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The role of the environment in the spatial dynamics of an extensive hybrid zone between two neotropical cats

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Abstract

Identifying factors that create and maintain a hybrid zone is of great interest to ecology, evolution and, more recently, conservation biology. Here, we investigated the role of environmental features in shaping the spatial dynamics of a hybrid zone between the southern tigrina, *Leopardus guttulus*, and Geoffroy's cat, *L. geoffroyi*, testing for exogenous selection as the main force acting on its maintenance. These Neotropical felid species are mainly allopatric, with a restricted area of sympatry in the ecotone between the Atlantic Forest and Pampa biomes. As both biomes have experienced high rates of anthropogenic habitat alteration, we also analysed the influence of habitat conversion on the hybrid zone structure. To do this, we used 13 microsatellite loci to identify potential hybrids and generated ecological niche models for them and their parental species. We compared the influence of variables on parental species and hybrid occurrence and calculated the amount of niche overlap among them. Parental species showed different habitat requirements and predicted co-occurrence was restricted to the forest-grassland mosaic of the ecotone. However, hybrids were found beyond this area, mainly in the range of *L. geoffroyi*. Hybrids demonstrated higher tolerance to habitat alteration than parental types, with a probability of occurrence that was positively related with mosaics of cropland areas and remnants of natural vegetation. These results indicate that exogenous

selection alone does not drive the dynamics of the hybrid zone, and that habitat conversion influences its structure, potentially favouring hybrids over parental species.

KEYWORDS
ecological niche modelling, habitat conversion, hybridization, *Leopardus geoffroyi*, *Leopardus guttulus*, niche divergence

1 | INTRODUCTION

Hybridization is a relatively common natural event with an important role in the evolutionary process (Barton, 2001; Menon et al., 2020; Seehausen, 2004). On the one hand, hybridization may introduce genetic novelty into a population, via the introgression of new variants, thus increasing genetic diversity and the possibility of adapting to changing environments (Menon et al., 2020; Shirk et al., 2020). On the other hand, it may lead to genetic homogenization of populations, loss of locally adapted genotypes and even displacement or extinction of parental species (Barton, 2001). It may also lead to the emergence of new taxa, with novel adaptations relative to their parental species (Abbott et al., 2013, 2016; Allendorf et al., 2001). Given these varied scenarios, the outcome of a hybridization event is difficult to predict and will depend on the ecological context in which the hybrid zone is established (Seehausen et al., 2008).

Currently, high rates of anthropogenic habitat conversion are altering species' ranges and ecological interactions (Blois et al., 2013; Haddad et al., 2015). As a consequence, in the last few decades, studies have described changes in the dynamics of hybrid zones and even the formation of new ones (e.g. Carney et al., 2000; Garroway et al., 2010; Heath et al., 2010; Seifert et al., 2010). These changes have significant consequences for species conservation and have fuelled a debate on whether and under what circumstances hybrids should be protected (e.g. Allendorf et al., 2001; Brennan et al., 2014; Grabenstein & Taylor, 2018; Stronen & Paquet, 2013).

According to Allendorf et al. (2001), natural hybridization events are part of the evolutionary legacy of the taxa involved, and thus, natural hybrids should generally be protected. In contrast, hybridization events with an anthropogenic origin often require management actions that remove hybrids or reduce their numbers to protect parental species (Allendorf et al., 2001). Therefore, identifying the factors promoting the formation and maintenance of a hybrid zone may help us understand the nature of this process, which can be crucial for management considerations targeting the involved species.

Hybrid zones are formed in areas where species with weak reproductive barriers co-occur (Barton & Hewitt, 1985). Often, these areas correspond to environmental gradients or ecotones that allow the secondary contact of related species adapted to different environments (Cullingham et al., 2012; Culumber et al., 2012; Kameyama et al., 2008; Lucid et al., 2020; Walsh et al., 2016). The genetic structure of hybrid zones is highly variable, and their maintenance in space and time is determined mainly by the type and strength of selection on hybrids (Barton, 2001). When the dynamics of a hybrid zone are primarily determined by endogenous selection, hybrids present a lower fitness than their parental species due to genetic, developmental, behavioural and/or morphological incompatibilities (Arnold & Hodges, 1995). In this case, selection decreases hybrid frequency, but continued dispersal from parental populations maintains the hybrid zone, in a model known as tension zone. If dispersal from one parental species decreases, due to lower population density or an environmental barrier, and dispersal from the other parental species is maintained or increases, the hybrid zone will be pushed towards the area of the former (Barton & Hewitt, 1985).

Environmental-based selection, or exogenous selection, can also influence the maintenance of hybrid zones (Anderson, 1948; Kruuk et al., 1999; Moore, 1977). In this case, parental species have different habitat requirements, and hybrid occurrence will be dictated mainly by their fitness in environments located within the hybrid zone. Therefore, exogenous selection acts on both parental and hybrid individuals, shaping the distribution of genotypes across the landscape (Rand & Harrison, 1989). Although most hybrid genotypes are expected to be less fit than parental types, hybridization might produce intermediate or transgressive phenotypes that outperform parental forms in transitional or heterogeneous zones (Arnold & Hodges, 1995; Moore, 1977).

In this study, we examine the influence of environmental factors on a hybrid zone between two species of small Neotropical cats, the southern tigrina (*Leopardus guttulus* Carnivora: Felidae) and Geoffroy's cat (*L. geoffroyi*). These two species exhibit mainly allopatric distributions and are associated with different environments (Eizirik et al., 2006; Trigo, Tirelli, et al., 2013) . *Leopardus guttulus* is considered a forest species, occurring almost exclusively in the Atlantic Forest of Brazil, Paraguay and northern Argentina (de Oliveira et al., 2016). *Leopardus geoffroyi*, on the other hand, occurs in more open environments in central and southern South America, occupying a broad range of vegetation types, such as savannas, shrublands, grasslands and dry forests (Pereira et al., 2015).

The two species overlap and hybridize in the southernmost Brazilian state of Rio Grande do Sul, in the transitional area between two biomes, the Pampa and the Atlantic Forest (Trigo et al., 2008, 2014; Trigo, Schneider, et al., 2013). This region consists of a mosaic of grassland vegetation and forest types and has been greatly altered by agricultural activities (Müller et al., 2012). In this area, admixture between the parental types is extensive and hybrids are frequently found. Despite the intense introgression, parental species remain

genetically and morphologically differentiated in most of their allopatric areas, which suggests that selective pressures restrict the dispersal of hybrids beyond the hybrid zone (Trigo et al., 2014).

Here, we investigated the role of environmental factors in the formation and maintenance of the hybrid zone between *L. guttulus* and *L. geoffroyi* by evaluating the influence of habitat features on the distribution of pure and hybrid individuals. Since the hybrid zone is presently embedded in an agricultural matrix, we also investigated the influence of human-dominated landscapes on the occurrence of hybrids and parental taxa. We hypothesized that different ecological preferences of parental species and hybrids, and the existence of an environmental mosaic in the contact area, play important roles in the distribution of the hybrid zone via exogenous selection. If environmental factors determined the location of the hybrid zone, we would expect parental species and their hybrids to exhibit different habitat preferences and produce habitat suitability models that closely match with each species' geographical distribution and the location of the hybrid zone (Culumber et al., 2012; Moore, 1977). In this case, we would expect higher suitability for the hybrids in the mosaic environment found in the contact zone. In contrast, if intrinsic factors predominantly shaped the hybrid zone, the predicted habitat suitability models should not match the distribution of parental species and hybrids, and niche divergence between parental types and hybrids should not be observed (Culumber et al., 2012; Swenson, 2008). To test these hypotheses, we applied species distribution modelling and niche divergence analysis to characterize habitat preferences of parental and hybrid individuals relative to environmental variation across the extent of the hybrid zone, as well as throughout most of the geographical range of each parental species.

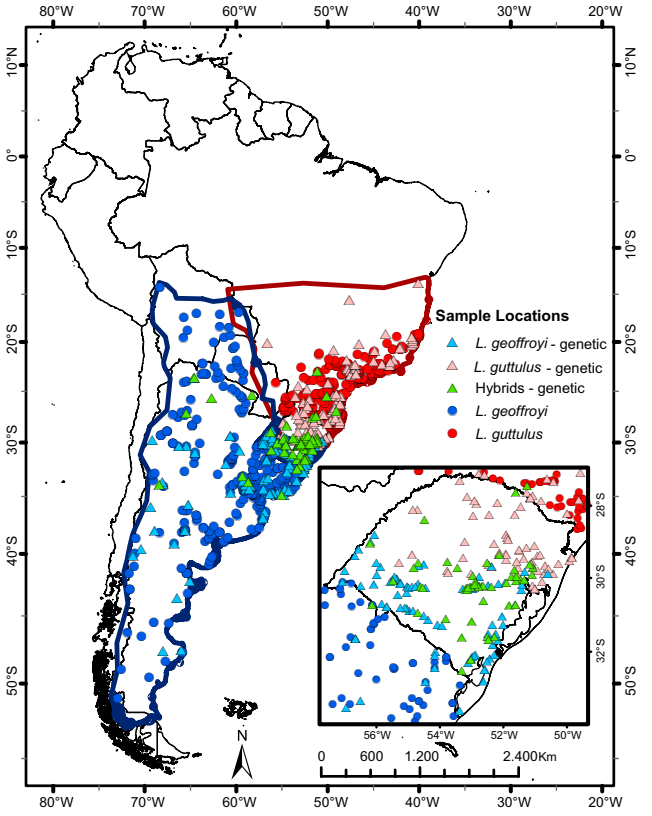

FIGURE 1 Study area and sample locations. The blue and red lines indicate the range maps of the parental species, modified from IUCN: *Leopardus guttulus* in red and *L. geoffroyi* in blue. Triangles indicate the genetic samples, whereas circles indicate presence records obtained from the literature, museum collections and databases. The inset shows a zoomed-in image of Rio Grande do Sul state, in southernmost Brazil, where the two species co-occur. Within this state, all individuals were genetically classified as *L. guttulus*, *L. geoffroyi* or hybrid based on the coefficient membership probability generated with the software Structure. Outside Rio Grande do Sul state, presence points from the literature and museum collections were also included (see Materials and Methods for more details)

2 | MATERIALS AND METHODS

2.1 | Study area and sampling effort

The study area encompasses almost the entire range of the parental species, including five South American countries: Brazil, Uruguay, Argentina, Paraguay and Bolivia (Figure 1). For *L. guttulus*, it comprises mainly two biomes, the Atlantic Forest and Cerrado, while for *L. geoffroyi*, it comprises different vegetation types that can be broadly categorized as grasslands, savannas, shrublands, dry and moist broadleaf forests. The hybrid zone is located in the transition zone between forested environments associated with the Atlantic Forest and grasslands associated with the Pampa biome in the southernmost Brazilian state of Rio Grande do Sul.

We collected blood and tissue samples from captive individuals of known origin and road-killed animals. Skin samples from museum collections were also used (Table S1). Preliminary specimen identification was made only on species level considering general pelage pattern and body size, with smaller individuals with a pelage where open rosettes predominates being identified as *L. guttulus*, and those individuals larger in size with predominantly solid black spots being identified as *L. geoffroyi*. Hybrids were not morphologically identified because, according to Trigo et al. (2014), they often did not present body sizes or pelage colorations that could be clearly distinguished from parental forms. Hence, the identification of hybrids was done exclusively through genetic analysis in this study. For road-killed samples, which lacked precise geographical coordinates, we estimated their locations by randomly placing them beside a road within the municipality where they were collected. The small imprecision in such sample locations is unlikely to affect our results due to the large extent of the study area (Graves et al., 2012). Only samples whose provenance was known at least to the municipality level were included.

To enhance our sampling effort, we also collected presence records from museum collections, including data derived from the Global Biodiversity Information Facility (GBIF, 2019) and SpeciesLink (http://www.splink.org.br/), and the literature. These records were located outside the state of Rio Grande do Sul,

since the current data indicate that hybridization events are restricted to this state (Trigo, Tirelli, et al., 2013; Trigo et al., 2014). Therefore, within the state of Rio Grande do Sul, we only considered records that could be genetically identified as pure or hybrid individuals (Figure 1).

2.2 | DNA extraction, genotyping and hybrid identification

We extracted DNA from tissue and blood samples using a standard phenol/chloroform protocol (Sambrook et al., 1989). The DNA extraction of the skin samples was performed in a room dedicated to work on low-concentration DNA, along with extraction controls, to monitor and minimize the risk of contamination. These samples were cleaned and hydrated following the protocol described by Moraes-Barros and Morgante (2007), and DNA extraction was performed using a modified phenol/chloroform protocol. We amplified the samples for 13 microsatellite loci, developed originally for the domestic cat (Menotti-Raymond et al., 1999, 2005): eight tetranucleotide (FCA391, FCA424, FCA441, FCA453, F42, F124, FCA742, FCA559), two trinucleotide (F98 and F146) and three dinucleotide repeat loci (FCA723, FCA730, FCA734). Each microsatellite locus was amplified individually with a polymerase chain reaction (PCR), as described in Trigo et al. (2008), Trigo, Schneider, et al. (2013). We visualized PCR products using an ABI3730XL sequencer and scored the genotypes using the software Peak Scanner v1.0 (Applied Biosystems). About 15% of the tissue and blood samples and 40% of the skin samples were genotyped twice as a quality-control step. Individuals with more than 20% of missing data were excluded from the analysis. We also added to our data set the genotypes for 122 individuals that were reported by Trigo, Schneider, et al. (2013). However, since their data set comprised a slightly different set of markers (not including loci FCA730, FCA734 and FCA559), we genotyped those samples for additional loci to allow comparison with all 13 markers used here. In addition, we repeated the genotyping of *ca.* 5% of the samples from Trigo, Schneider, et al. (2013) to standardize genotype binning across the whole integrated data set.

To identify parental types and putative hybrids, we applied the Bayesian clustering method implemented in the program Structure v.2.3.4. (Pritchard et al., 2000), with a varying number of clusters (K). We ran the analyses under the admixed and correlated allele frequency model. We conducted 10 independent runs for each value of K (1–6), using 1,000,000 MCMC iterations following a burn-in period of 500,000 steps, without putative population information. The best-fit number of clusters ($K = 2$) was defined using the DK method (Evanno et al., 2005), in Structure Harvester (Earl & vonHoldt, 2012). We used the coefficient membership probability (q) for $K = 2$ generated by Structure, to classify individuals into one of the three groups (*L. guttulus*, *L. geoffroyi* or hybrid). We considered as potential hybrid individuals those with a coefficient membership probability $0.2 < q < 0.8$, and as putatively pure individuals those with $q \geq 0.8$ to their phenotype-based (species-level) group of origin, as in Trigo et al. (2014). Finally, we analysed the genetic diversity within the three groups with the programs FSTAT (Goudet, 2002) and Arlequin (Excoffier et al., 2005).

2.3 | Landscape variables

To analyse patterns of habitat use by our three groups, we assembled geospatial data for a set of environmental variables that has been demonstrated to influence habitat choice in other carnivorans (Gómez-Fernández et al., 2020; Macdonald et al., 2018; Vergara et al., 2016): mean annual temperature, mean annual precipitation, annual amount of solar radiation (Worldclim—http://www.worldclim.org/), elevation (Lehner et al., 2008), NDVI (USGS—https://lpdaac.usgs.gov/), per cent of tree canopy cover (USGS—https://www.usgs.gov/), 13 categories of landcover (Table S2) (GlobCover—http://due.esrin.esa.int/page_globcover.php) and three categories of perennial river width (small: ≤200 m; medium: 200<>500 m; large: ≥500 m—Allen & Pavelsky, 2018). Because a large proportion of the habitat within and around the hybrid zone has been converted into pasture and agricultural fields (Andrade et al., 2015), we also analysed the influence of the amount of livestock per km^2 (Robinson et al., 2014) and the presence of cropland areas on the occurrence of both species and hybrids. We added a more recent cropland layer (USGS) to the landcover layer to have a more up-to-date evaluation of cropland areas, and then classified them into two categories: (a) cropland areas with remnants of natural vegetation, which included all cropland categories considered to represent areas with some percentage of simultaneous presence of native vegetation and cropland; and (b) cropland areas containing only agricultural land uses, without remnants of natural vegetation. The cropland classes were not differentiated by the crop product, including even forest plantations areas. Owing to the large extent of the study area, all layers were projected at 1-km^2 resolution.

2.4 | Habitat suitability modelling

To generate habitat suitability models and to investigate which variables were most related to each parental species and hybrid occurrence, we used the Random Forest algorithm, implemented in the randomForest package (Liaw & Wiener, 2002) in R. Random Forest is a classification and regression tree method (CART) (De'ath & Fabricius, 2000) that is not prone to common issues that can negatively affect other statistical methods, such as over-fitting and multicollinearity (Breiman, 2001; Cutler et al., 2007; Evans et al., 2011). It has been shown to have higher predictive power than the logistic regression, producing more accurate maps of patterns of occurrence (Cushman et al., 2017; Cushman & Wasserman, 2018). The response variable comprised presence records (1) and pseudo-absence data (0). The presence data were consisted of the genetic data and published records, as described above.

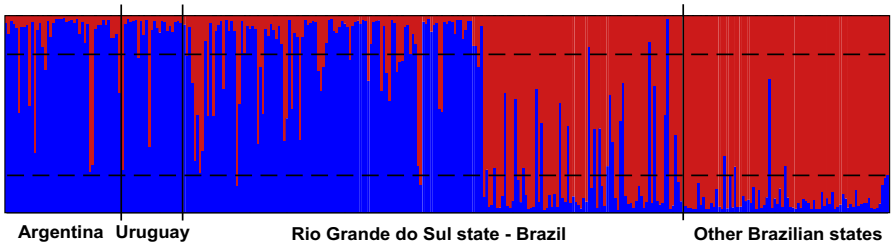

FIGURE 2 Genetic structure of *L. guttulus*, *L. geoffroyi* and hybrids estimated with the program Structure using $K = 2$. Each vertical bar represents an individual, with the colours indicating the proportion of its genomic membership (q) in the two clusters: *L. guttulus* (red) and *L. geoffroyi* (blue). The horizontal dashed lines indicate the threshold of 0.8 used to assign individuals to each genetic group

Since the data were unbalanced due to an unequal sampling effort across the range, we subsampled the presence records to keep a similar density of points in all areas, so as to minimize the bias of an overprediction of habitat suitability in areas with higher concentrations of points (Boria et al., 2014; Kramer-Schadt et al., 2013). Therefore, we calculated the density of presence points within an area of 100km^2 across the entire study area with the 'point density' tool in ArcMap and then used the area with the lowest density to standardize the rest of the distribution. We used a bootstrap procedure to randomly remove a certain number of points in all areas with higher point concentration, such that all areas achieved a similar density. We repeated this procedure 10 times to reduce possible biases (e.g. Wan et al., 2017). We thus had 10 subsets of presence data (i.e. 10 bootstrapped samples) after this step.

The pseudo-absence points were created randomly across the species' ranges with the 'Create Random Points' tool in ArcMap. We generated 10,000 random points and erased any point that fell within an 8-km radius around the presence points. We chose an 8-km radius scale considering an average home range of 8 km^2 for *L. geoffroyi* (Pereira et al., 2015) and also that this value is within the home range interval reported for *L. guttulus* by de Oliveira et al. (2016). Moreover, studies on several carnivoran species have shown habitat selection at relatively broad spatial scales (e.g. Mateo Sánchez et al., 2014; Vergara et al., 2016; Hearn et al., 2018; Macdonald et al., 2019). We thus took a random subset of the remaining points proportional to the number of presence points. We used a ratio of 1:1 for presence and pseudo-absence (e.g. Evans & Cushman, 2009; Wan et al., 2017).

For each predictor variable, we calculated the focal mean of the raster value around each presence and pseudo-absence point on an 8-km radius scale, with the Focal Statistics tool in ARCMap. We checked for multicollinearity by computing Pearson's correlation coefficient for each pair of predictor variables. When two variables were correlated (i.e. $r > 0.5$), we retained the variable that was most related to the response variable, based on the out-of-bag (OOB) error from the univariate random forest model, and excluded the other one from further analysis. To identify the variable subset that resulted in the most parsimonious model, we applied the Model Improvement Ratio (MIR) (Murphy et al., 2010). We compared the models and selected the one that had the lowest total OOB error and lowest maximum within-class error. We validated the model utilizing the OOB error rate, sensitivity (proportion of observed positives that were correctly predicted), specificity (proportion of observed negatives that were correctly predicted) and Kappa. The final habitat suitability model was an ensemble derived from the combination of all 10 models (i.e. based on the 10 bootstrapped samples). All procedures for the habitat suitability modelling were conducted separately for each parental species and their hybrids.

2.5 | Niche divergence

To quantify the amount of niche overlap among parental species and their hybrids, and to analyse how their ecological requirements differ from each other, we used three approaches. First, we calculated the spatial difference of the probability of occurrence obtained from the random forest models. Specifically, we subtracted the predicted habitat suitability map of one species from the predicted map of the other species in the entire studied area. Second, we used density profiles of occurrence points to assess single variable niche overlap using the R package SM (Bowman & Azzalini, 2014) for the most important predictor variables. Finally, we calculated pairwise niche overlap based on logistic probabilities of occurrence in each grid cell according to Schoener's D and Hellinger's I metrics with the ENMTools package (Warren et al., 2019). Both metrics range from 0 (no overlap) to 1 (complete overlap). As parental species have mainly allopatric distributions, niche differences might be due to different environmental conditions available in their ranges (Warren et al., 2008). Therefore, we tested if niches are more or less divergent than expected given the environmental background of their geographical ranges with the Background Test in ENMTools. Briefly, this test contrasts the observed niche overlap to a distribution of niche overlap values created by comparing the habitat suitability model of one species with habitat suitability models generated from random points drawn within the background of the other species. For each comparison, we calculated 100 random models, with random points equal to the number of presence records and the background of each genetic

group delimited by a minimum convex polygon created around the data points. Tests were conducted for all pairwise groups and in both directions (i.e. asymmetric test). Rejection of the null hypothesis indicates that ecological niches differ from random and are a function of habitat selection and/or suitability. Failure to reject the null hypothesis indicates that niche differences might be simply due to the background environmental differences available in their ranges (Warren et al., 2008, 2010).

3 | RESULTS

3.1 | Genetic identification of hybrids and parental taxa

We genetically identified 135 individuals as pure *L. guttulus*, 140 as pure *L. geoffroyi* and 60 as potential hybrids (Figure 1 and Figure 2). Of the individuals identified as hybrids, 78% were from Rio Grande do Sul state in southernmost Brazil. Thirteen individuals identified as hybrids were located in areas previously not described as hybrid zone by Trigo, Schneider, et al. (2013): two in Paraná state and two in Santa Catarina state, within the limits of the Atlantic Forest in Brazil; two in Uruguay and seven in Argentina, within the range of *L. geoffroyi*. Interestingly, of the seven hybrids identified in Argentina, four were from the northern region of the country, characterized by the presence of open forests.

Only 20 of the 60 identified hybrids demonstrated a similar ancestry from both parental species (*i.e.* $0.4 < q < 0.6$) (Figure 2). Of the remaining hybrids, 17 demonstrated a higher genetic similarity with *L. guttulus* and 23 with *L. geoffroyi*, suggesting the occurrence of backcrosses with both parental species. Half of the identified hybrids were spatially concentrated in a 160 km range (north-south) surrounding the centre of the contact zone, a lower percentage than the one found by Trigo et al. (2014) (Figure 1). Whereas only about 13% of the hybrids were located to the north of this range, more than 36% were identified to the south, showing a greater concentration of hybrids in this region. The three groups presented similar genetic diversity (Table 1), with hybrids presenting slightly higher values of allelic richness and observed heterozygosity, but with a lower number of alleles than both parental species.

TABLE 1 Genetic diversity of hybrids and parental taxa considering all samples

Group	N	N_a	A_r	H_e	H_o
Leopardus guttulus	135	147	9.473	0.708	0.644
Leopardus geoffroyi	140	145	9.473	0.732	0.640
Hybrids	60	125	9.544	0.759	0.687

Note: Abbreviations: A_r, Allelic richness; H_e, Expected heterozygosity; H_o, Observed heterozygosity; N, number of individuals; N_a, number of alleles.

3.2 | Habitat suitability models

After removing individuals with imprecise location, we obtained 944 presence records for the three groups defined here: 396 records for *L. guttulus*, 488 for *L. geoffroyi* and 60 for hybrids (including data genetically identified and data from other sources) (Figure 1). After filtering the presence points to obtain similar densities across the study areas, each subset of presence data contained 211, 234 and 39 records for *L. guttulus*, *L. geoffroyi* and hybrids, respectively. For each group, the 10 models generated were highly correlated with each other ($r > 0.91$, $p < .001$), although some variables selected in each model varied (Supporting information, Table S3). The models of the parental species were well supported, with an OOB error rate around 20% and a Kappa index ~ 0.55, whereas the models of the hybrids were somewhat less supported (OOB ~ 25%, Kappa ~ 0.47) (Table S4). This difference in performance is probably due to the smaller number of presence data used in the hybrid analysis.

For *L. geoffroyi*, the most important variable was precipitation, with the highest probability of occurrence in areas with low annual precipitation (<1,000 mm). Temperature, solar radiation, elevation and livestock population density were also important for the model. Occurrence frequency was high at mean annual temperature between 13 and 25°C, solar radiation indices above 16,000 kWh/m^{2}a and low elevations (<500 m). For livestock, the model presented high probability of occurrence when livestock population density is around zero, and medium probability when livestock population density is between 70 and 170 individuals/km^2. Cropland and shrubland were also selected in most of the models: the species presents high probability of occurrence in shrubland and areas with low cropland indices (Figure S1).

For *L. guttulus*, solar radiation, temperature, precipitation and elevation presented the largest contributions to the models. This species had high probability of occurrence at solar radiation indices between 13,000 and 15,000 kWh/m^{2}a, annual precipitation levels above 1,800mm and with increasing elevation. Areas with mean annual temperatures between 18 and 21°C had the highest probability of occurrence, and the probability of occurrence dropped dramatically in areas with mean annual temperatures higher than 21°C. Cropland and deciduous forest were positively related with *L. guttulus* habitat selection, whereas the species showed a medium to high probability of occurrence with values of NDVI between 0.45 and 0.55 and increasing probability of occurrence when NDVI values were higher than 0.7 (Figure S2).

Finally, for hybrids, 'cropland areas including remnants of natural vegetation' was the variable with the highest contribution to all models, showing a positive relationship with their probability of occurrence. Tree cover, shrubland and elevation were also selected as important variables. Hybrids had a low probability of occurrence in shrubland areas or in altitudes higher than 500m. The models also indicated that the presence of at least 10% tree canopy cover is essential for hybrid occurrence. However, above this percentage, the probability of occurrence remained very high regardless of the amount of tree canopy cover (Figure S3), suggesting a nonlinear

threshold in canopy cover (e.g. Cushman & Lewis, 2010). Overall, the hybrid random forest models indicated high habitat suitability in the hybrid zone. However, the model predicted that suitable habitat is not restricted to this area, as a high probability of occurrence was also predicted within parental ranges. According to the model, areas suitable to hybrids extend to the south of the hybrid zone, into Uruguay and northeastern Argentina, within the *L. geoffroyi* range; and to the north, surrounding areas predicted as suitable for *L. guttulus*, entering the Cerrado biome, in central Brazil (Figure 3).

3.3 | Niche divergence

Predicted suitable habitats differed between the three groups. The spatial difference in the probability of occurrence demonstrated high divergence in habitat requirements between the parental species, with a small overlapping area. When predicted suitability maps were compared between hybrids and parental species, hybrids presented equal or higher suitability values than parental species within the hybrid zone and in parts of the parental ranges (Figure 4).

Based on the density profile of occurrence points, parental species presented significantly different niches for all the analysed variables. Interestingly, hybrids presented tolerance ranges that were more similar to *L. guttulus* than to *L. geoffroyi*. Of the analysed variables, hybrids presented significantly different niches from both parental species for 'cropland with natural remnants', 'livestock', 'precipitation', 'solar radiation' and 'tree cover'. For 'cropland with natural remnants' and 'livestock', hybrids presented a higher density of occurrence points in higher values of these variables than parental species, which was not observed for the other variables. For the remaining variables, hybrid niches were significantly different from *L. geoffroyi* for three variables (cropland, shrublands and temperature), and from *L. guttulus* for one variable (elevation) (Figure 5).

The background test highlighted the niche difference between parental species, which presented low to moderate levels of niche overlap and niches significantly more divergent than expected by the background environment of either species. The comparison between hybrids and *L. geoffroyi* also indicated significantly divergent niches, but with a slightly higher value of niche overlap. Interestingly, comparisons between hybrids and *L. guttulus* indicated that the niche of hybrids is more divergent from that of *L. guttulus* than expected by the latter's environmental background. However, the niche of *L. guttulus* is neither more nor less divergent from that of hybrids than expected by hybrids' background (Table 2).

4 | DISCUSSION

4.1 | Habitat suitability models for hybrids and parental species

Our results showed parental species and hybrids occupying at least partially different habitat niches and are projected to occur in substantially different geographical areas (Figure 4). Furthermore, our results show that hybrids seem to have a much stronger association with human-altered environments, suggesting they may have higher tolerance and greater ability to persist in anthropogenically modified ecosystems.

For *L. guttulus*, our model indicated that it prefers habitats with high levels of precipitation and high elevation, medium to high levels of solar radiation and medium levels of mean annual temperature, whereas it seems to avoid areas with higher temperatures. These

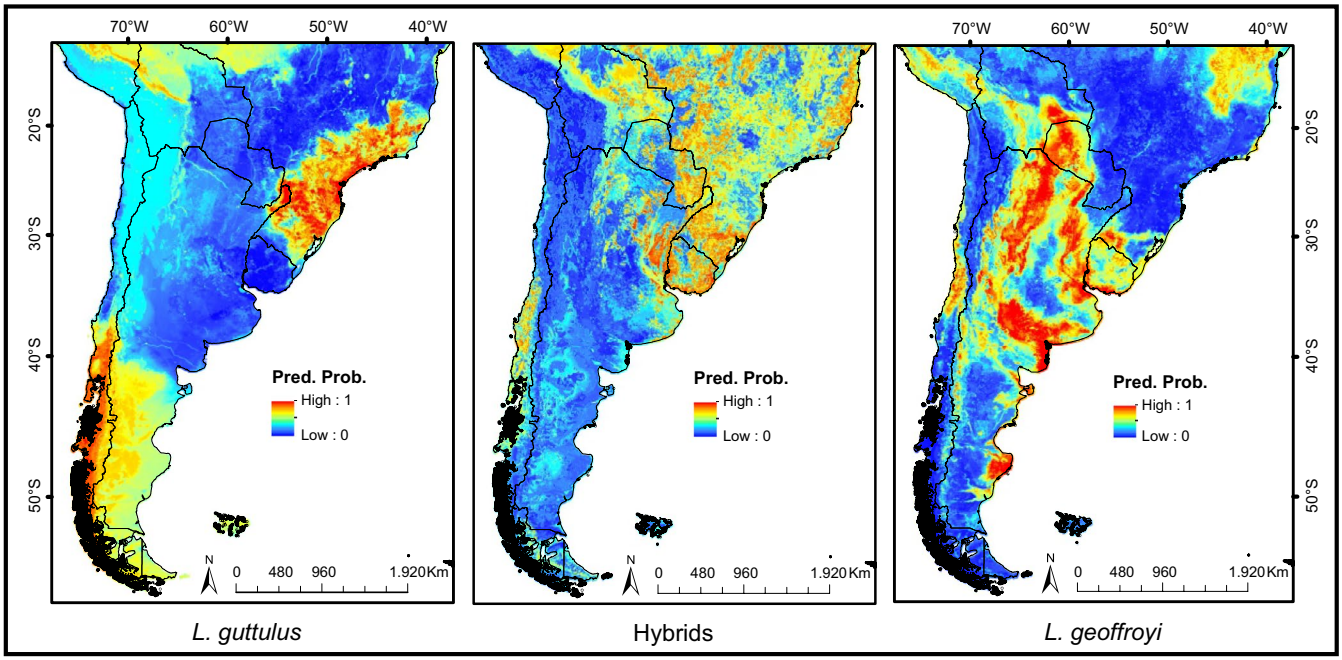

FIGURE 3 Predicted habitat suitability model for *L. guttulus*, *L. geoffroyi* and hybrids generated with the Random Forests algorithm

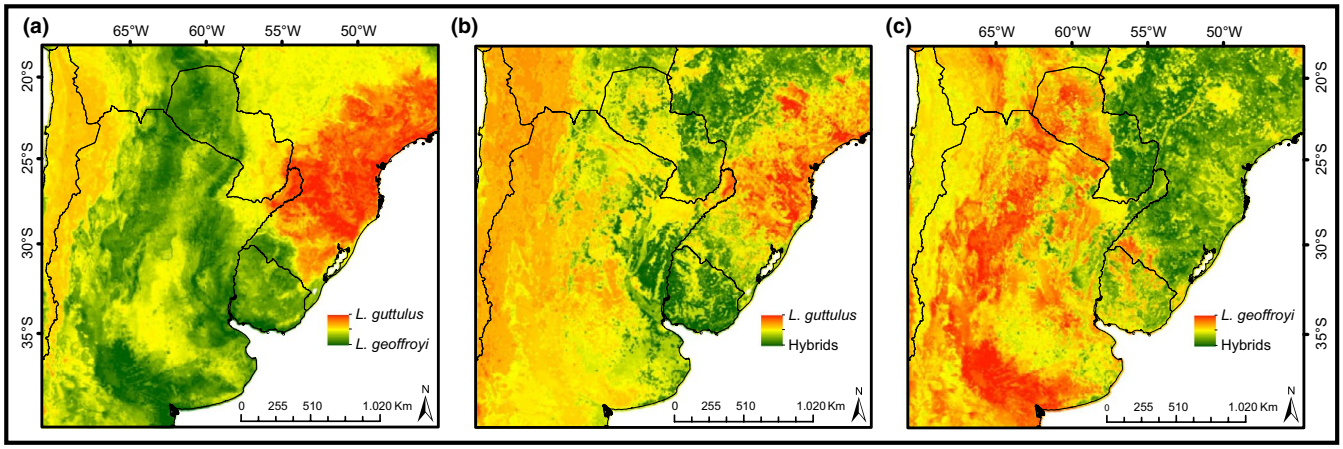

FIGURE 4 Spatial difference of habitat suitability between parental species and hybrids in the hybrid zone and adjacent areas in both directions. Maps were produced by the subtraction of: (a) the predicted probability of *L. geoffroyi* from the predicted probability of *L. guttulus*; (b) the predicted probability of hybrids from the predicted probability of *L. guttulus*; and (c) the predicted probability of hybrids from the predicted probability of *L. geoffroyi*. In (a) darker red colours indicate where the predicted probability of *L. guttulus* is higher than that of *L. geoffroyi*, whereas greener colours indicate where the predicted probability of *L. geoffroyi* is higher. Intermediate colours indicate similar suitability values for both of them. In (b) and (c), darker red indicates areas where parental species have higher occurrence probability than hybrids, whereas greener colours indicate areas that are more suitable for hybrids. Intermediate colours again indicate similar suitability values between the compared groups

attributes highly correlate with the characteristic of the Atlantic Forest (da Fonseca, 1985), suggesting the strong association between this cat and this biome. Although *L. guttulus* is known to tolerate some degree of habitat alteration (de Oliveira et al., 2010), the species does not seem to go far into agricultural lands, being limited by the presence of natural cover (de Oliveira et al., 2016). It is possible that the positive relationship with cropland areas identified here is reflecting the rapid habitat fragmentation in this region as a large proportion of the areas where individuals of *L. guttulus* had been detected have been transformed into cropland. Alternatively, the observed positive correlation may be induced by the presence of forestry areas within the cropland layer used here. It is also possible that *L. guttulus* may is benefiting from the higher prey density along the border areas between croplands and forests, as long as there is sufficient forest cover in the adjacent areas to provide shelter for the felid species (Facure-Giaretta, 2002; Jerosch et al., 2018). Future studies with occupancy models and stable isotopes analyses on whiskers within these areas may help clarify this relationship.

For the other parental species, *L. geoffroyi*, our results suggest that its probability of occurrence is correlated with low levels of annual precipitation, high levels of solar radiation and medium to high annual mean temperatures. Furthermore, the probability of occurrence is correlated with low extent of cropland, although the species seems to tolerate moderate levels of these human-altered environments. The model presented an interesting relationship with livestock, with a very high probability of occurrence when livestock population density is around zero, but with a medium probability of occurrence when cattle population density is between 70–170 individuals/km^2. This result may demonstrate some degree of tolerance by this species to the presence of this human disturbance and is consistent with recent studies that suggest *L. geoffroyi* is tolerant to some degree of habitat alteration, with greater ecological plasticity than many other felid species (Caruso et al., 2016; Cuyckens et al., 2016; Pereira et al., 2011, 2012).

The model obtained for hybrids predicted relatively high suitability in areas far beyond the hybrid zone, and high tolerance to human-altered environments. However, the existence of remnants of natural vegetation within this agricultural matrix, even if small, seems to be essential for hybrid occurrence. This is supported by the selection of tree cover by the model. Specifically, hybrid individuals need at least 10% of tree canopy cover within 8km focal extents to occur, which demonstrates that there is likely a nonlinear relationship between species occurrence and tree cover, with occurrence depending on the presence of at least a low amount of tree cover (e.g. Cushman and Lewis, 2010).

4.2 | Niche divergence

Our results suggest that environmental features play an important role in shaping the spatial dynamics of this hybrid zone. As expected, parental species exhibited different ecological requirements. For both species, precipitation, elevation, solar radiation and temperature were the most important predictors of occurrence, although the direction of influence of the variables on each species was the opposite, suggesting niche divergence in these species' ranges is driven largely by climatic factors. Predicted suitability models of both parental species exhibited suitable areas in the hybrid zone. In this area, the habitat suitability models of parental species seem to partition space such that *L. guttulus* has higher suitability values in the northern and eastern areas of the hybrid zone, and *L. geoffroyi* in the southwestern areas. In addition, the areas of overlap between

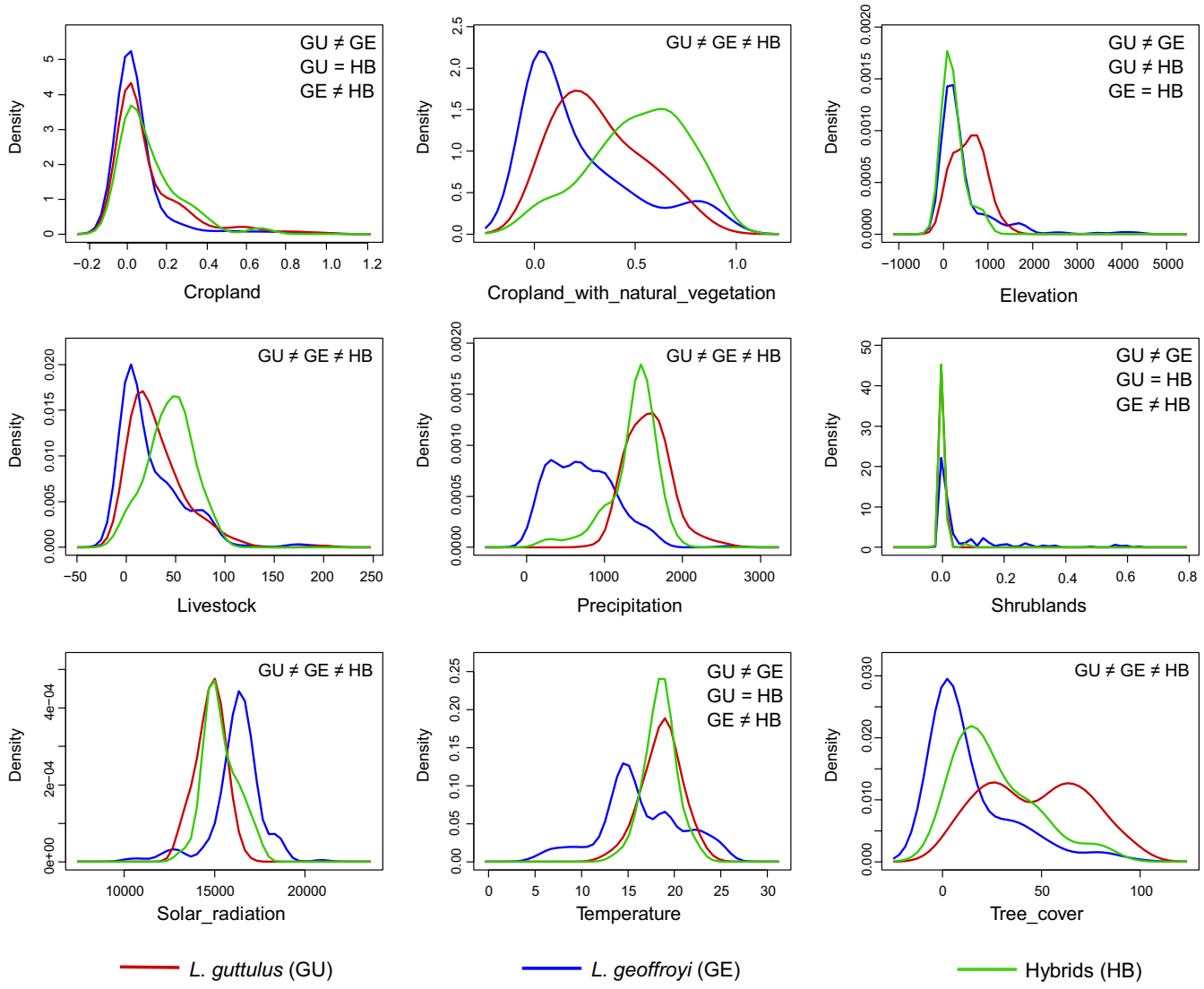

FIGURE 5 Density profile of occurrence points for the three groups (*L. guttulus*, *L. geoffroyi* and hybrids) across the environmental gradient of the most important predictor variables selected in the habitat suitability analysis. Niche differentiation between all pairwise groups is indicated in each plot. Niches were considered significantly different (≠) when $p < .05$

Observed group	Background group	D metric	I metric	More or less similar than expected
L. guttulus	*L. geoffroyi*	0.303	0.623	Less similar ($p < .05$)
L. guttulus	Hybrids	0.609	0.856	NS (D metric: $p = .515$; I metric: $p = .317$)
L. geoffroyi	*L. guttulus*	0.312	0.655	Less similar ($p < .05$)
L. geoffroyi	Hybrids	0.445	0.772	Less similar ($p < .05$)
Hybrids	*L. guttulus*	0.666	0.913	Less similar ($p < .05$)
Hybrids	*L. geoffroyi*	0.630	0.888	Less similar ($p < .05$)

TABLE 2 Comparative niche overlap and tests of background similarity among *Leopardus guttulus*, *Leopardus geoffroyi* and hybrids

Note: D and I correspond to Schoener's and Hellinger's metrics, respectively.

parental species were restricted to the forest-grassland mosaic found in the region. The diversity of habitats within this contact area probably facilitates the coexistence of the parental species, creating opportunities for hybridization. This result, added to the niche divergence detected, offers some support to our hypothesis of exogenous selection shaping the location of the hybrid zone.

On the other hand, according to Swenson (2006, 2008), if the suitable habitat of one of the parental species expands beyond its

occurrence, the hybrid zone is likely maintained by endogenous selection. Our *L. guttulus* model predicted suitable areas further south than its known occurrence, reaching the southeastern portion of Rio Grande do Sul state. Although this region is usually considered a part of the Pampa biome (Oliveira et al., 2017), it presents some areas with similar characteristics to the Atlantic Forest biome, with the presence of forest formations, medium levels of mean annual temperature and higher elevations (Pillar et al., 2009). In fact, this region is sometimes recognized as part of the Atlantic Forest biome due to its characteristics (Muylaert et al., 2018; Ribeiro et al., 2009), and so it is not surprising that it presents favourable environmental conditions for *L. guttulus* occurrence. However, to our knowledge, the only record of the species in the southeastern portions of the state is possibly the result of a wildlife release operation (Eizirik et al., 2006), and therefore, must be considered with caution. As there is no other confirmed record for the species in this region, we believe that environmental factors alone may not limit the occurrence of this cat and the location of the hybrid zone. Intrinsic factors, such as competition between parental species or even with the hybrids and other carnivorans, likely interact with habitat preferences, preventing range expansions and limiting their overlap. This pattern was already suggested by Trigo, Tirelli, et al. (2013) when analysing parental species' prey preferences. They had found considerable overlap between the two species' diet, but with an apparent association with different prey items indicating the possible existence of competitive exclusion.

In addition, the high predicted suitability for hybrids of areas within the range of *L. geoffroyi* and the higher proportion of potential hybrids identified in this region suggest that introgression is being favoured towards the south of the contact zone and may indicate a potential for expansion in this direction. Hybrids presented a different set of environmental requirements from both parental species, with their associations with climatic features resembling those of *L. guttulus* more than those of *L. geoffroyi*. In spite of competition, it is possible that *L. guttulus* individuals, or backcrossed hybrids, disperse to the south of the hybrid zone, favoured by areas with suitable conditions such as those found in southeastern Rio Grande do Sul. In this region, hybrids may occupy areas with characteristics that resemble *L. guttulus* preferences and might even competitively exclude them.

Although hybrids present an environmental niche that is more similar to that of *L. guttulus* than to that of *L. geoffroyi*, the reverse direction of dispersal appears to be uncommon, since neither hybrid occurrence nor its habitat suitability model predicts highly suitable areas within the *L. guttulus* range beyond the hybrid zone. It is possible that the higher elevations in the *L. guttulus*' range contribute to make this area unsuitable for hybrids, since this was the only variable that strongly differentiates *L. guttulus* and hybrids. The lack of suitable conditions for *L. geoffroyi* and hybrids in the northern portion of the hybrid zone seems to limit their dispersal into the *L. guttulus* range. In contrast, the higher habitat suitability for hybrids in a broader area of *L. geoffroyi* occurrence, including southeastern Rio Grande do Sul and other parts of the state as well as Uruguay, may increase the number of hybrid individuals and the strength of genomic introgression in this region. Further studies with genomic data will reveal more information on the extent of hybridization, admixture and integration, which will help us understand the real magnitude of the introgression in this complex hybridization process.

Our models provide evidence to postulate that the hybrid zone is more likely to expand southward because of the high predicted habitat suitability for hybrids in this area. According to our density profile analyses, hybrids showed higher tolerance to habitat alteration than parental species. Previous studies have demonstrated hybrid capacity to occupy altered environments (Grabenstein & Taylor, 2018; Otis et al., 2017), and this seems also to be the case in this hybrid zone. The Brazilian Pampa is a highly fragmented biome, with less than 50% of its natural vegetation remaining, being the conversion of natural areas into livestock or cropland production the main cause of habitat loss (Andrade et al., 2015; Oliveira et al., 2017). Within most of the Brazilian and Uruguayan Pampa, hybrids presented higher suitability values than parental species. In addition, the selection of cropland areas with remnants of natural vegetation as the most important variable in predicting hybrid occurrence might indicate that hybrids are favoured by habitat conversion. Therefore, it is possible that human activities modified the structure of the hybrid zone, increasing hybrid occurrence and the potential of the hybrid zone to expand towards the south.

Overall, our results suggest that exogenous selection is not the only force shaping the spatial dynamics of the *L. guttulus-L. geoffroyi* hybrid zone, and that some level of endogenous selection also may be acting upon it. More importantly, our results suggest that human-induced landscape change appear to be modifying the dynamics of this hybrid zone and widening its occurrence. This has potentially significant consequences for species conservation. Increased hybridization rates might lead to the formation of hybrid swarms, with further dilution of parental groups in areas surrounding the hybrid zone. The selection of hybrids over parental species might also lead to the introgression of hybrid genes into pure populations through backcrossing, reducing parental adaptability to their environment (Allendorf et al., 2001; Grabenstein & Taylor, 2018). Although less likely, it is also possible that increased landscape change leads to the expansion of the hybrid zone towards the area of one of the parental species, or even a new habitat, such as the Cerrado biome, as demonstrated by the hybrids' suitability model.

Finally, due to the high fragmentation of the Pampa biome and its great economic value for agricultural and cattle production, conservation strategies of this biome are focused on the preservation of remnants of natural vegetation within agricultural matrices (Carvalho & Batello, 2009; Oliveira et al., 2017). However, as hybrids seem to be more tolerant of this habitat than *L. geoffroyi*, the preservation of these areas within the agricultural matrix could favour hybrid occurrence, potentially posing an additional challenge to the conservation of *L. geoffroyi's* genomic integrity in the Pampa biome. Thus, restoring neighbouring habitats could be one of the few solutions to reduce or contain the hybrid population. On the other hand, the higher adaptability of hybrids may enable them to persist in regions that are unsuitable for either parental species due to increasing human footprint. Therefore, it may be that maintaining hybrids in

these areas as representatives of the ecological functions performed by parental species may be a relevant conservation strategy, at least in the short term.

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DATA AVAILABILITY STATEMENT

Genotype file has been submitted to FigShare Repository, accessible at https://doi.org/10.6084/m9.figshare.13235537.v1.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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