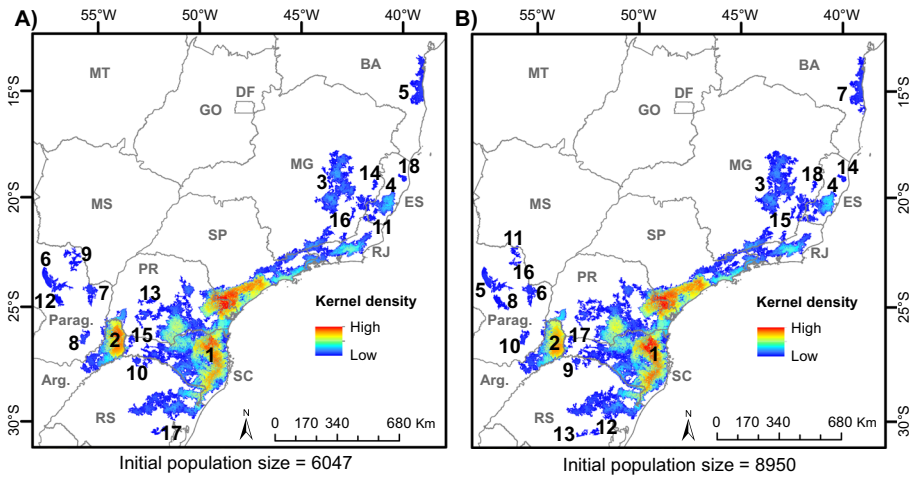


Fig. 2 Identified core areas larger than 1000 km² and ranked values of their importance for conservation considering a dispersal threshold of 200,000 cost units and an initial population size of **A** 6047 and **B** 8950 individuals. Brazilian states are identified as follows: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Espírito Santo (ES), Bahia (BA), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS) and Federal District (DF). *Arg.* Argentina, *Parag.* Paraguay. (For color figures, please refer to the online version)

thresholds. While in some analyses the fifth most important core area was located in the Brazilian state of Bahia, the northernmost area of the species' distribution, on others, it was identified in the westernmost portion of Paraguay (Fig. 2A, B; Supplementary Information, Fig. S7).

The factorial least cost path identified a similar pattern of movement density as predicted by the resistant kernel analysis, with higher values concentrated in the south-south-eastern portions of Brazil and the Misiones province (Fig. 3A, B; Supplementary Information, Figs. S8 and S9). As the analysis generated a network map with paths interwoven with each other, we decided to split them into groups of corridors as in Kaszta et al. (2021). We identified 14–29 groups of corridors between the core areas. However, unlike the kernel analysis, the corridors' location and rank varied greatly depending on the initial population size and dispersal threshold considered (Supplementary Information, Tables S7, S8 and S9).

Population size and genetic diversity in the future

The results from CDPOP simulations varied with the initial population size, the mean number of offspring per female and the dispersal threshold considered. Despite that, simulations predicted genetic diversity values similar to the current ones across all scenarios (Table 1). Our analysis showed that simulated population size for the species, as well as its projected area of distribution, decreased in all parameterization scenarios. The species is projected to go extinct in most core areas, with only Core area 1 maintaining an extant population across all scenarios. The populations of Core areas 2 and 3 also survived in most of the scenarios, but populations from Core area 4 only survived in the most optimistic scenario (i.e., the one with the highest initial population size, dispersal

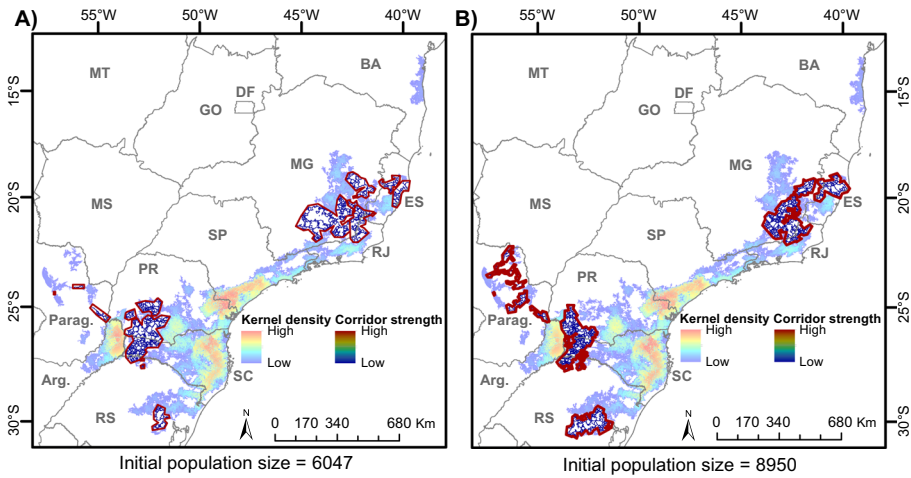


Fig. 3 Identified corridors connecting the core areas larger than 1000 km² considering a dispersal threshold of 800,000 cost units and an initial population size of **A** 6047 and **B** 8950 individuals. Groups of corridors are delimited by red polygons. Brazilian states are identified as follows: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Espírito Santo (ES), Bahia (BA), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS) and Federal District (DF). *Arg* Argentina, *Parag* Paraguay. (For color figures, please refer to the online version)

Table 1 Genetic diversity and population size estimated for future scenarios simulated with CDPOP considering different initial population sizes, mean number of offspring per female and dispersal thresholds values

Initial population size	Mean offspring per female	Dispersal threshold (cost units)	Number of individuals	Number of alleles	Expected heterozygosity	Observed heterozygosity
Current			6047/8950	148	0.70971	0.64830
6047	2.1	200,000	0	–	–	–
		300,000	3286	300.24	0.72100	0.70832
	2.2	200,000	3871	336.02	0.73592	0.68639
		300,000	5493	388.15	0.74668	0.70536
	2.3	200,000	4865	370.5	0.74432	0.67217
		300,000	6403	406	0.74207	0.68994
8950	2.1	200,000	0	–	–	–
		300,000	3578	327.6	0.73315	0.71884
	2.2	200,000	3853	336.14	0.74072	0.69409
		300,000	5660	391.75	0.74730	0.70484
	2.3	200,000	5008	373.06	0.74254	0.66767
		300,000	6559	408.5	0.74499	0.69189

Simulation values represent the mean of 50 runs. Current genetic diversity was estimated with 13 microsatellite loci

threshold and mean number of offspring per female; Fig. 4A, B; Supplementary Information, Figs. S10 and S11). In addition, these core areas had their area reduced. Although the distributions predicted for the different scenarios were similar, the number of remaining individuals varied from ~3200 to 6500. Still, it is worth mentioning that in the scenario with 2.1 mean offspring per female and a dispersal distance of 200,000 cost units, the species went extinct in the entire Atlantic Forest region, regardless of the initial population size considered. It is also important to note that, at a fecundity level of 2.0 mean offspring per female (which is commonly used as the fecundity parameter in landscape genetic simulation modeling, e.g., Landguth et al. 2020; Kaszta et al. 2019), the population was projected to go extinct regionally in all scenarios.

Discussion

In this study, we prioritized key areas for *L. guttulus* conservation and analyzed the impact of habitat alteration on species connectivity by integrating empirical connectivity modeling with individual-based genetic simulations. Our analysis demonstrated that the connectivity of the species decreased significantly with the increase in the presence of areas of anthropogenic use. In addition, some of the core areas identified may not be viable in the long term and our simulations suggest that the smaller subpopulations are likely to go extinct across the edges of the species' range if conservation measures are not taken.

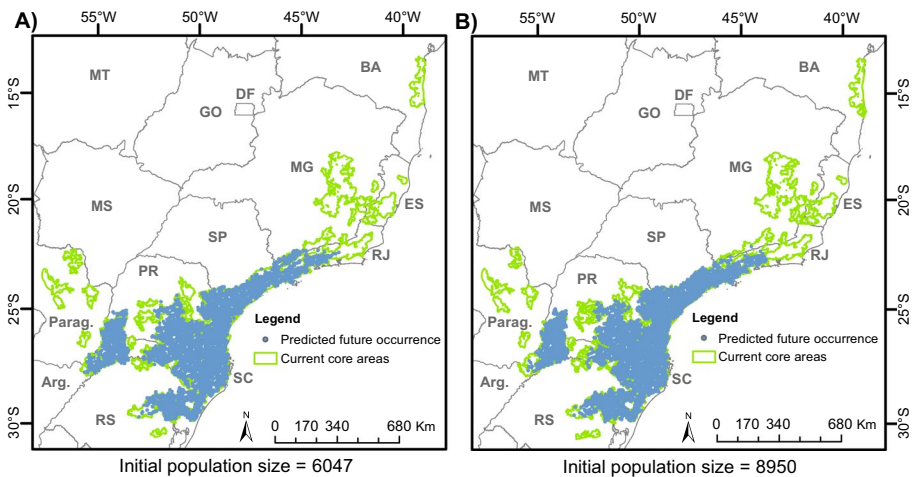


Fig. 4 Predicted areas with remaining populations in the future based on the current landscape resistance, considering a mean of 2.2 offspring per female, a dispersal threshold of 200,000 cost units and an initial population size of **A** 6047 and **B** 8950 individuals. Brazilian states are identified as follows: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Espírito Santo (ES), Bahia (BA), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS) and Federal District (DF). Arg Argentina, Parag Paraguay. (For color figures, please refer to the online version)

Current genetic diversity and connectivity

Despite fragmentation, our microsatellite analysis demonstrated that the species still retains moderate to high genetic diversity values and constitutes one genetic population. It seems that some individuals can still migrate between forest patches, allowing gene flow between forest remnants. It is also possible that our analysis did not show signs of genetic structure due to the small sample set for the northern portion of the species' range and the absence of samples from Argentina and Paraguay, which are the farthest and most isolated areas within its distribution. Alternatively, the relatively high genetic diversity and low genetic substructure may be attributed to a time lag effect resulting from rapid landscape change—the biome has lost over 10% of its natural vegetation in the last 40 years (Proyecto MapBiomias 2022)—and population decline that has affected this species in the past several decades (Landguth et al. 2010). Therefore, we recommend future research to increase genetic sampling in these regions to better evaluate the genetic structure of *L. guttulus* across its entire distribution.

Our genetic analyses suggest that *L. guttulus* population size likely ranges from 8950 to 15,190 individuals (assuming approximately 10 times the estimated effective population size). Although these values are higher than the estimate produced by de Oliveira et al. (2016), if we considered the same area of occupancy of 473,254 km² employed by those authors, our estimates would be within the range expected for the common population density of 1–5 individuals/100 km² recognized for this species (4732.54–23,662.7 individuals), demonstrating congruence between the genetic-based estimate and existing demographic projections.

Among the core areas identified, Core areas 1 and 2 were by far the strongest areas from the resistance kernel analysis and the most critical for species conservation. Core area 1 encompasses the three largest fragments of Atlantic Forest in Brazil, accounting for more than 20,000 km² of forest (Ribeiro et al. 2009). This area has the highest densities recorded for the species (Tortato and Oliveira 2005; Oliveira-Santos et al. 2012), but in recent years, these values seem to have dropped considerably (de Oliveira et al. 2016). This reduction in population density, however, is more likely attributed to the loss of habitat quality or insufficient protection of forest fragments, rather than habitat loss itself, as the native forest cover has remained relatively stable in this area over the last few decades (Proyecto MapBiomias 2022). Indeed, this area presents a small number of protected areas, especially in Santa Catarina state, which encompasses the largest portion of Core area 1 (ca. 95,000 km² or 30% of Core area 1) but has only 7 protected areas larger than 100 km², which protects ca. 3.5% of the core area in the state (Supplementary Information, Tables S4, S5 and S6 and Fig. S12).

Core area 2 is mostly located in the Misiones province of Argentina and has the largest remaining area of continuous Atlantic Forest (Izquierdo et al. 2011), retaining around 50% of the original native forest of the province (Izquierdo and Clark 2012; Zuleta et al. 2015). In addition to Misiones' fragments, two Brazilian parks, Iguaçu National Park and Turvo State Park, connected to them, increase the area of the patch. Although this core area is much smaller than Core area 1, it has a higher relation of summed kernel values/km², showing the high predicted density of the patch. Nonetheless, despite the apparent high quality of the habitat in this area, studies with relative abundance of individuals demonstrated low detectability for *L. guttulus* (Di Bitetti et al. 2010; Kasper et al. 2016; Cruz et al. 2018). It is possible that the high abundance of *L. pardalis* in this area limits the numbers of *L. guttulus*, increasing, even more, the importance of Core area 1.

Core area 1 and 2 are still linked to each other, with a group of corridors identified between them. Yet, these corridors were weak and located in a matrix of agricultural and urban areas, with small fragments of forest. Seventeen protected areas are found in this region, but 12 of them have less than 8 km², and only one spans more than 25 km² (Estação Ecológica de Mata Preta, with 82 km²), indicating the vulnerability of the connection between these two core areas.

In contrast, Core areas 3 and 4 do not present high-density values of predicted movement densities but are important for the maintenance of the species in the northern portions of its distribution. Their importance is further demonstrated by the number of corridors we identified between them and smaller patches. The states that encompass these two core areas, Minas Gerais and Espírito Santo, have also retained a relatively stable forest cover (Proyecto MapBiomias 2022), but they have few protect areas. This is specially the case of Espírito Santo state, which safeguards only about 3.5% of the core area within protected areas greater than 100 km² in extent (Supplementary Information, Tables S4, S5 and S6 and Fig. S12). Moreover, the few available studies demonstrate a low abundance of the species in the region (Hatakeyama 2015; Massara et al. 2016), and our models predicted low movement density values for these core areas. However, this region is an important connectivity hub, linking the main core area to patches in the north of the species distribution and therefore has great importance for species viability in the area.

It is worth mentioning the two core areas identified as the fifth most important, depending on the evaluated scenario. One of these core areas is located in the state of Bahia and represents the northernmost known area of the species' distribution. Despite this patch having one of the highest percentages of protected areas within the core area (Supplementary Information, Tables S4, S5 and S6), it appears to be completely isolated, as no corridor was identified linking this area with any other, regardless of the scenario considered. Finally, the other core area identified as the fifth most important is in Paraguay. This country has lost approximately 30% of the biome's forest cover within its territory in the last four decades (Proyecto MapBiomias 2022) and thus, this patch may harbor one of the last populations of *L. guttulus* in the country. Furthermore, there is no protected area with more than 100 km² within this core area or in its surroundings (Supplementary Information, Fig. S12). Therefore, the isolation of the Bahian patch and the lack of protection, coupled with high deforestation rates in the Paraguayan region, underscores the threat of regional extinction for this species on the edges of its distribution.

Simulations for the future

Our simulations of future population dynamics predicted a possible concerning prospect for *L. guttulus* conservation, with species extinction in all core areas but Core areas 1 and, occasionally 2, 3 and 4, depending on the assessed scenario. Considering the core areas identified here, around one third of them do not contain protected areas of more than 100 km² (Supplementary Information, Tables S4, S5 and S6; Fig. S12). However, even in the core areas that are most well protected (with >40% of their area protected), it appears that the small size of individual protected areas, low initial population size and low density of individuals, do not enable the maintenance of long-term viable populations, leading to regional population extinction. In addition, the current connectivity between these areas seems not to be strong enough to overcome this result. This demonstrates the importance of promoting forest regeneration and creating a network of connected protected areas to

keep viable populations in the long term (e.g., Cushman et al. 2018; D'Aloia et al. 2019; Kaszta et al. 2019, 2020).

Despite this concerning result, it is imperative to highlight that using a larger initial population size than the ones we used in our analysis could increase the estimations of population size and decrease extinction risk. That said, we tested two different initial population sizes based on the best existing information and they produced similar predictions of species occurrence, demonstrating that even larger plausible populations sizes will probably not avoid the extinction of the populations in the smaller core areas. Still, some simulations showed an increase in the geographic distance between Core areas 1 and 2, with the extinction of the species in some intervening patches. These two areas have the largest fragments of Atlantic Forest and the highest density of movement, and the isolation between them would probably have great impact on species genetic diversity and population numbers.

Furthermore, only ~15–20% of Core areas 1 and 2 are under protection (Supplementary Information, Tables S4, S5 and S6). Although both Brazil and Argentina have kept relatively stable percentages of forest cover of the Atlantic Forest (Proyecto MapBiomias 2022), considering the importance of these core areas for the species' survival, the small number of protected areas in them is concerning. Hence, management plans should focus on the creation and/or effectiveness of protected areas inside these core areas and the maintenance of natural fragments outside them. The Brazilian Forest Code mandates the conservation of a certain percentage of native vegetation on rural private lands (Soares-Filho et al. 2014). These fragments of habitat may work as connecting corridors but also as vital areas for the conservation of the species considering that unprotected areas seem to be important for this species' due to the general absence or low abundance of *L. pardalis* (de Oliveira et al. 2016). Therefore, measures to incentivize landowners to adhere to the Forest Code are essential for the conservation of this species. Moreover, Core area 2 and the northern portion of Core area 1 are considered important units for jaguar (*Panthera onca*) conservation in the Atlantic Forest. However, jaguar populations in these areas do not seem to be connected (Paviolo et al. 2016). Thus, the creation and protection of corridors between these areas would be beneficial for other species.

Interestingly, the decrease in population size was not followed by the loss of genetic diversity in our simulation. This suggests a time lag effect in the simulated data (Kaszta et al. 2019), indicating that the surprisingly high current genetic diversity in the observed extant population is likely not reflective of an equilibrium between population size and gene flow with the landscape, and will decline with drift over time (e.g., Landguth et al. 2010). It is also possible that the size and connectivity of Core area 1 slowed the decline of global genetic diversity, even with the species going extinct in the smaller patches (Gibbs 2001).

Conclusions

The maintenance of landscape connectivity is crucial for the viability of populations in the long term. However, high rates of habitat loss and fragmentation pose a challenge for biodiversity conservation (Cushman 2006; Haddad et al. 2015). The Atlantic Forest has undergone intense landscape change for over 500 years (Morellato and Haddad 2000). As a result, species from this biome are now typically restricted to small isolated patches. This is particularly critical since the Atlantic Forest area is inhabited by 70% of the Brazilian human population, and many areas still face increasing rates of deforestation (SOS Mata

Atlântica and INPE 2021). The approach we presented here provides a useful basis for *L. guttulus* conservation actions, with the spatial identification and prioritization of core areas and corridors that maintain connectivity among populations. Our analyses suggest that populations of this species are fragmented and connectivity between forest patches is low. The simulations of future connectivity and population size demonstrate that current linkages among most core areas are not strong enough to prevent population extinction in all small core areas and reduction of population size in the remaining ones. Still, it is imperative to highlight that the scenarios generated for the future were simulated over the current landscape and did not consider further habitat conversion. Therefore, without conservation actions, population reduction may be even more severe. Nonetheless, considering the species' moderate to high levels of genetic diversity and the consistent forest cover percentage in the Atlantic Forest biome of Brazil and Argentina in recent decades, it may be possible to avert these extinction scenarios. By establishing a well-connected network of protected areas and ensuring effective conservation of natural vegetation on private rural lands, we can enhance the species' prospects for long-term persistence.

Finally, the conservation of core areas and corridors identified here will probably benefit other species, as they encompass the most significant remaining fragments of the Atlantic Forest. We recommend that future research should focus on conservation planning that considers human population growth and development to minimize negative ecological impacts in the area. We hope our study will motivate the implementation of management actions, increasing the protection of Atlantic Forest fragments and creating effective linkage corridors that allow the dispersal of individuals among them.

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Data availability Genotype file is available from the FigShare Repository, accessible at <https://doi.org/https://doi.org/10.6084/m9.figshare.13235537.v1> (Sartor et al. 2020). Sample locations are provided in the Supporting Information of Sartor et al. (2021).

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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