FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA
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ANÁLISES ECOLÓGICAS DE DUAS ESPÉCIES DE FELÍDEOS (*LEOPARDUS GEOFFROYI* E *L. COLOCOLO*) EM ÁREAS ANTROPIZADAS DA SAVANA URUGUAIA

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Pontifícia Universidade Católica
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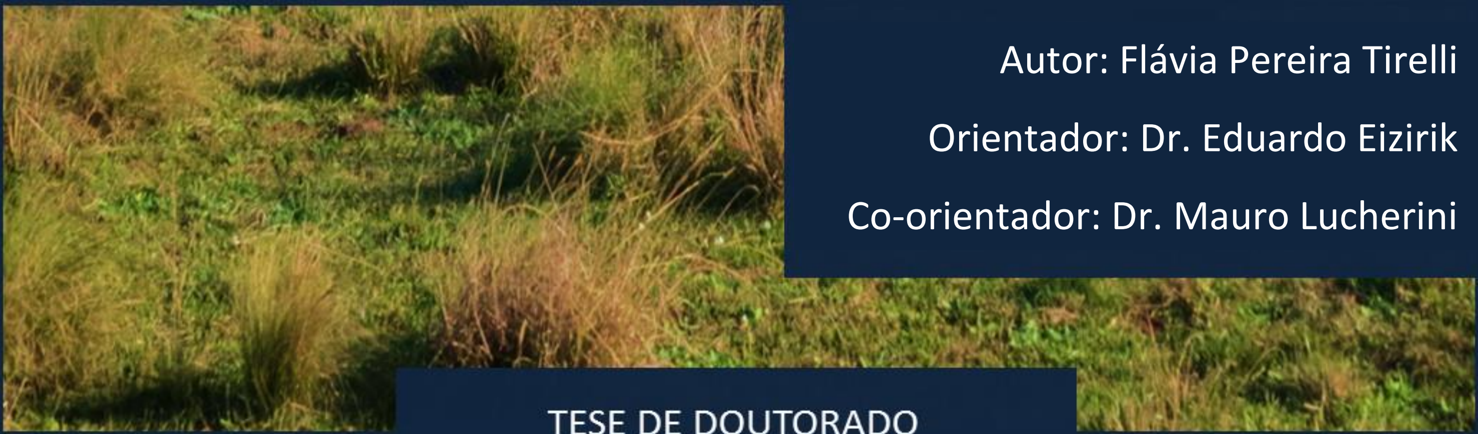

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Tese apresentada como requisito para obtenção do grau de Doutor pelo Programa de Pós-Graduação em Zoologia da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Aprovada em:_____de______________de__________.

Banca examinadora:

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Prof. Dr. Javier Pereira

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Profa. Dra. Maria João Pereira

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Profa. Dra. Sandra Hartz

PORTO ALEGRE – RS- BRASIL
2017

Sumário

À conservação dos felídeos.

Agradecimentos

"Quem caminha sozinho pode até chegar mais rápido, mas aquele que vai acompanhado, com certeza vai mais longe.”

Clarice Lispector

A realização desta tese de doutorado não seria possível sem a participação de inúmeras pessoas, esta é uma conquista de muitos. E sou imensamente grata a cada uma dessas pessoas que em grandes ou pequenas ações contribuíram para tal.

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Resumo

As espécies *Leopardus geoffroyi* e *L. colocolo* são felídeos neotropicais de pequeno porte, típicos de ambientes abertos, que possuem distribuição simpátrica na ecorregião da Savana Uruguaia. Nesta região, *L. geoffroyi* é considerada uma espécie abundante e *L. colocolo* um felídeo raro. O presente estudo teve por objetivo avaliar essas proposições e caracterizar aspectos ecológicos das duas espécies nesta ecorregião ameaçada. O trabalho de campo foi conduzido entre novembro de 2013 e setembro de 2015, em propriedades rurais, na Serra do Caverá, sul do Brasil. Foram instaladas e monitoradas 26 armadilhas-fotográficas e foram realizadas oito campanhas de captura visando à colocação de radio-colares VHF, bem como a coleta de amostras de sangue dos indivíduos de ambas as espécies. Como resultados, foram realizados 516 registros fotográficos de *L. geoffroyi* e foram capturados 12 indivíduos desta espécie, sete dos quais puderam ser monitorados com radio-telemetria. Não foram encontrados registros de *L. colocolo*, indicando a escassez de indivíduos desta espécie na área de estudo. Em relação aos dados de *L. geoffroyi*, observou-se que os machos possuem áreas de vida, de uso e peso corporal maiores do que as fêmeas. Os resultados indicaram que o peso corporal dos machos influenciou positiva e significativamente no tamanho das áreas de vida dos mesmos, relação esta que não ocorreu entre as fêmeas. Também foi observada uma extensa sobreposição de áreas de vida e de uso, sem grandes mudanças sazonais, indicando algum grau de sociabilidade nesta população. Os resultados da análise de parentesco indicaram que a maioria dos indivíduos capturados desta população não eram parentes. Ao mesmo tempo, a relação genética parece não influenciar a sobreposição de área de vida, o que sugere que esta população, na escala estudada, possui uma estrutura sócio-espacial aparentemente distante da proposição tradicional na qual os machos tenderiam a dispersar por maiores distâncias e as fêmeas seriam filopátricas. Além dos padrões sócio-espaciais, este estudo investigou fatores que influenciam na persistência desta espécie na Savana Uruguaia. Foi possível observar que esta população selecionou significativamente mais as áreas de vegetação ripária e evitou as áreas de campos com gado. Cabe ressaltar que a proteção da vegetação ripária é exigida pela legislação nacional brasileira. Assim, este habitat pode fornecer abrigo para *L. geoffroyi* e hipoteticamente poderia servir como corredor ecológico, uma questão importante que precisa ser aprofundada no futuro. Esta população também apresentou padrões noturnos significativos que, consequentemente, evitam o contato humano. A estimativa de densidade foi considerada razoável em comparação com a observada em outras áreas, concordando com a proposição inicial para a espécie na região. Os resultados desses estudos auxiliaram na compreensão das características ecológicas da espécie e dos fatores que auxiliam sua persistência nesta região de paisagens dominadas por humanos. Tais esclarecimentos não foram possíveis para *L. colocolo*, que não foi encontrado na área de estudo. A aparente ausência nesta área, bem como em outras áreas dentro da ecorregião, sugerem preocupação em termos da conservação desta espécie, o que torna-se ainda mais relevante tendo em vista que esta população é considerada uma "Unidade Evolutivamente Significante" (UES) distinta. Assim, o quarto capítulo da tese objetivou estimar a distribuição espacial atual e o *status* de conservação da UES de *L. colocolo* presente na Savana Uruguaia. Foram coletados 107 registros da espécie e foram construídos dois modelos (incluindo ou não variáveis antrópicas), cada deles sendo gerado com dois algoritmos (Maxent e Maxlike). Os modelos resultaram em distribuições similares, indicando as áreas de campo com altitudes entre o nível do mar e 400m como aquelas apresentando mais elevada adequabilidade ou maior probabilidade de ocorrência (PO). Os modelos que apresentaram melhor desempenho foram os que incluíam as variáveis antrópicas. Esses modelos geraram distribuições mais restritas das áreas de maior adequabilidade e PO, apoiando a conclusão que essa população de *L. colocolo* é afetada por perturbações humanas. A estimativa do tamanho populacional presente nas áreas de maior adequabilidade e PO resultaram, respectivamente, nas categorias "Criticamente em Perigo" e "Em Perigo" para esta UES. Destaca-se, portanto, a necessidade urgente de planos de conservação para esta unidade populacional de *L. colocolo*. O estudo, em sua totalidade, observou que espécies de felídeos com características tradicionalmente similares respondem de forma diferente aos impactos antrópicos. Os resultados obtidos podem contribuir para o desenho de ações de manejo e conservação a fim de assegurar a sobrevivência, em longo prazo, das duas espécies nesta ecorregião ameaçada.

Palavra-chave: conservação, felídeos, Pampa.

Abstract

The Geoffroy's cat (*Leopardus geoffroyi*) and the pampas cat (*L. colocolo*) are small Neotropical felids that typically occur in open landscapes. These species are distributed simpatricaly in the Uruguayan Savannah ecoregion. In this region, Geoffroy's cat and pampas cat are considered, respectively, to be abundant and rare species. The present study aimed to evaluate these propositions and to characterize ecological aspects of these species in this threatened ecoregion. The study was conducted from November 2013 to September 2015 in privately owned areas in "Serra do Caverá", southernmost Brazil. We installed and monitored 26 camera-traps and we performed eight capture campaigns aiming to fit individuals of both species with VHF radio-collars and to collect blood samples. As a result, we obtained 516 image records of Geoffroy's cat and captured 12 different individuals of same species, seven of which were monitored by radio-telemetry. We did not find any record of pampas cat, which demonstrated the rareness or absence of the species in the study area. From the Geoffroy's cat data, we observed that males had home-range (HR), core-area and body weight that were larger than those of females. We also observed that male body weight significantly and positively influenced HR size, whereas in females such relationship was not significant. We observed extensive HR and core area overlap, with minor changes in overlap between seasonal partitions. These results indicate some degree of sociability in this population. Interestingly, our kinship analyses indicated that the majority of the cats sampled in this population were unrelated, and that genetic relatedness did not significantly influence the levels of HR and core area overlap. This indicated that the population, in the studied scale, has a socio-spatial structure that departs from what could be expected based on a model assuming male dispersal and female philopatry. Additionally to the socio-spatial patterns, we also investigated factors that influence the persistence of the species in the threatened Uruguayan Savannah. We observed that this population significantly selected riparian vegetation and avoided open grassland with cattle. It is noteworthy that the protection of riparian vegetation is required by Brazilian national law. Hence, this habitat may provide shelter for Geoffroy's cats, and hypothetically it could serve as an ecological corridor for the species, an important issue that should be tested deepened in the future. This population also presented significantly nocturnal activity patterns, which consequently avoids human contact. The density estimation was considered reasonable in comparison to other areas within the Geoffroy's cat range, agreeing with the initial estimate for the species in the region. In these studies, we obtained insights that help us understand some ecological characteristics of the species, and to assess the bases for its persistence in these human-dominated landscapes. Such insights could not be obtained for the pampas cat, as it seems to be currently absent from the study site. Its absence or rarity at this site and several others within the ecoregion is a worrisome observation, from a conservation perspective. This is especially the case given that the Uruguayan Savannah contains a distinct 'Evolutionarily Significant Unit' (ESU) of pampas cats. Therefore, in the fourth chapter, we aimed to assess the current spatial distribution and conservation status of the pampas cat in the Uruguayan Savannah. We collected 107 spatial records and constructed two models (with and without anthropogenic variables), each of them built separately with two different algorithms (Maxent and Maxlike). All models were very similar, indicating higher suitability (or probability of occurrence [*PO*]) in grassland areas from sea level to 400m of altitude. The best-fit models were those including anthropogenic variables, which also yielded a more restricted distribution of higher suitability and higher PO areas, supporting the conclusion that this pampas cat population is affected by human disturbance. The estimation of the population size for high-suitability and high-PO areas resulted in assignment to the "Critically Endangered" and "Endangered" categories, respectively. Based on these results, we highlight the urgent need for conservation plans targeting pampas cats in the Uruguayan Savannah. In the global study, we observed that similar felid species seem to respond differently to human impacts, and obtained results that can contribute to the design of conservation actions aiming to ensure the long-term survival of these two species in this threatened ecoregion.

Key-words: conservation, felids, Pampa.

Apresentação

Este estudo teve por objetivo compreender questões ecológicas básicas de duas espécies de felídeos (*Leopadus geoffroyi* e *L. colocolo*) na Savana Uruguaia. A estrutura geral da tese foi baseada nas normas da revista científica ‘Animal Conservation’. Cada capítulo desta tese de doutorado, com exceção dos ‘Capítulo 1 – Introdução Geral’ e ‘Capítulo 5 – Conclusões Gerais’, é constituído de um artigo a ser submetido para revistas científicas distintas. Assim, cada capítulo referente a um manuscrito está de acordo com as normas das revistas às quais estes serão submetidos (Apêndices 1, 2 e 3). O ‘Capítulo 2 – *Spatial structure and social dynamics of Geoffroy's cat individuals in the Brazilian pampas*’ será enviado para a revista ‘Journal of Zoology’ (Apêndice 1), o manuscrito referente ao ‘Capítulo 3 - *An assessment of factors underlying the persistence of Geoffroy's cat populations in human*-*dominated landscapes in the Brazilian pampas*’ será submetido para ‘Animal Conservation’ (Apêndice 2) e o ‘Capítulo 4 - *Distribution modelling and conservation assessment of the Pampas cat (Leopardus colocolo) in the Uruguayan Savannah'* será enviado para a revista “Oryx – The International Journal of Conservation’ (Apêndice 3).

Capítulo 1 – Introdução Geral

Felídeos: características & conservação

A família Felidae (Mammalia: Carnivora), atualmente, é dividida em 11 gêneros e 38 espécies (Johnson *et al*., 2006; Macdonald, Loveridge & Nowell, 2010; Trigo *et al*., 2013; Kitchener *et al*., 2017). Esta família de carnívoros obrigatórios ocorre naturalmente em quase todos os continentes do planeta, com exceção da Austrália e Antártida. No entanto, mesmo em escala global, as áreas de distribuição geográfica e o tamanho populacional das espécies de felídeos estão diminuindo (IUCN Red List of Threatened Species, 2017). Isto se deve ao aumento demográfico humano, que implica a crescente utilização de ambientes naturais para implementação de agricultura, rodovias e centros urbanos, criando uma matriz antrópica com fragmentos isolados de remanescentes naturais (Butchart *et al*., 2010; Rands *et al*., 2010; Loveridge *et al*., 2010; Li *et al*., 2016). Esta fragmentação de hábitats influencia no aumento dos conflitos entre felídeos e humanos. A proximidade destas espécies pode ocasionar, por exemplo, esporádicas predações por parte dos felídeos aos animais domesticados, sendo a consequência disso normalmente a caça por retaliação (Loveridge *et al*., 2010). A caça comercial e esportiva e os atropelamentos também são motivo de diminuição populacional e extinção local de espécies de felídeos (Sunquist & Sunquist, 2002; Loveridge *et al*., 2010). Estas pressões antrópicas sobre as populações de felídeos podem ocasionar a extinção destas espécies predadoras e, como consequência, causar grandes desequilíbrios no ambiente. Assim, o entendimento de como as populações de felídeos estão respondendo aos processos de degradação do ambiente e a compreensão dos tipos de conflitos com humanos existentes em diferentes regiões podem auxiliar na mitigação dos impactos antropogênicos e contribuir para a sobrevivência das espécies pertencentes a esta família.

As espécies de felídeos apresentam características distintas em relação aos tamanhos corporais, às presas, à área de vida e às densidades populacionais. A maior espécie de felídeo do mundo é o tigre (*Panthera tigris*), com peso entre 121 e 225 kg;

sua base alimentar é compreendida por ungulados, sendo capazes de predar até rinocerontes e elefantes asiáticos adultos (Sunquist & Sunquist, 2002); a área de vida pode variar entre 20 e 1.379 km², e a densidade entre 0,13 e 11,65 indivíduos por 100 km²; atualmente, existem entre 3.500 – 5.000 tigres na natureza (Macdonald *et al.*, 2010). Já a menor espécie desta família é o gato-vermelho-malhado (*Prionailurus rubiginosus*) que possui peso corporal variando entre 0,8 e 1,6 kg; alimenta-se de pequenos roedores e anfíbios; seu tamanho de área de vida e a densidade são desconhecidos (Macdonald *et al.*, 2010), e estima-se que existam menos de 10.000 indivíduos na natureza (Khan & Mukherjee, 2008). Estes dois extremos exemplificam a diversidade e o conhecimento científico acerca das espécies na família. Aquelas de grande porte tendem a receber maior atenção nas pesquisas, possivelmente devido ao seu apelo carismático, aos conflitos com humanos levando a um alto grau de ameaça de extinção, à sua mais fácil observação, elementos estes que facilitam a obtenção de financiamento para estudos científicos. Por sua vez, os felídeos de pequeno e médio porte, mais de 86% das espécies que constituem esta família, possuem um número consideravelmente menor de publicações científicas por espécie. Em particular, os gatos de menor porte (<10 kg) da América do Sul e Ásia, representando cerca de 45% das espécies, são os menos estudados mundialmente (Macdonald *et al.*, 2010).

O gênero *Leopardus* é exclusivo da região neotropical e é constituído por oito espécies reconhecidas atualmente (Johnson *et al.*, 2006; Trigo *et al.*, 2013; Kitchener *et al.*, 2017). Dentre estas, encontram-se *L. geoffroyi* e *L. colocolo*, felídeos de pequeno porte que apresentam características ecológicas relativamente similares. Ambos possuem simpatria em parte de suas distribuições geográficas, e são caracterizados por ocorrer tipicamente em ambientes abertos. No entanto, estas espécies apresentam particularidades que afetam seus *status* de conservação e sua sobrevivência na matriz antrópica onde habitam.

Leopardus geoffroyi (d'Orbigny and Gervais, 1844)

O gato-do-mato-grande, *L. geoffroyi*, possui porte semelhante ao gato-doméstico (Fig. 1a), apresentando um peso médio de 4,26 kg (Lucherini *et al.*, 2006), porém com cauda mais curta e cabeça mais robusta. Sua pelagem de fundo é constituída de tonalidades diferentes de cinza até uma coloração mais amarelo-ocráceo, possuindo pintas que não formam rosetas. Formas melânicas são comuns nesta espécie (Sunquist & Sunquist, 2002; Oliveira & Cassaro, 2006; Schneider *et al.*, 2015). A espécie distribui-se desde a Bolívia e o Chaco paraguaio até o sul do Chile, cobrindo praticamente toda a Argentina, o Uruguai e parte do Rio Grande do Sul (Sunquist & Sunquist, 2002; Macdonald & Loveridge, 2010; Cuyckens *et al.*, 2015; Pereira, Lucherini & Trigo, 2015) (Fig. 1b). Este felídeo, como citado anteriormente é associado a ambientes abertos, como campos (Sunquist & Sunquist, 2002). Apesar disso, *L. geoffroyi* pode ser encontrado em áreas de mais densa vegetação (Johnson & Franklin, 1991; Manfredi *et al.*, 2012), existindo ainda dúvidas sobre a real preferência de hábitats desta espécie.

Este felídeo parece possuir hábitos noturnos (Johnson & Franklin, 1991; Manfredi *et al.*, 2011); contudo, em períodos de escassez de presas, a espécie pode alterar seus hábitos (Pereira, 2010). A densidade pode variar de 9 – 42/100 km^2, no Chaco boliviano (Cuellar *et al.*, 2006) a 16 – 45/100 km^2, na Argentina Central (Caruso *et al.*, 2012). Em região próxima, neste mesmo país, a densidade pode se tornar extremamente alta (100 – 290/100 km^2) devido à presença de indivíduos transientes (Pereira *et al.*, 2012). O tamanho de área de vida e a relação com o peso corporal tanto em machos quanto em fêmeas parece variar entre os estudos; entretanto, isto nunca foi comparado estatisticamente. Os estudos com esta espécie sugerem que ela parece tolerar, em certo nível, áreas antropizadas rurais (Pereira *et al.*, 2011, 2012), demonstrando um comportamento plástico (Pereira *et al.*, 2012).

Historicamente, indivíduos dessa espécie foram caçados em grande escala. Na década de 70, uma média anual de 116.000 peles eram exportadas da Argentina, reduzindo, durante os anos 80, para o ainda impressionante número de 55.000 peles/ano, até que, em 1992, tenha se dado a proibição da caça comercial naquele país (Nowell & Jackson, 1996). Entretanto, o gato-do-mato-grande ainda é morto devido à sua predação sobre aves domésticas, além de sua pele ser ainda possivelmente comercializada de forma ilegal (Macdonald & Loveridge, 2010). Esta espécie é considerada globalmente como apresentando “Menor Preocupação” por ser ainda relativamente abundante e amplamente distribuída (Pereira *et al.*, 2015).

Figura 1. *Leopardus geoffroyi*. **a.** Imagem de um exemplar da espécie. **b.** Distribuição geográfica definida pela cor azul.

Leopardus colocolo (Molina, 1782)

O gato-palheiro, *L. colocolo*, é morfologicamente semelhante ao gato-doméstico, devido ao seu tamanho (peso médio de 4 kg) (Silveira *et al*., 2005), pelos longos, orelhas pontiagudas e cabeça com face mais larga. Apresenta pelagem de coloração variada, com seis padrões diferentes, desde tons acinzentados a tons marrom-avermelhados, podendo ou não apresentar rosetas. Porém, a principal característica da pelagem encontra-se nas patas (membros), com listras escuras e largas (duas ou três nas anteriores e três a cinco nas posteriores) e pés parcialmente ou totalmente negros (Fig. 2a) (Oliveira & Cassaro, 2006); também apresenta forma melânica, com forte evidência de seleção natural favorecendo o melanismo no Brasil central (Silveira *et al*., 2005; Schneider *et al*., 2015). A espécie ocorre dos Andes do Equador e Peru até o extremo sul do continente. A distribuição no território brasileiro ainda é incerta, porém sabe-se que ocorre nos biomas Pampa, Cerrado e Pantanal (Fig. 2b) (Sunquist & Sunquist, 2002; Oliveira & Cassaro, 2006). Encontra-se em uma variedade de ambientes, desde savanas alagadas (Pantanal) até regiões de clima frio semiárido (deserto da Patagônia) (Sunquist & Sunquist, 2002) e mesmo em ambientes extremos como nas montanhas andinas, onde registros evidenciam ocorrência desta espécie a 5000m de altitude (Nowell & Jackson, 1996).

Este pequeno felídeo é considerado tradicionalmente uma espécie de hábito noturno (Sunquist & Sunquist, 2002; Oliveira & Cassaro 2006), porém um trabalho realizado com radiotelemetria no Cerrado brasileiro verificou hábito variado nos espécimes estudados, com picos de atividade entre 8 - 10h e entre 18 - 20h; este mesmo estudo estimou a área de vida média como sendo 19.47 km² (Silveira *et al.*, 2005). Nos Andes, um estudo apresentou maior proporção de atividade (71,1%) durante a noite (Lucherini *et al.*, 2009) e a média da área de vida para esta região foi estimada em 14.90 km² (Tellaeche, 2015). Quanto à densidade, esta variou de 1 a 5 indivíduos/ 100 km² no pampa brasileiro (Oliveira *pers. comm.* in Queirolo *et al.* 2013); 2 a 10 indivíduos/ 100 km² no Cerrado (Silveira, em prep.); 11 a 17 indivíduos/ 100 km^2, no centro da Argentina (Caruso *et al.*, 2012); e 74-79 indivíduos/ 100 km², nos Andes (Gardner *et al.*, 2010). A espécie parece ter padrões ecológicos e comportamentais distintos em cada região; além dos fatores externos (incluindo competição com outros felídeos), isto poderia estar relacionado com características genéticas próprias de cada população.

A classificação de *L. colocolo* vem sendo discutida ao longo das últimas duas décadas. Dados moleculares apoiam a existência de uma única espécie com alta estruturação geográfica, que pode ser interpretada como compreendendo várias subespécies (Masuda *et al.*, 1996; Johnson & O’Brien ,1997; Pecon-Slattery & O’Brien, 1998; Johnson *et al.*, 1999; Johnson *et al.*, 2006; O’Brien & Johnson, 2007; Napolitano *et al.*, 2008; Cossíos *et al.*, 2009; Santos, 2012; Sartor, 2016) ao passo que dados morfológicos sugerem que o grupo compreenda três espécies distintas, *L. colocolo, L. pajeros e L. braccatus* (Garcia-Perea, 1994), ou até mais espécies (Nascimento 2010). Santos (2012), realizou um estudo filogeográfico de *L. colocolo* com base em genes do DNA mitocondrial, demonstrando a existência de diferenciação genética entre as populações do centro-oeste brasileiro e a população sul-brasileira-Uruguaia, com ausência de fluxo gênico matrilinear (DNAmt) recente entre estas unidades. Além disso, um estudo realizado com marcadores de microssatélites (Sartor, 2016) suportou os resultados de Santos (2012). Estes estudos moleculares sugerem que população de *L. colocolo* existente na ecorregião da Savana Uruguaia (Olson *et al*., 2001) - sul do Brasil (estado do Rio Grande do Sul) e Uruguai - é uma Unidade Evolutivamente Significativa (‘Evolutionarily Significant Unit’ [ESU]). Este termo é relevante no contexto de planos de conservação para populações geneticamente distintas, independentemente de controvérsias acerca de sua classificação taxonômica.

A perda de hábitat e a caça são os maiores fatores de ameaça da espécie *L. colocolo*. Entre 1977 e 1979, foram exportadas 78.239 peles deste felídeo partindo de Buenos Aires, Argentina (Sunquist & Sunquist, 2002). Além disso, a caça em retaliação à predação de aves domésticas é uma cultura tradicional nos Andes (Macdonald & Loveridge, 2010). O gato-palheiro é listado globalmente como “Quase Ameaçado” (Lucherini *et al*., 2016).

Figura 2. *Leopardus colocolo* **a.** Imagem de um exemplar da espécie. **b.** Distribuição geográfica definida pela cor verde.

A ecorregião da Savana Uruguaia é caracterizada por campos subtropicais (WWF, 2016) constituídos por uma grande diversidade de espécies de gramíneas e de outras famílias florais. Os campos são muitas vezes divididos pela vegetação ripária, que pode ser composta por vegetações arbustivas ou por matas mais densas. Apesar de parecer uniforme, a ecorregião é formada por um mosaico complexo de formações vegetacionais resultantes dos distintos relevos, pluviosidades, tipos de solo, entre outros fatores (Hasenack *et al*., 2010). Devido a algumas destas características, a Savana Uruguaia é uma região altamente explorada pela economia humana, tendo como principais atividades a pecuária, a agricultura e a silvicultura (estas últimas, normalmente, sendo utilizadas em sistema de monocultura) (Martino, 2004; MMA, 2007). Em decorrência disto, esta ecorregião apresenta poucas áreas de fragmentos naturais remanescentes, sendo atualmente considerada “Crítica/ Em Perigo” (Loyola *et al*. 2009; WWF, 2016). Além disso, de acordo com os “padrões de distribuição de espécies de vertebrados terrestres” a Savana Uruguaia é indicada como uma das áreas de mais alta prioridade para conservação na região Neotropical (Loyola et al., 2009).

Os felídeos *L. geoffroyi* e *L. colocolo* ocorrem em simpatria na Savana Uruguaia. A primeira espécie é considerada relativamente comum e abundante (T. Trigo *pers. obs*. 2014, in Pereira *et al*., 2015), enquanto a segunda é considerada rara (Oliveira *pers. comm*. in Queirolo *et al.* 2013) na região. Ao se observar a compilação de dados sobre cada uma das duas espécies, é possível constatar que quase nada se sabe sobre sua ecologia nesta ecorregião. São necessários estudos ecológicos com focos comportamentais, espaciais e de estimativas populacionais para que sejam abordadas questões importantes para o entendimento destas espécies e a viabilização de sua conservação na região. Esta carência de informação se reflete na avaliação do *status* de conservação destas espécies. Apesar de constarem em listas de espécies ameaçadas, é difícil dizer ao certo, atualmente, quais são as suas principais ameaças e seu verdadeiro *status* na natureza. Neste sentido, o presente estudo inclui a investigação de questões complexas sobre processos ecológicos envolvendo cada uma das espécies, com direta aplicação na conservação e manejo destes táxons. Os dados gerados no presente estudo servirão de subsídios para o delineamento de estratégias de manejo para garantir a sobrevivência destes felídeos em longo prazo.

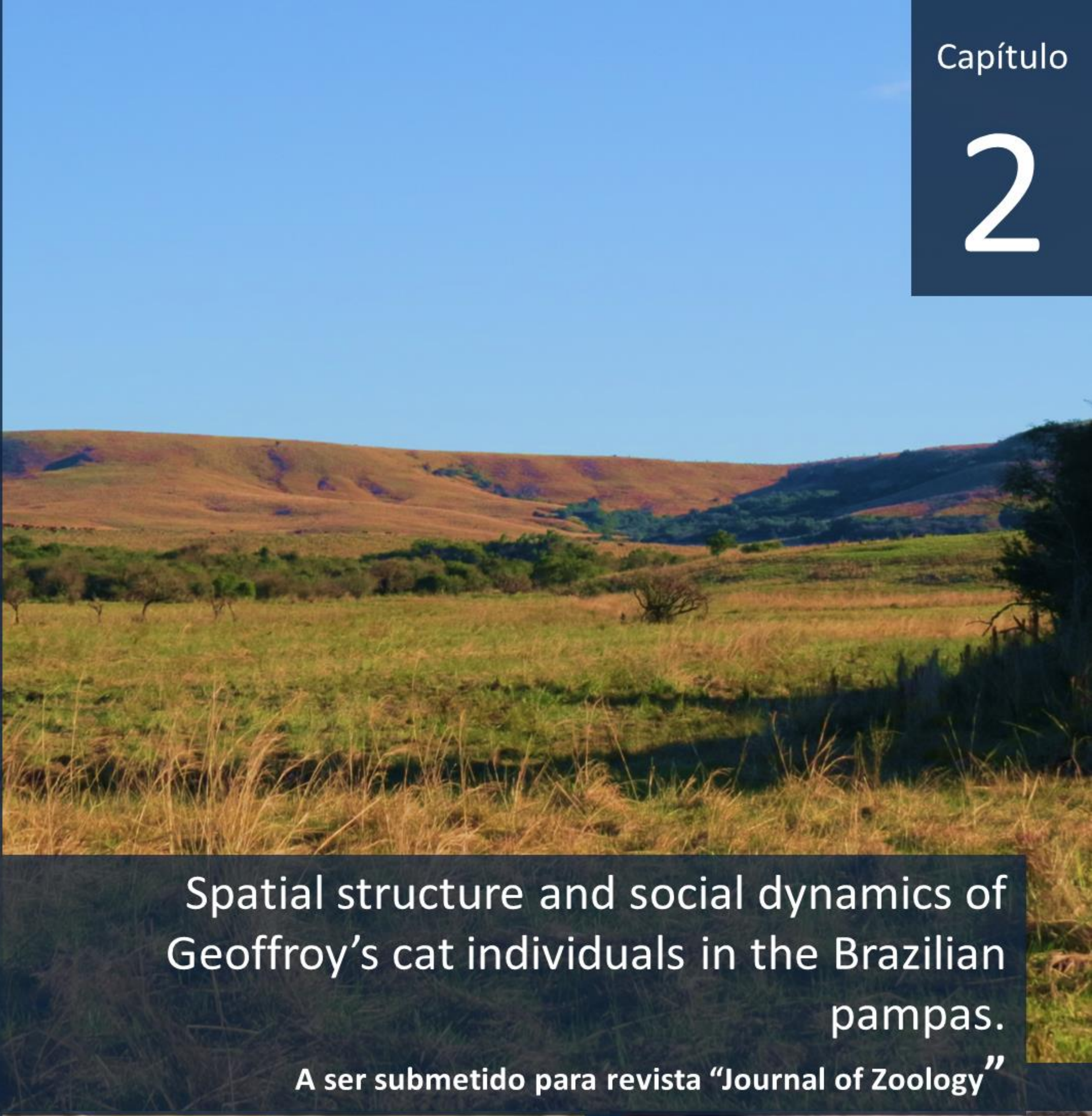
Objetivos

Objetivo geral

Caracterizar aspectos ecológicos de *Leopardus geoffroyi* e *L. colocolo* na ecorregião da Savana Uruguaia, preenchendo lacunas básicas no conhecimento sobre estas espécies nesta região, bem como realizando análises avançadas com focos específicos em cada uma delas.

Objetivos específicos:

1. Investigar a presença de *Leopardus geoffroyi* e *L. colocolo* em uma área de estudo focal, localizada próximo ao centro da Savana Uruguaia.
2. Realizar uma análise detalhada do tamanho da área de vida e estrutura sócio-espacial da população de *Leopardus geoffroyi* presente na área focal.
3. Avaliar a densidade populacional de *Leopardus geoffroyi*, bem como o seu uso da paisagem e o padrão de atividade de indivíduos presentes na área focal.
4. Estimar a distribuição potencial de *L. colocolo* na Savana Uruguaia, analisando sua relação com variáveis de paisagem naturais e antropogênicas, a fim de embasar estratégias de conservação regionais com foco nesta espécie.



Capítulo

2

Spatial structure and social dynamics of Geoffroy’s cat individuals in the Brazilian pampas.

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Spatial structure and social dynamics of Geoffroy's cat individuals in the Brazilian pampas

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Abstract

Geoffroy’s cat (*Leopardus geoffroyi*) is a small solitary Neotropical felid whose social behaviour remains poorly known. In the present study, based on simultaneous radio-telemetry and camera-trapping data, we examined the spatial structure and dynamics of a Geoffroy’s cat population located in the Uruguayan Savannah ecoregion, including the first assessment of inter-individual genetic relatedness for this species. Additionally, we compared our results on home range (HR) sizes and body weight with those reported in previous studies. We found that male HRs and core areas were larger than those of females (a significant difference with five out of 10 estimators), and that males were also significantly heavier than females. Linear regression analyses indicated that male body weight significantly influenced HR size, whereas in females such relationship was not significant. When we integrated our data with those reported previously for other study sites, we observed no significant relationship between male body weight and HR size when different regions were compared, suggesting that the underlying process acts most locally. These findings support the conclusion that sexual dimorphism driven by male-male competition is an important component of the biology of this species. Interestingly, we observed extensive HR and core area overlap among most of the monitored individuals, with no clear gender-based pattern. Moreover, our molecular data indicated that most of the sampled individuals were unrelated, and that the levels of HR and core area overlap were not significantly influenced by genetic relatedness. These results suggest some degree of sociability in this species, beyond what could be expected based on a model assuming female philopatry and male-biased dispersal, at least on the assessed scale. We detected only minor changes in HR and core area size and overlap between seasonal partitions, indicating that the spatial structure we observed is temporally stable. On a broader perspective, our comparisons illustrate the usefulness of performing multiple ecological studies employing comparable methods at different sites, to better understand the ecology of wild felid populations worldwide.

Keywords: *Leopardus geoffroyi*, home range overlap, body weight, kinship.

Introduction

The spatial structure and dynamics of related and unrelated individuals of terrestrial Carnivora species are often influenced by intraspecific competition (Macdonald, 1983). Within carnivores, individuals may have exclusive territories or overlap considerably in their use of space, depending on the abundance and distribution of resources (Johnson *et al*., 2002; Duncan *et al.,* 2015), on fitness benefits or costs of defending these resources (Macdonald, 1983), as well as on inbreeding avoidance strategies (Schmidt *et al*., 2016). Kinship dynamics of both solitary and group living species are usually based on female philopatry and male-biased dispersal (Waser & Jone, 1983; Gompper & Wayne, 1996) and may strongly affect their spatial organization (Macdonald, 1983). For solitary carnivores, kinship influence on spatial patterns has been observed in raccoons (*Procyon lotor*; Ratnayeke *et al*., 2002), bobcats (*Lynx rufus*; Janecka *et al*., 2006), black bears (*Ursus americanus*; Costello *et al*., 2008), and ocelots (*Leopardus pardalis*; Rodgers *et al*., 2015). However, some studies with pumas (*Puma concolor*; Nicholson, Krausman & Munguia-Vega, 2011; Elbroch *et al*., 2015) failed to find an effect of relatedness on spatial organization. Given the scarcity of studies performed so far, and the variability of their approaches and results, it is early to draw conclusions on general patterns, and necessary to pursue such analyses in additional species.

Most felids seem to present non-cooperative breeding strategies, with a social organization centred on individual territoriality (Caro, 1989; Sunquist & Sunquist, 2000). The majority of studies on felid social structure has so far focused on large cats, with very little information presently available for the more numerous small-bodied species (Macdonald, Loveridge & Nowell, 2010). The absence of combined spatial and kinship data is even worse, with 86.5% of extant felid species remaining completely unexplored in this regard (Macdonald, Mosser & Gittleman, 2010). This lack of knowledge hampers our understanding of the evolutionary dynamics of most felids globally, and is also an issue in the context of identifying, monitoring and mitigating the threats imposed on them by human activities.

Among the small Neotropical felids, Geoffroy's cat (*Leopardus geoffroyi*) has been the focus of a relatively large amount of studies addressing spatial dynamics (Johnson & Franklin, 1991; Manfredi *et al.*, 2006; Pereira *et al.*, 2006; Castillo *et al.*, 2008; Pereira *et al.*, 2012; Manfredi *et al*., 2012). Although comparing these studies may allow some of the first insights into regional variation in spatial patterns in a Neotropical felid, much of the biology and spatial ecology of this species remains to be characterized. Geoffroy's cat is distributed from Bolivia and southernmost Brazil to the southern tip of Chile and Argentina (Macdonald & Loveridge, 2010; Cuyckens *et al*., 2015), having been recorded in 22 different ecoregions (Fig. 1a). Of the six studies published so far that reported home range sizes for this species (Johnson & Franklin, 1991; Manfredi *et al.*, 2006; Pereira *et al.*, 2006; Castillo *et al*., 2008; Pereira *et al.*, 2012; Manfredi *et al*., 2012), five were performed in Argentina and one in Chile, altogether covering three ecoregions (Magellanic subpolar forest, Humid Pampas and Low Monte-Espinal) (Olson *et al*., 2001) (see Fig.1a).

Geoffroy's cat is considered a solitary felid (Ximenez, 1973), and previous studies have indicated that its home range size may vary considerably, likely influenced by sex, body size, landscape features and/or prey availability. Males tend to have larger home ranges and move farther than females (Johnson & Franklin, 1991; Manfredi *et al*. 2006, 2012). Interestingly, some studies have reported that males maintained their home ranges for 3–5 months before abandoning them (Johnson & Franklin, 1991; Pereira *et al*., 2006). In certain areas, no overlap was found among male home ranges (Johnson &

Franklin, 1991), while in others such overlap was extensive (Manfredi *et al.*, 2006). According to Manfredi *et al.* (2006) and Pereira *et al.* (2006), female home range sizes varied according to prey availability; hence, the degree of home range overlap fluctuated seasonally. Even with this fluctuation, females seemed to maintain their general home range in the same area (Johnson & Franklin, 1991). Pereira *et al.* (2006, 2012) observed that the level of spatial overlap was high in a protected area, with some individuals partially sharing core areas, whereas in privately owned ranches only a few individuals showed home range overlap. According to those authors, these discrepancies may reflect a reaction to human-induced habitat alteration, which was considered intense in the ranches (with 9 to 21 cattle heads per km^2) and probably affected negatively prey abundance; in response to that, males would have increased their level of territorial exclusion of other males.

Although variation in body mass has been reported to influence home-range (HR) size in this species (Pereira *et al.*, 2006), the connection between these variables has so far not been tested statistically for any site, precluding in-depth assessments of local and regional variations in their relationship. Previous studies (Johnson & Franklin, 1991; Lucherini *et al.*, 2006, Pereira *et al.*, 2006) have reported Geoffroy's cat body masses from several sites (Fig. 1a), and provided strong evidence for sexual size dimorphism in this species in all the surveyed areas (Lucherini *et al.*, 2006; Pereira *et al.*, 2006). Some regional differences in body mass and their relationship to HR size variation have been observed and discussed (Lucherini *et al.*, 2006; Pereira *et al.*, 2006), laying out interesting hypotheses that can be further explored with additional data. Furthermore, kinship relationships have never been investigated in this species, hampering an assessment of their influence on spatial dynamics.

Therefore, in the present study, we examined the spatial structure of a Geoffroy’s cat population in the Uruguayan Savannah ecoregion, and compared it with previous studies focusing on this species. Specifically, we studied the size and inter-individual overlap of Geoffroy’s cat home ranges at our study site, aiming to understand whether they are affected by sex and body weight, as well as by the genetic relatedness among individuals. Additionally, we reviewed all the information available on the spatial dynamics of Geoffroy’s cat populations, aiming to achieve more general conclusions about the factors driving the observed patterns.

Material and Methods

Study area

We conducted our study from November 2013 to September 2015, in two contiguous, privately owned ranches, located in the “Serra do Caverá” region (30°04’S, 55°31’W), Rio Grande do Sul state, southernmost Brazil, within the Uruguayan Savannah ecoregion (Fig. 1a). The study area comprised ~30km^2 and consisted of a mosaic of remaining natural habitats, cattle pastures and agriculture. The native vegetation is characterized by riparian forests (Guadagnin *et al.*, 2015), which are considered permanent preservation areas by Brazilian legislation (Brazilian Federal Law 12.651/2012), surrounded by a diversity of grasslands (Boldrini *et al.*, 2009) undergoing light to moderate levels of livestock grazing (~0.01 cattle per km^2). In addition to native vegetation in protected and non-protected areas, agriculture is common, usually consisting of rice, soybean, oat and ryegrass croplands. Climate is subtropical humid (categorized as ‘Cfa’ in the Köppen climate classification), and weather conditions change considerably across seasons, with hot summers (average temperature of 24°C) and mild to cold winters (average temperature of 13°C). The average annual rainfall is 1500 mm (Nimer, 1989).

Capture and sample collection

From May 2014 to August 2015, we conducted eight live trapping campaigns in the four different seasons. We used tomahawk box traps modified using a technique aimed at preferentially capturing felids (relative to other co-occurring carnivores). We attached an external wooden compartment to the end of the trap opposite to the entrance, and used it to safely house a live bait (domestic chicken). This enclosure enticed the felids' curiosity, as they could smell and hear the chicken from the outside, but could only see it after entering the trap. After being captured, cats could not reach the baits, avoiding the death of the chickens and allowing fasting of the felid, which ensured the safety of the sedation procedure. In addition, this extra compartment helped care for the chicken's welfare by better controlling temperature and humidity conditions. Throughout the capture campaigns, traps were checked and maintained daily, including the cleaning of bait enclosures and feeding of chickens.

Trapped Geoffroy's cat individuals were immobilized with an intramuscular injection of Zoletil® (Virbac) at the intentional dose of 8-10 mg/kg (Manfredi *et al.*, 2006). During handling, we used earplugs and bands to reduce sound and visual stimuli, and moistened the cats' eyes with Paralube® to prevent dryness of the cornea. Every 5-10 minutes, we monitored their body temperature and respiratory and heart rates. We conducted all captures under the supervision of an expert wildlife veterinarian, and followed the recommendations of the Manual of Capture and Handling of the American Society of Mammalogists (Sikes *et al.*, 2011). The PUCRS institutional animal care and use committee approved all animal capture and handling procedures, which were also approved by the Brazilian Ministry of the Environment (permit SISBIO-36803).

For each captured individual, we recorded the sex, weight, body measurements, age class, spotting patterns (photographically) and health status. In addition, we fitted healthy adult individuals captured between May 2014 and February 2015 with VHF radio-collars with mortality and activity switches (MOD-080-2; Telonics, Mesa, Arizona); collars weighed 45 g, which represents 1.5% of the body weight (3 kg) of the lightest captured individual. Finally, to perform genetic analyses, we collected blood and cheek swab samples of each individual, and kept them in screw-cap tubes with the buffer TES (100 mMTris, 100 mM EDTA, 2% SDS) at -20^{o}C.

Telemetry data

We monitored the radio-collared cats from May 2014 to September 2015. All data points (including location and activity) were collected by a single person in a vehicle or on foot. We estimated the positions of each specimen by triangulation from the ground (White & Garrott, 1990), using a hand-held H-antenna (RA-23K; Telonics) and a portable receiver (TR-4K; Telonics), based on at least three bearings for each location. The majority of bearings were taken relatively near the cat (<300 m of distance), and we used only azimuths that differed by 60º–120º to reduce location errors (White & Garrott, 1990). We georeferenced the punctual fixes (including visual sightings) of radio-collared animals using an eTrex Legend® global positioning system (GPS) device (Garmin International Inc., Olathe, Kansas) and included them in the analysis of home range sizes. We spaced successive locations by at least three hours, which according to Manfredi *et al.* (2006) was long enough for cats to cross their entire home ranges at their study site. We acquired locations for each individual at least six times per month, aiming to distribute the effort homogeneously across the 24-hr cycle. In addition to triangulation bearings, we included in each record complementary information such as date, time, weather characteristics and level of cat activity.

Camera-trap data

We included in our study camera-trap data, comprising photographic records collected from November 2013 to September 2015 (except for March to May 2014). We deployed 26 digital camera-traps (20 Scout Guard, 4 Bushnell and 2 Moultrie) on trails and other sites with Geoffroy' cat signs (*e.g.* faeces, tracks). We spaced cameras at *ca.* 600 m from each other across the surveyed area (~30 km²), and each camera position was georeferenced using GPS. We did not use any type of lure, and we positioned each camera at *ca.* 30 cm above the ground. We checked the camera-traps once a week to replace memory cards and batteries, and confirm that they were functioning properly. We programed cameras in video mode (20s) and to be active 24h per day, with date and time recorded. We then used all videos of radio-collared individuals to increase the number of fixes for HR analyses and to record as many as possible of the individuals present in the area, including those that were not captured and/or not monitored by telemetry. These individuals could be reliably identified based on their unique spotting pattern (*e.g.* forehead markings, flank spots, or tail stripes).

Genetic data

We extracted genomic DNA from blood samples of each individual using the QIAamp DNA Stool Mini Kit® (Qiagen). DNA extracts were assessed on 1% agarose gels stained with GelRed (Biotium) and quantified with a Nanodrop® (Thermo Scientific) spectrophotometer. We used these extracts to genotype 14 microsatellite loci (nine tetranucleotides [F53, FCA391, FCA559, FCA742, FCA441, FCA453, F42, F124 and FCA740], four trinucleotides [A04, CO5, F98, F146] and one dinucleotide [FCA723]) originally developed for the domestic cat (Menotti-Raymond *et al.*, 1999, 2005), and modified to contain an M13-tailed forward primer to allow flexible fluorescent labeling (Boutin-Ganache *et al.*, 2001). Each microsatellite locus was amplified individually by PCR (Saiki *et al*., 1985), in 10-mL reactions containing 1X PCR buffer (Invitrogen), 200 mM dNTPs, 2.5 mM $MgCl_2$, 0.2 mM of the reverse primer and the M13-fluorescent primer (labeled with NED, FAM or HEX fluorophores), 0.0133 mM of the M13-tailed forward primer, 0.1–0.5 unit of Platinum Taq DNA Polymerase (Invitrogen), and 10–50 ng of genomic DNA. The reaction profile for all loci was: 94°C for 3 min, 10 cycles of 94°C for 45s, 60-50°C for 45s, 72°C for 1 min 30s, 30 cycles and a final extension of 72°C for 30 min. Negative controls were included in each PCR batch to check for contamination. Reaction products were genotyped using a 3730xl DNA analyzer (ThermoFisher Scientific), using internal size standards following the manufacturer's protocols.

Data analysis

We measured the capture rate by dividing the number of captures by the capture effort (total number of trap-days) and multiplying it by 100. We calculated capture rate for each Geoffroy's cat individual, for the species as a whole, and for all the other carnivores captured during the same campaigns.

Spatial ecology

Home range sizes were calculated using two data sets: (i) telemetry data only; and (ii) telemetry plus camera-trap data. For each data set, we applied a kernel estimation of the utilization distribution (UD) (Worton, 1989) [incorporating 95% ('full kernel' HR) and 50% ('core area' HR) of the sampled points] (Calenge, 2006). In these estimates, we used a smoothing parameter (*h*) with two different approaches: hA) the Least Square Cross Validation (hLSCV) (Calenge, 2006); and hB) the reference bandwidth (href) (Wand & Jones, 1995). In addition to the kernel estimates, we also estimated HR sizes using a Minimum Convex Polygon (MCP) approach, considering 50% and 100% of the sampled points, to allow comparisons with previously reported HR sizes.

To test if male HRs and core areas were larger than those of females, we performed a one-tailed Wilcoxon-Mann-Whitney test (*U*). These analyses were carried out for all estimators (kernel hA, kernel hB and MCP) and for the two data sets (telemetry only and telemetry + camera-trap).

To estimate the spatial overlap among monitored cats, we used the combined data (telemetry + camera trap), since it increased our location sample size. As an additional piece of information, we calculated camera-trap-based HRs for individuals that were captured but not monitored with radio-telemetry. We did not include these results in the estimation of average HR sizes, but used them to measure HR overlap. The overlap was estimated as the proportion of animal *i*'s home range that is overlapped by animal *j*'s HR (Kernohan *et al.*, 2001). The analyses were carried out with kernel hB 95% and 50%. The values of HR overlap range from 0 (no overlap) to 100% (total overlap). All estimations were carried out with the package 'adehabitatHR' (Calenge, 2006, 2011) in R 3.2.3. (R Development Core Team, 2015). To test whether there were differences in the degree of overlap among different types of dyad (female–female [F–F], female–male [F–M], male–male [M–M]) we used a Kruskal-Wallis test (*H*) in R. We also visualized the overlap spatially by creating and exporting the HR shapefiles with packages 'sp'(Pebesma, 2005) and 'maptools' (Bivand, 2016). These shapefiles were then plotted onto a supervised classified vegetation map using the software

ArcGIS 10.4.1. (see Tirelli *et al.*, in prep. [Chapter 3] for more details of the image reference and map generation).

Finally, we measured HR sizes and levels of overlap in two seasonal partitions: spring-summer (October to March) and fall-winter (April to September). We estimated HR size with the three estimators mentioned above (kernel 95% hA, hB, and 100% MCP) using the joint telemetry + camera-trap data set, and only including individuals for which we had collected more than 20 fixes per seasonal partition. To assess whether there was a statistically significant difference in spatial overlap between the seasonal partitions, we compared the observed values for each pair of individuals in spring-summer *vs.* fall-winter using a one-sample paired Wilcoxon-Mann-Whitney test (*V*). We performed this test separately for each type of dyad (F–F, F–M, and M–M).

Comparison of HR size and body weight with previous studies

To assess geographical patterns of variation in Geoffroy's cat HR, we compared our estimates for males and females (using only telemetry data and the 100% MCP method) with those reported by previous studies (Johnson & Franklin, 1991; Manfredi *et al.* 2006, 2012; Pereira *et al.*, 2006, 2012) (Appendix S1). We did not include in the analyses the data from Castillo *et al.* (2008), since that study did not report HR estimates for both sexes.

We compared the HR sizes using a Kruskal–Wallis test (*H*). If the result was significant, then we performed Dunn's multiple comparison post-hoc z-test (*Z*), which is appropriate for groups with unequal numbers of observations (Zar, 2010). We performed this test separately for males and females. We also compared the HR of males and females for each different field site using a one-tailed Wilcoxon-Mann-Whitney test (*U*).

We used the body weight data from captured adult males and females to assess sexual dimorphism and the relationship between weight and HR in this area. We calculated the mean and standard deviation (*SD*) of male and female weights, and compared them using a one-tailed Student *t*-test. In addition, to compare our results with mean values reported previously for other sites (Ximenez, 1973; Johnson & Franklin, 1991; Lucherini *et al*., 2006, Manfredi 2006; Pereira *et al*,. 2006, 2012) (Appendix S2), we performed a two-way ANOVA test from summary data (number of samples, mean and standard deviation for each site) for males and females. If the result was significant, we performed post-hoc analyses with Tukey's HSD (Honest Significant Difference). For these analyses, we also did not include the data from Castillo *et al.* (2008), as they were derived from a single male individual.

To understand if home range size is influenced by body weight in Geoffroy's cat, we generated a linear regression model for each sex using the results of HR sizes from each estimator (MCP 100%; kernel 95% hA; and kernel 95% hB). These analyses were also performed with data from other studies (Johnson & Franklin, 1991; Manfredi *et al.*, 2006, 2012; Pereira *et al.*, 2006, 2012) to assess if any common pattern could be discerned. We did not include data from Manfredi *et al*. (2012) and Castillo *et al.* (2008) since these studies did not report averages for both sexes. Body weight was assumed to be the explanatory variable, while the HR estimators were treated as the dependent variables. The analyses were performed in R 3.2.3.

Genetic diversity and genetic relatedness among individuals

We measured allele sizes and called genotypes with the software GeneScan® (Applied Biosystems). To estimate genetic diversity indices, including the number of alleles and allele frequencies, as well as the observed and expected heterozygosities per locus, we used the computer programs ML-Relate (Kalinowski *et al.*, 2006) and Genepop 4.5 (Rousset, 2008). We tested for Hardy-Weinberg equilibrium using 10,000 randomization steps with ML-Relate. The identification of null alleles was carried out with both ML-Relate and Micro-Checker 2.2.3 (Van Oosterhout *et al.*, 2004). The inbreeding coefficient (F_{IS}) for the study population was estimated with FSTAT 2.9.3.2 (Goudet, 2001). Relatedness coefficients (r) between the sampled individuals were estimated with ML-Relate, after exclusion of inferred null alleles. These results were then used to assign to each pair of individuals one of four possible pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO).

Relationship of spatial overlap and genetic relatedness

We employed generalized linear models to test if the proportion of HR overlap was influenced by the relatedness coefficient (r) estimated for each dyad, for both the 95% and 50% kernel hB. We performed separate tests for each type of dyad (F–F, F–M, and M–M, and also for M–M including a male kitten). The analyses were carried out in R 3.2.3. Finally, we combined relatedness information with home range data for each individual and generated a spatial map with pedigree structure, so that this relationship could be assessed visually.

Results

Data collection

The total capture effort was 583 trap/nights. We concluded that the use of the new trap compartment was successful as a selective strategy, since the capture rate of Geoffroy's cats was 3.94%, and the capture rate of different individuals of this species was 2.06%, both higher than the rate observed for other Carnivora species during the same period (0.85%) (Appendix S3). We captured 12 different Geoffroy's cat individuals (5 females and 7 males) (Table 1). Male M2 was recaptured six times and male M3 four times; two females (F2 and F4) were recaptured three and two times, respectively. During the period in which we were fitting Geoffroy's cats with VHF collars, we captured nine specimens, and seven of them (3 females and 4 males) were monitored by telemetry. We did not monitor the remaining two individuals due to the fact that one was a sub-adult and the other was killed by domestic dogs two days after the capture (Table 1). One of monitored females (F1) also died (in this case by poisoning) after two months of monitoring.

Table 1 Individuals captured in the study (ID), age category of each specimen (A=adult, J=juvenile); monitored period of telemetry (TL), total days and total data points (fixes) collected using this method. Telemetry and camera-trap combined period (TL + CT) (since first record of the individual in its HR area until last day recorded), days of monitoring, and number of location records (fixes) of each individual with both methods combined.

		TL			TL + CT		
ID	age	period tracked	days	fixes	period tracked	days	fixes
F1	A	2 May 2014 - 23 Jun 2014	53	35	27 Jan 2014 - 23 Jun 2014	148	41
F2	A	28 Jun 2014 - 10 Jul 2015	378	133	28 Jun 2014 - 08 Aug 2015	407	186
F3	A	11 Nov 2014 - 17 Sep 2015	311	69	13 Aug 2014 - 17Sep 2015	401	102
F4*	A	09 and 10 Jul 2015	0	2	26 Nov 2014 - 12 Jul 2015	229	50
F5*	A	11 Jul 2015	0	1	14 Aug 2014 - 11 Jul 2015	332	21
M1	A	19 Jun 2014 - 9 May 2015	325	59	ND	ND	ND
M2	A	15 Sep 2014 - 19 Jan 2015	127	54	15 Sep 2014 - 05 Aug 2015	325	103
M3	A	18 Dec 2014 - 13 Feb 2015	58	23	29 Nov 2013 - 28 Jul 2015	607	53
M4	A	16 Feb 2015 - 17 Sep 2015	214	62	12 Dec 2013 - 17 Sep 2015	645	87
M5*	J	20 Feb 2015	0	1	19 Feb 2015 - 28 Jul 2015	160	13
M6*	J	13 Jul 2015	0	1	11 Apr 2015 - 13 Jul 2015	94	7
M7*	A	22 Jun 2014 - 24 Jun 2014	2	3	ND	ND	ND

*Individuals not monitored by telemetry. F4 and F5 were captured in the last field campaign, during the recapturing campaign to remove the radio-collars of the monitored cats (no collar was fitted and consequently no telemetry monitoring were carried out in these two individuals); M6 and M7 were juveniles; and M7 was kill by dogs.

We recorded 516 independent photographs of Geoffroy's cats during 8,845 camera-trapping days (Tirelli *et al.*, in prep. [Chapter 3]). These data include cats that were captured and monitored with radio-telemetry, others that were captured and not radio-collared (since those captures occurred towards the end of the survey period, outside the window of radio-collar fitting), and 10 additional individuals that were present in the area and not captured during the study (Table 2). With this camera-trap data set, we could increase the total number of fixes of captured cats from 442 to 663 (which improved the sampling of all captured individuals except for M1, not recorded in camera-traps). Since many images were recorded before the capture of an individual or after the end of its telemetry-based monitoring, they also helped expand the period of data collection, from a per-individual average of 122 days to 345 days (see Table 1).

Table 2 Individuals recorded only by camera-trap in the study (ID: F= female; M= male; Un= unidentified sex), age of each specimen (A=adult, J=juvenile, K=kitten); period of camera-trap monitoring (from the first record to the last image of the specimen), total days and total data points collected using this method.

ID	age	Camera-trap (CT) period tracked	Days	Fixes
F6	A	04 Dec 2013 - 24 May 2015	537	13
F7	A	23 Dec 2014 - 18 Jul 2015	208	24
F8	A	08 Mar 2015 - 17 Jul 2015	132	18
F9	J	13 Feb 2015 – 25 Jun 2015	133	50
M8	A	13 May 2014 - 29 Jul 2014	48	12
M9	A	18 Nov 2014	1	1
M10	A	26 Apr 2015 - 16 Jun 2015	49	7
Un1	K	05 Jun 2014	1	1
Un2	A	01 Jul 2014 - 01 Nov 2014	124	3
Un3	J	29 Apr 2015 - 20 Jul 2015	83	2

Home range size

The average home range size for the species (including males and females) at our study site, using telemetry data only, was (mean ± *SD*) 239.43 ± 229.28 ha (95% kernel hA), 306.84 ± 291.37 ha (95% kernel hB) and 208.65 ± 193.14 ha (100% MCP).

When we combined radio-telemetry and camera-trap data (Fig. 1b), the average home range size was 378.66 ± 302.05 ha (95% kernel hB) and 273.31 ± 272.89 ha (100% MCP). The average core area, using telemetry data only, was 44.95 ± 48.49 ha (50% kernel hA), 77.55 ± 58.53 ha (50% kernel hB) and 39.56 ± 38.77 ha (50% MCP). When the two sexes were analysed separately, we observed a strong trend, apparent across all estimators, for male HRs to be larger than those of females (Table 3). This difference between the sexes was found to be statistically significant for five out of ten estimators, indicating the occurrence of dimorphism in both HR and core area size (Table 3).

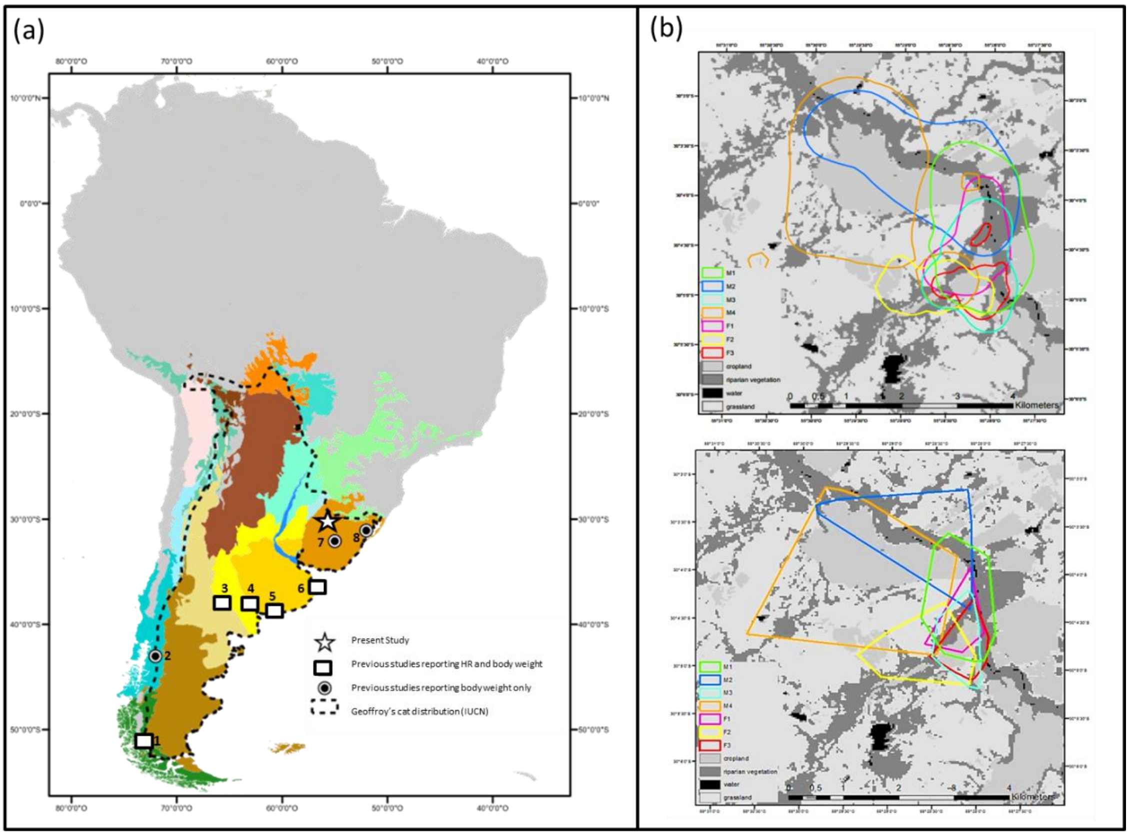

Figure 1 (a) Geoffroy's cat geographical distribution in South America, with colours indicating different ecoregions. Sites for which HR and/or body weight data are available are indicated by numbers: 1 – Torres del Paine (Johnson & Franklin, 1991 [n=9 individuals]); 2 – Los Aleceres (Lucherini *et al*,. 2006 [n=2]), 3 – Lihué Calel (Pereira *et al.*, 2006, 2012 [n=18]); 4 – E. Torniquist (Manfredi, 2006; Lucherini *et al.*, 2006; Manfredi *et al.*, 2012 [n=3]); 5 – Coronel Dorrego (Castillo *et al.*, 2008 [n=1]); 6 – Campos del Tuyú (Manfredi, 2006; Lucherini *et al.*, 2006; Manfredi *et al.*, 2006 [n=4]); 7 – Northen Uruguay (Ximenez 1973, Lucherini *et al.*, 2006 [n=2]); 8 – Southern Brazil (Lucherini *et al.*, 2006 [n=23]); and Present study (star

symbol [n=12 body weight; n=7 HR]). (b) Home ranges of Geoffroy’s cat individuals monitored at our study site in Rio Grande do Sul state, southern Brazil. The top panel represents the HRs using the 95% kernel hB estimator, while the bottom panel represents HRs based on the 100% MCP estimator.

Table 3 Estimation of home range and core area sizes for each individual monitored using different estimators and 2 data sets (TL=telemetry only and TL+CR=telemetry plus camera-trap). Wilcoxon-Mann-Whitney test (*U*) was performed to compare HR sizes of males and females, *P* is the *P-value* observed in each test.

	Home range (TL)						Home range (TL + CR)					
	MCP		kernel hA		kernel hB		MCP		kernel hA[1]		kernel hB	
id	50%	100%	50%	95%	50%	95%	50%	100%	50%	95%	50%	95%
F1	15.6	75.0	25.8	102.7	39.2	155.9	20.0	86.2	56.4	212.8	56.4	212.8
F2	21.0	187.0	18.8	99.3	38.6	183.2	15.9	205.8	-	-	28.4	168.1
F3	2.5	62.8	3.5	21.4	9.7	66.4	3.6	90.2	-	-	13.9	101.1
$\bar{X}$(F)	**13.0**	**108.3**	**16.0**	**74.5**	**29.2**	**135.2**	**13.2**	**127.4**	**-**	**-**	**32.9**	**160.7**
SD	9.5	68.5	11.4	46.0	16.9	61.1	8.5	67.9	-	-	21.6	56.2
M1	78.8	246.1	47.7	211.6	119.3	466.2	78.8	246.1	47.7	211.6	119.3	466.2
M2	30.3	186.1	46.7	194.2	60.9	279.0	86.9	355.1	-	-	150.7	654.3
M3	20.4	86.1	23.1	102.4	94.1	342.4	36.4	112.7	-	-	72.9	285.2
M4	108.4	617.6	149.1	704.3	180.8	894.7	107.9	789.8	-	-	157.4	850.5
$\bar{X}$(M)	**59.5**	**284.0**	**66.7**	**303.1**	**113.8**	**495.6**	**77.5**	**375.9**	**-**	**-**	**125.1**	**564.1**
SD	41.4	232.0	56.1	271.7	50.7	277.2	30.0	293.2	-	-	38.5	243.3
U	11.0	10.0	11.0	11.0	12.0	12.0	12.0	11.0	-	-	12.0	12.0
P	0.06	0.11	0.06	0.06	0.03*	0.03*	0.03*	0.06	-	-	0.03*	0.03*

* Statistically significant (*P*<0.05).

[1] The estimation with kernel hA (hLSCV) did not yield interpretable results for most individuals. using the combined data, since it over-fragmented the estimated HRs and core areas.

HR and core area overlaps occurred for all types of dyad (F – F, F – M, and M – M) (Appendices S4, S5). The mean ± SD (max-min values) of F – F HR overlap with the 95% kernel estimator was 30 ± 19% (12 – 95%); for F – M it was 36 ± 18% (6 – 81%) and for M – M it was 34 ± 24% (5 – 92%) (Fig. 2a). For the core areas, the overlap was 8 ± 12% (0 – 50%) for F – F; 4 ± 7% (0 – 25%) for F – M, and 8 ± 14% (0 – 45%) for M – M (Fig. 2b). There was no significant difference in overlap among the different types of dyads, for both HR (*H* = 1.84, *df* = 2, *P* = 0.40) and core area (*H* = 3.98, *df* = 2, *P* = 0.14).

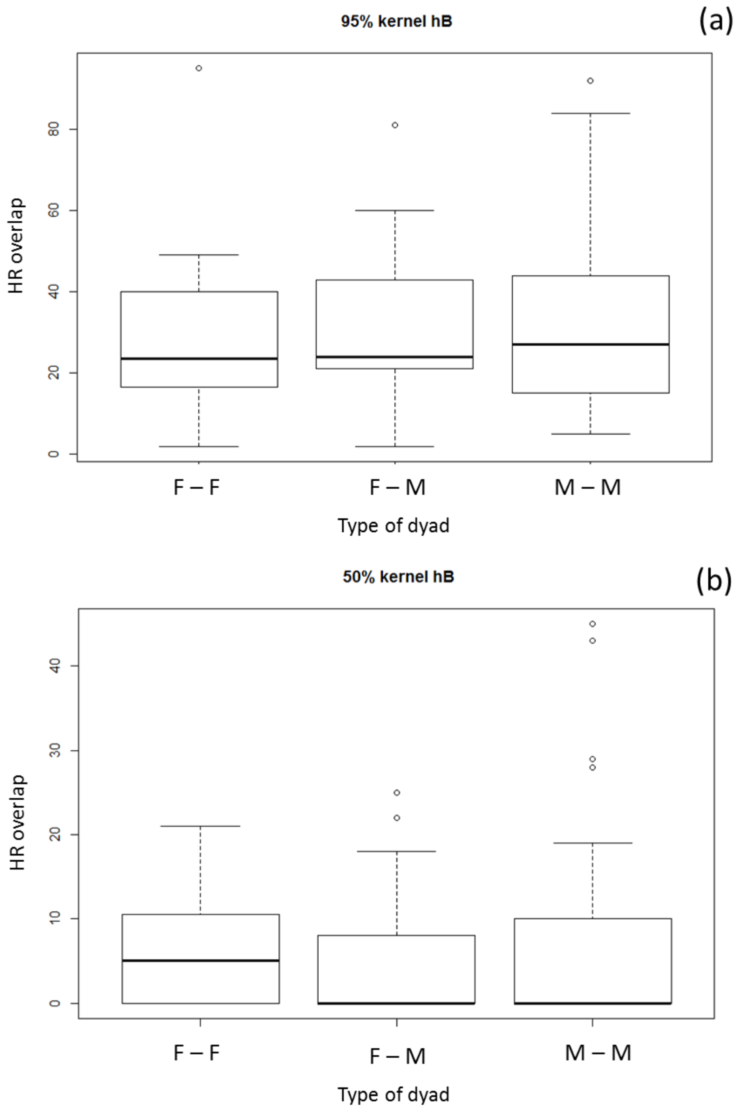

Figure 2 Spatial overlap for each type of dyad (F – F, F – M, and M – M). (a) Home range (95% kernel hB). (b) core area (50% kernel hB). The bottom and top limits of the box are the upper bounds of the first and third quartiles (1Q and 3Q), and the band inside the box is the median. The ends of the whiskers are the minimum and maximum values, excluding outliers. Circles are outliers.

With respect to HR size in seasonal partitions, we observed that the average HR and core area sizes tended to be larger in winter than summer for both sexes, except HR with 95% kernel hB for females and HR size using 100% MCP for males (Appendices S6 and S7). When we visually assessed the HR distributions in the two partitions, we observed that they changed somewhat between Spring-Summer and Fall-Winter

(Appendix S6). However, when we compared the extent of overlap for each pair of individuals in Spring-Summer (Appendix S8) and Fall-Winter (Appendix S9), we observed no significant difference for F – F (n=6 pairs, *V*=14, *P* =0.56) and F – M (n=24 pairs, *V* = 93, *P* = 0.10), whereas we found some difference for M – M (n=12 pairs, *V* = 14, *P*-value = 0.05).

Comparison of HRs and body weight in different regions

The Kruskal-Wallis test (*H*) indicated a significant variation in HR size for males from different sites (*H*=15.8, *P*<0.05). Our post-hoc analysis defined that HR sizes of males from Torres del Paine (Chile) (Johnson & Franklin, 1991) were significantly different from those from Lihué Calel (Argentina) (Pereira *et al*., 2006, 2012); all other comparisons did not yield significant differences, possibly because of limited sample sizes (Table 4). For females, the HR size was not significantly different among regions (*H*=5.9, *P*=0.21) (Table 4). The HR size did not vary significantly between males and females (Fig. 3a) at any site: Torres del Paine (*H*=9, *P*=0.09); Lihué Calel (*H*= 57, *P*=0.29); E. Tornquist (*H*= 2, *P*=0.33); Campos del Tuyú (*H*=4, *P*=0.17); and present study (*H*=10 *P*=0.11 [Table3]).

Table 4 Multiple pairwise comparisons between HR sizes of male Geoffroy's cats at each field site using the Kruskal-Wallis test (post-hoc Dunn test [*Z*]). The *P*-values were adjusted for multiple comparisons with the Benjamini-Hochberg method.

Study locations	Males		Females	
	Z	*P*-value	*Z*	*P*-value
Torres del Paine (Chile) - Lihué Calel (Argentina)	3.33	0.01*	1.31	0.47
Torres del Paine (Chile) - E. Tornquist (Argentina)	-0.05	0.96	-0.55	0.65
Lihué Calel (Argentina) - E. Tornquist (Argentina)	-2.35	0.09	-1.61	0.36
Torres del Paine (Chile) - Campos del Tuyú (Argentina)	0.56	0.82	0.89	0.62
Lihué Calel (Argentina) - Campos del Tuyú (Argentina)	-1.68	0.19	-0.19	0.84
E. Tornquist (Argentina) - Campos del Tuyú (Argentina)	0.5	0.77	1.28	0.40
Torres del Paine (Chile) - Present Study (Brazil)	2.29	0.07	1.80	0.36
Lihué Calel (Argentina) - Present Study (Brazil)	-0.35	0.81	0.84	0.57
E. Tornquist (Argentina) - Present Study (Brazil)	1.82	0.17	2.00	0.45
Campos del Tuyú (Argentina) - Present Study (Brazil)	1.24	0.36	0.82	0.52

* Statistically significant (P<0.05).

We weighed ten adult Geoffroy's cats (five males and five females). Males significantly (t = 3.84, df = 6.6, P <0.05) outweighed females (4.63 ± 0.57 kg *vs.* 3.48 ± 0.35 kg). Interestingly, the observed averages were almost identical to those previously reported (using a non-overlapping set of individuals) for this same geographic region (Appendix S2) (Lucherini *et al.*, 2006). In the comparison across field sites, we did not observe a significant difference in body mass for males ($F_{5,38}$ = 13.20, P = 0.18), or females ($F_{5,23}$ = 4.00, P = 0.12) (Fig. 3b). However, when we included both sexes simultaneously in the analyses, we observed a significant difference ($F_{1,5}$ = 18.00, P<0.05). The post-hoc test indicated that this result was driven by the difference between the sexes, with the Torres del Paine site in Chile being the only area in which body weight was not significantly different between males and females (P=0.09) (Fig. 3b).

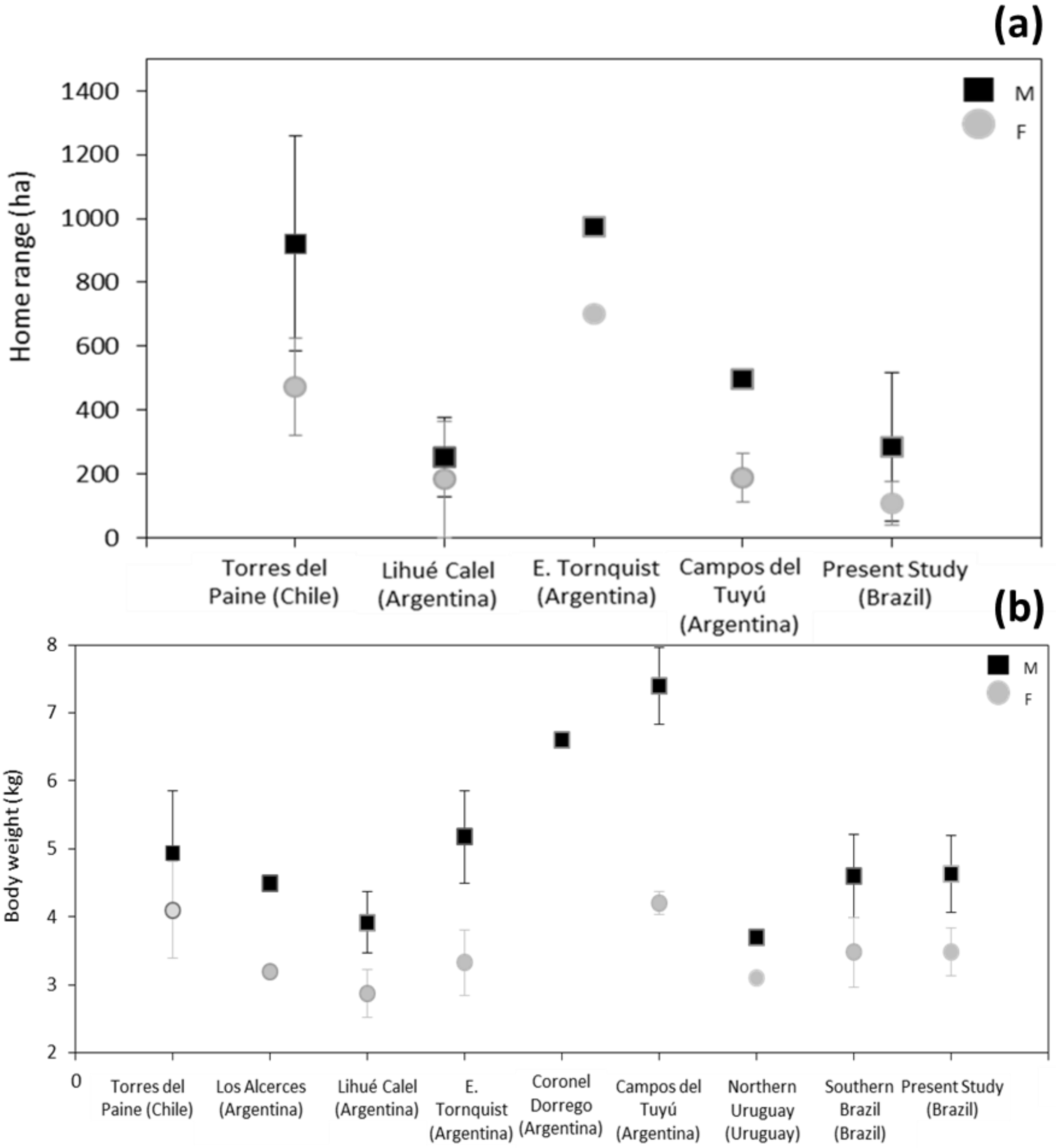

Figure 3 Geographical variation in home range size (100% MCP) and body weight of Geoffroy's cats. (a) Average and standard deviation of home range sizes. The single male individual reported for location 5 (Castillo *et al.*, 2008) is not shown in the plot due to its extreme outlier home range size (2,696.4 ha) relative to the other studies. (b) Average and standard deviation of body weight of adult Geoffroy's cats. Sites Los Alcerces (Lucherini *et al.*, 2006), Coronel Dorrego (Castillo *et al.*, 2008), and northern Uruguay (Ximenez, 1973, in Lucherini *et al.*, 2006) are shown in the figure, but were not included in the analysis since their standard deviation was not available.

With respect to the influence of body weight on HR size, the results differed between sexes. For males, the coefficient of linear regression (r^2) was always positive, with two out of three estimates being statistically significant: for the 95% kernel hA, r^2=0.99 ($F_{1,2}$=400, P<0.05); for the 95% kernel hB, r^2=0.16 ($F_{1,2}$=4.94, P=0.71); and for 100% MCP, r^2=0.94 ($F_{1,2}$=32.75, P<0.05). For females, the coefficient was also positive, but never significant: with the 95% kernel hA, r^2=0.03 ($F_{1,1}$=0.03, P=0.70); for the 95% kernel hB, r^2=0.26 ($F_{1,1}$=0.35, P=0.66); and for the 100% MCP, r^2=0.66 ($F_{1,1}$=1.96, P=0.39).

We then assessed this relationship across field sites, using the 100% MCP estimator and analyzing males and females separately (see Methods). The results were very similar for the two sexes, in both cases yielding positive, non-significant *P*-values (males [six field sites] r^2=0.10, $F_{1,4}$=0.44, P=0.54; females [five field sites]: r^2=0.13, $F_{1,3}$=0.46, P=0.55).

Genetic variability

Genetic variation (*e.g.,* number of alleles, allelic richness, and observed heterozygosity) in the Geoffroy's cat population assessed here was quite high, when compared, for example, to the values reported by Trigo *et al.* (2008). We observed a mean of 6.14 ± 2.5 *SD* alleles per locus (ranging from three to 10 alleles), and the average gene diversity per locus was 0.74 ± 0.15 *SD*. The mean inbreeding coefficient (F_{IS}) was estimated to be 0.03 (Table 5). Of the fourteen loci tested, one (FCA723) was estimated to harbour null alleles based on the Micro-Checker results; using ML-Relate, heterozygote deficiency patterns suggestive of null alleles were observed at two loci (FCA723, P<0.05; and C05, P<0.05). Conservatively, we did not include these two loci in the relatedness analyses. We found 15 cases of potential (non-zero) relatedness (r) between individuals in this population, four of which were inferred to represent interpretable relationships (Appendix S10). Overall, the observed levels of relatedness between individuals were very low (female-female pairs: mean r=0.093±0.24; male-male pairs: mean r=0.03±0.06; female-male pairs: mean r=0.043±0.11), indicating that this local population was outbred.

Table 5 Assessment of genetic variation at 14 microsatellite loci in the surveyed Geoffroy's cat population (n=12), including the number of alleles, allelic richness, observed (Ho) and expected (He) heterozygosities, and inbreeding coefficient (F_{IS}).

Locus	N of alleles	Allelic richness	Ho	He	F_{IS}
FCA453	5	4.91	0.27	0.76	0.12
FCA391	7	6.91	0.07	0.85	0.11
F98	3	3	1.00	0.57	-0.46
FCA441	4	4	0.88	0.77	-0.19
FCA723	10	9.66	0.00	0.9	0.44
F146	4	3.91	0.40	0.38	0.11
FCA742	8	7.83	0.13	0.84	0.2
F53	10	10	1.00	0.89	-0.12
F42	7	6.99	0.53	0.88	-0.05
FCA740	2	2	0.97	0.5	-0.33
F124	5	4.92	0.66	0.75	0
A04	9	8.74	0.10	0.86	0.23
F559	5	5	0.20	0.78	0.15
C05	7	6.66	0.01	0.69	0.27
$\bar{X}$	6.14	6.04	0.45	0.74	0.03

Relationship between spatial overlap and genetic relatedness

To understand if the proportion of HR overlap is influenced by genetic relatedness, we tested two linear regressions for each type of dyad (Appendix 11). For F – F dyads, the results were not significant: for the 95% kernel hB, the r^2 was 0.06 ($F_{1,18}$=1.09, P=0.31), while for the 50% kernel hB, the r^2 was 0.07 ($F_{1,18}$=1.41, P=0.25). For F – M dyads, there was also no significant relationship: the r^2 using the 95% kernel hB was 0.04 ($F_{1,28}$=1.30, P=0.26), and using the 50% kernel hB it was 0.003 ($F_{1,28}$=0.09, P=0.76). For M – M dyads, the results were also non-significant: for the 95% kernel hB, the r^2 was 0.03 ($F_{1,18}$=0.58, P=0.45), and for the 50% kernel, the r^2 was

0.02 ($F_{1,18}$=0.38, P=0.54). However, when M – M dyads were assessed including a male kitten, the linear regression results were different between the 95% kernel hB and the 50% kernel estimator: r^2=0.06 ($F_{1,28}$=1.83, P=0.19) for the 95% estimator, and r^2=0.17 ($F_{1,28}$=5.87, P<0.05) for the 50% estimator. When we analyzed the spatial structure of related animals (Fig. 4), we observed extensive overlap for all cases of inferred genetic relationship. However, we also observed a similar level of overlap for unrelated pairs of animals (see Figure 4).

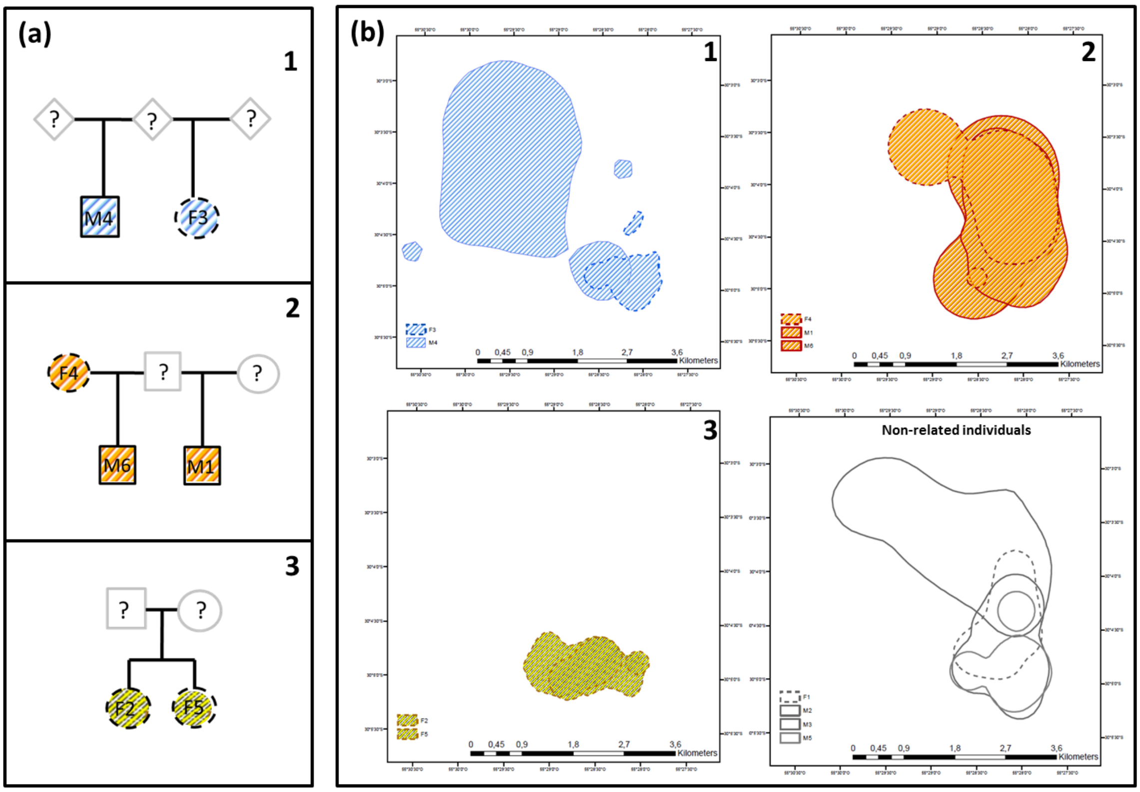

Figure 4 Genetic relationships and spatial overlap of Geoffroy's cat individuals sampled at our study site. (a) Pedigrees of related individuals; question marks indicate presumed individuals that were not captured in the study. (b) HR overlaps (95% kernel hB); individuals with the same colour are genetically related. (b1) M4 and F3; (b2) M1, M6 and F4; (b3) HR overlap of F2 and F5; and HR overlap of all other individuals captured indicated as non-related individuals.

Discussion

This study describes the spatial structure and dynamics of a Geoffroy's cat population, including the first assessment of inter-individual relatedness for this species. We also compared our results on HR sizes and body weight of the surveyed population with those reported in previous studies, aiming to assess whether patterns of variation were consistent or varied among different field sites.

The camera-trap records were important for this study, since they increased the number of locations of captured individuals, the monitoring period of cats, and allowed us to include additional individuals relative to those that were live-trapped. With these images, we were able to observe three females with their kittens on different occasions. One of the females (F2) was recorded on many opportunities during its pregnancy (October 2014) and then until its kitten (a female) was approximately nine months old. In this population, we observed that each pregnant female appeared to raise only one kitten per litter, in contrast to other regions of southern Brazil, where litters more often comprised two or three offspring (F. Mazim, *pers. comm.*). Camera-trap records also allowed us to observe individuals hunting rodents and birds, scent-marking and having an inter-specific encounter (with an armadillo). All these records improve our knowledge of their behaviour, space use and the interactions among individuals at a fine scale. We thus strongly recommend the use of this approach in studies of felid behavioural ecology, even when radio-telemetry data is collected.

All adult individuals that were captured and telemetry-monitored in the present study were considered residents (>100 days) in the area. Adult males stayed in the same area for at least 11 months, and one male (M4) was in same area for almost two years (Table 1). These results differed from those reported for other regions (Johnson & Franklin, 1991; Pereira *et al.*, 2006), in which some males stayed in an area for three to five months and then abandoned it. Although the four captured males were residents, we observed the presence of three transient adult males in the camera-trap records (Table 2). While males can be residents or transients, all adult females recorded in the present study were considered residents.

Male HRs and core areas appeared to be larger than those of females. Specifically, we observed a significant difference in the HR sizes using one of the three HR estimators (the 95% kernel hB), and on a finer scale, for two of three core area size estimators.

No individual had an exclusive HR and the mean overlap for the different types of dyad (F – F, F – M and M – M) was not significantly different, suggesting there is no gender-based pattern in HR overlap in this population. According to Macdonald, Mosser & Gittleman (2010), spatial arrangements in felids may be non-random and involve various types of social interactions. Those authors proposed four possible social group arrangements for felids, based on two categories (exclusive [E] or overlapping [O]) of inter-sexual and intra-sexual home range overlap: EE (no overlap or <10% overlap), OE (males overlap and females do not), EO (females overlap and males do not), and OO (HRs of both sexes overlap). In our study, the arrangement was “OO”, and we observed the same pattern in three previous Geoffroy’s cat studies (Manfredi *et al.*, 2006, 2012; Pereira *et al.*, 2012). The social group arrangement “EO” was also found for the species in two previous studies (Johnson & Franklin, 1991; Pereira *et al.*, 2006). In our study area, the overlap in core areas also occurred in all types of dyads (F – F, F – M and M – M) with no significant difference. Additionally, there were ten additional individuals in the study area for which we could not assess overlap. However, the records of these individuals were always inside the HRs of the captured cats, suggesting that the overall degree of overlap was larger than what we estimated. These results suggest some degree of sociability, since home range overlap appears to be common, substantial (even encompassing core areas) and relatively widespread in the majority of Geoffroy's cat sites.

Although it is also important to note that other variables could influence overlap, observing the HR and core area overlaps in the classified maps, it appears that it typically occurred in the riparian vegetation, a permanently protected area within farmlands. Our results were similar to those reported by Pereira *et al.* (2006, 2012), who observed a greater spatial overlap in a protected area, with some individuals overlapping even in core areas. Pereira *et al.*'s studies were conducted in privately owned ranches and in a protected area; our study was carried out in farmland areas with permanent protected areas; thus, even though the spatial scales differ, a comparison between these studies may be valuable. The cattle density in our study area (~0.01 individuals per km^2) is lower than in the ranches assessed by Pereira *et al.* (2006, 2012) (with 9 to 21 cattle per km^2). This may influence both the variety and abundance of resources (as *e.g.* prey, water, shelter) which may in turn affect Geoffroy's cat spatial dynamics. Further studies are necessary to test the influences of these resources on spatial dynamics of this felid.

HRs were slightly different in size and overlap between seasonal partitions. Home-ranges tended to be larger in fall-winter than in spring-summer. Previous studies in Argentina reported that the degree of home range overlap between females can fluctuate across seasons (Manfredi *et al.*, 2006; Pereira *et al.*, 2006), and suggested that this may be associated to prey availability and abundance. In southern Brazil, although we observed a certain seasonal difference in HR size, this variation did not appear to have a large influence on the HR overlap, which suggests that the spatial dynamics of this population do not change substantially between seasons.

The statistical analyses including our results and previous assessments of Geoffroy’s cat HR sizes showed variation for males, although a significant difference was found in only one out of ten tested pairs. Female HR sizes did not vary geographically. Although HRs were larger for males than females, there was no statistical significance. The results described here may suggest that home range in Geoffroy’s cat as a species does not vary considerably among regions, especially for females. This may be related to the body weight results, which indicated no significant difference in either sex across different regions. This lack of variation in body weight may be connected to results of previous molecular studies, which suggested that the species has high level of genetic diversity with no geographic structure (Johnson *et al.*, 1999; Eizirik & Johnson, 2006). Additionally, Nascimento (2014), studding morphological variation of Geoffroy’s cat, agreed with the results obtained by molecular data. That author concluded that no subspecies should be recognized, since there seems to be a large panmictic population with no significant barriers to gene flow (Johnson *et al.,* 1999; Eizirik & Johnson, 2006; Nascimento, 2014).

We also failed to find any geographical variation in body weight for males or females. However, males were significantly heavier than females at the majority of sites (except Torres del Paine). These results support those of Lucherini *et al*. (2006), reporting the existence of body weight dimorphism in this small cat.

In our study area, the body weight of males was positively related to HR size, whereas body weight in females did not appear to influence their HR sizes. Sexual dimorphism is explained by the theory of sexual selection (Darwin, 1871). In the majority of mammals, sexual selection acts differently on males and females, with the former being driven to mate with as many females as possible, and the latter being driven to promote offspring survival. This induces male-male competition for access to females (Freeman & Herron, 2009). This may explain why HR sizes are influenced positively by body mass in males and not in females in this study. Hypothetically, larger males with larger HR sizes have more opportunities to breed than smaller ones. Females do not necessary need to increase their HRs if these provide sufficient resources to maintain themselves and their kittens. When we performed the same analysis using the average of HR and body weight for each study site of Geoffroy's cat distribution (using the 100% MCP estimator), the results were positive, but not significant for either sex. These results are consistent with the notion that competition among males occurs within each population, and is not substantially different across sites.

The genetic variation within this population was considerably high; the inbreeding coefficient value indicated that it is an outbred population, implying that it is connected to other areas. This result is important from a conservation point of view, indicating that Geoffroy's cats can still disperse among local populations in this region. As riparian vegetation seems to be a very important habitat for the species in this human-dominated landscape (see also Tirelli *et al.*, in prep. [Chapter 3]), it may serve as a corridor enabling connections among populations in the region. Testing this hypothesis should be the target of future studies addressing these populations.

The extent of HR and core area overlap was not influenced (in any of type of dyad) by genetic relatedness in this Geoffroy's cat population. Here we did not find support for the hypothesis that more closely related females overlapped more in space than unrelated ones. These results were similar to those reported by two studies on *Puma concolor* (Nicholson *et al.*, 2011; Elbroch *et al.*, 2015). According to these studies, these results might support arguments that relatedness fails to influence spatial organization in carnivore populations. Alternatively, Elbroch *et al.* (2015) suggested that their results may have been biased by human hunting pressure, and that in hunted populations territoriality may be weaker and female immigration stronger than in natural puma populations, because of a destabilization of matrilineal lineages (Beausoleil *et al*., 2013; Stoner *et al*., 2013). Both arguments could explain the results found for Geoffroy's cats in our study. In Brazil, hunting native wildlife is illegal, but there are frequent reports of native animals being killed by domestic dogs, as was observed in our study. Additionally, because human-dominated areas represent 97% of the Brazilian Pampas biome (MMA, 2007), human disturbance may have a similar effect on territorial boundaries in Geoffroy's cat populations as hunting may do for pumas. Testing these hypotheses in future Geoffroy's spatial studies should shed light onto the processes underlying the observed patterns.

A noteworthy result was the observation that the core area overlap was positively influenced by relatedness in M – M pairs when a male kitten was included. This could indicate that an adult male may tolerate a kitten male that is its half-sibling within its core area. This is an interesting hypothesis that can be tested with larger sample sizes and long-term monitoring of the same population.

The largest gap in knowledge on wild felids is the lack of studies focusing on small cats from South America and Asia. This is important since ~45% of extant felids are small (<10 kg) and occur in these two geographic regions (Macdonald, Loveridge & Nowell, 2010). In this context, the understanding of the spatial dynamics of this Geoffroy's cat population will aid in the development of regional management plans for this species in this biome. Additionally, understanding patterns of variation in Geoffroy's cat biology and ecology across its range should help design effective conservation strategies that allow the long-term persistence of this felid in the variety of habitats in which it occurs.

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Supporting Information

Appendix S1. Home ranges (HR) of male and female Geoffroy's cats in hectares (ha), as reported by previous studies.

Appendix S2. Mean body weight (kg) of males and females (mean ± *SD*) from our study and previous home range studies of Geoffroy's cat.

Appendix S3. Capture efforts of Geoffroy's cat and other Carnivora species at our study site.

Appendix S4. Home range overlap for pairs of captured individuals (including two male juveniles [M5 and M6]) using 95% kernel hB; values range between 0 (no overlap) and 100% (complete HR overlap).

Appendix S5. Core area overlap of captured individuals (including two male juveniles [M5 and M6]) using 50% kernel hB; values range between 0 (no overlap) and 100% (complete HR overlap).

Appendix S6. Home range size of Geoffroy's cat in two different seasonal partitions (spring-summer and fall-winter) using two distinct estimators (95% kernel and 100% MCP) at our study site.

Appendix S7. Home range (HR) and core area (CA) size of Geoffroy's cats at our study site, estimated for two different seasonal partitions (spring-summer and fall-winter), using two distinct estimators: 95%(HR) and 50% (CA) kernel and 100% (HR) and 50% (CA) MCP.

Appendix S8. Overlap of home range from Geoffroy's cat individuals at our study site in the spring-summer seasonal partition. Values range between 0 (no overlap) and 100% (complete HR overlap).

Appendix S9. Overlap of home range from Geoffroy's cat individuals at our study site in the fall-winter seasonal partition. Values range between 0 (no overlap) and 100% (complete HR overlap).

Appendix S10. Pairwise relatedness index (*r*) values for Geoffroy's cat individuals sampled at our study site, estimated from microsatellite data using the software ML-Relate. All results were indicative of unrelated pairs, except in the cases with $r \geq 0.19$, whose relationships were inferred to be as follows: PO: parent-offspring; FS: full-sibling; HS: half-sibling.

Appendix S11. Generalized linear models testing if the proportion of HR overlap was influenced by the relatedness coefficient (*r*). The estimates are shown for both the 95% and 50% kernel hB. We performed separate tests for each type of dyad (F–F, F–M, and M–M, and also for M–M including a male kitten).

Appendix S1. Home ranges (HR) of male and female Geoffroy's cats in hectares (ha), as reported by previous studies.

Site	HR Study	Males/ Females ID	N fixes	HR 100% MCP (ha)
Torres del Paine (Chile)	Johnson & Franklin 1991	M210	22	794
Torres del Paine (Chile)	Johnson & Franklin 1991	M483	45	1089
Torres del Paine (Chile)	Johnson & Franklin 1991	M502	62	392
Torres del Paine (Chile)	Johnson & Franklin 1991	M511	42	1241
Torres del Paine (Chile)	Johnson & Franklin 1991	M555	45	1089
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM1M	24	371.1
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM2M	18	60.8
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM3M	25	176.5
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM20M	42	212.7
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM21M	20	218.8
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM22M	37	305.2
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM23M	76	400.05
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM24M	29	79.9
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM25M	19	169.3
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM46M	18	62.8
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM30M	21	225.3
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM36M	54	404.05
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM39M	38	242.7
Lihué Calel (Argentina)	Pereira *et al.* 2012	OGM40M	27	307.3
E. Tornquist Provincial (Argentina)	Manfredi *et al.* 2012 (Manfredi 2006)	M5	54	979
E. Tornquist Provincial (Argentina)	Manfredi *et al.* 2012 (Manfredi 2006)	M7	110	972
Campos del Tuyú (Argentina)	Manfredi *et al.* 2006	M1	102	503
Campos del Tuyú (Argentina)	Manfredi *et al.* 2006	M2	49	490
Coronel Dorrego (Argentina)	Castillo *et al.* 2008	M1	40	2696.4

Serra do Caverá (Brazil)	Present Study	M1	59	246.1
Serra do Caverá (Brazil)	Present Study	M2	54	186.1
Serra do Caverá (Brazil)	Present Study	M3	23	86.1
Serra do Caverá (Brazil)	Present Study	M4	62	617.6
Torres del Paine (Chile)	Johnson & Franklin 1991	F402	109	282
Torres del Paine (Chile)	Johnson & Franklin 1991	F472	115	516.33
Torres del Paine (Chile)	Johnson & Franklin 1991	F508	33	347
Lihué Calel (Argentina)	Pereira *et al.* 2012	OG04F	70	25.75
Lihué Calel (Argentina)	Pereira *et al.* 2012	OG42F	19	68.9
Lihué Calel (Argentina)	Pereira *et al.* 2012	OG29F	63	151.15
Lihué Calel (Argentina)	Pereira *et al.* 2012	OG37F	46	434.05
Lihué Calel (Argentina)	Pereira *et al.* 2006	07F	26	130.1
Lihué Calel (Argentina)	Pereira *et al.* 2006	09F	23	214
Lihué Calel (Argentina)	Pereira *et al.* 2006	12F	28	622.9
E. Tornquist Provincial (Argentina)	Manfredi *et al.* 2012 (Manfredi 2006)	F6	135	701
Campos del Tuyú (Argentina)	Manfredi *et al*. 2006	F1	110	243
Campos del Tuyú (Argentina)	Manfredi *et al*. 2006	F2	42	134
Serra do Caverá (Brazil)	Present Study	F1	35	75.0
Serra do Caverá (Brazil)	Present Study	F2	133	187.0
Serra do Caverá (Brazil)	Present Study	F3	69	62.8

Appendix S2. Mean body weight (kg) of males and females (mean ± *SD*) from our study and previous home range studies of Geoffroy’s cat.

	Site	Body weight Studies	M	SD (M)	N (M)	F	SD (F)	N (F)
1	Torrel del paine (Chile)	Johnson & Franklin 1991	4.94	0.92	5	4.1	0.71	4
2	Los Alerces (Argentina)	Lucherini et al. 2006	4.5	-	1	3.2	-	1
3	Lihué Calel (Argentina)	Pereira et al. 2006, 2012	3.92	0.45	14	2.88	0.35	4
4	E. Tornquist Provincial (Argentina)	Manfredi 2006; Lucherini et al. 2006	5.18	0.68	4	3.33	0.48	4
5	Coronel Dorrego (Argentina)	Castillo et al. 2008	6.6	-	1	-	-	0
6	Campos del Tuyú (Argentina)	Manfredi 2006; Lucherini et al. 2006	7.4	0.57	2	4.2	0.17	3
7	Northern Uruguay (Uruguay)	Ximenez, 1973; Lucherini et al. 2006	3.7	-	5	3.1	-	5
8	Southern Brazil (Brazil)	Lucherini et al. 2006	4.6	0.61	14	3.48	0.51	9
9	Serra do Caverá (Brazil)	Present Study	4.63	0.57	5	3.48	0.35	5

Appendix S3. Capture efforts of Geoffroy’s cat and other Carnivora species at our study site.

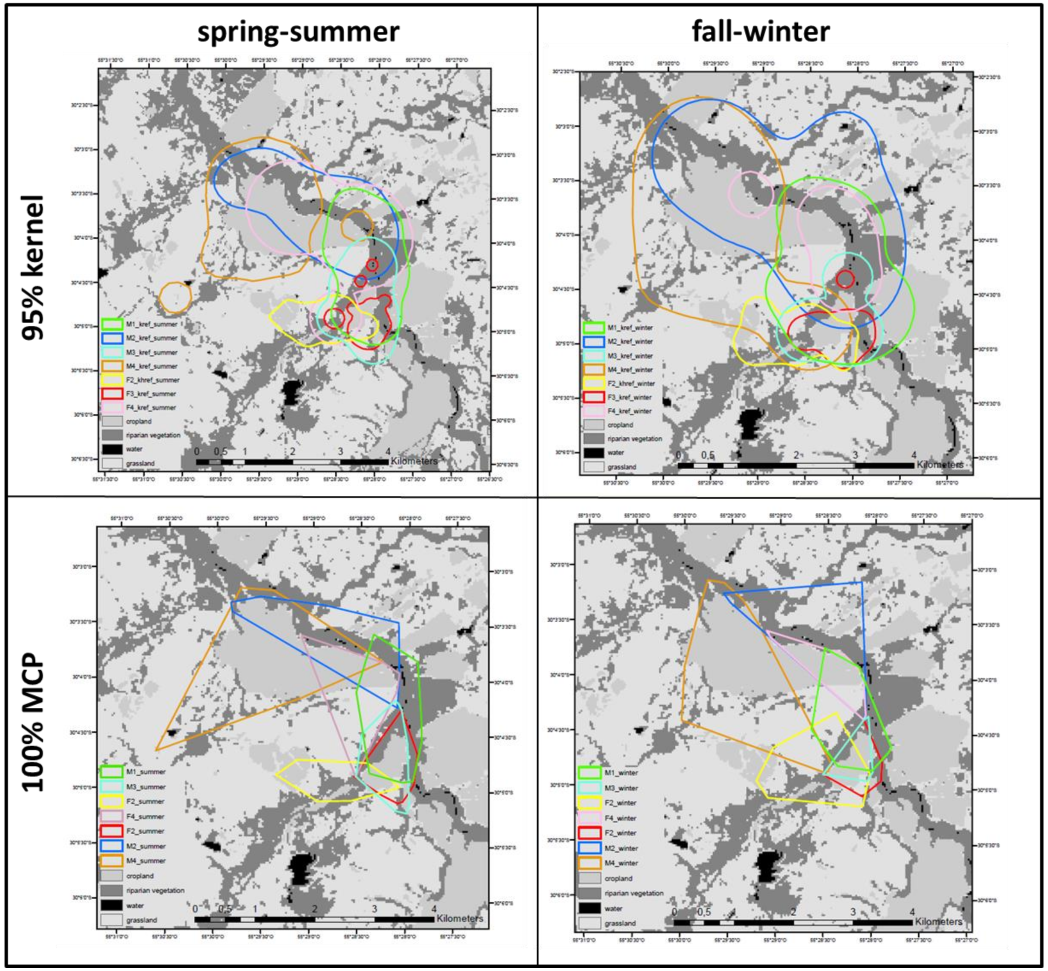
Year	Day-trap	Different individuals of Geoffroy's cats captured	Total Geoffroy's cats captured	Total Carnivora cats captured
May 05 to 10, 2014	30	1F	1F	1 *Cerdocyon thous*
June 17 to 29, 2014	168	2M e 1F	2M e 1F	1 *Cerdocyon thous*
September 09 to 17, 2014	56	1M	2M	1 *Lycalopex gymnocercus*
November 03 to 12, 2014	63	1F	2F e 2M	0
December 12-19, 2014	49	1M	1M	1 *Lycalopex gymnocercus*
February 11-20, 2015	63	2M	5M	0
July 06 to 18, 2015	84	2F e 1M	4F e 2 M	1 *Lycalopex gymnocercus*
July27 to August 06, 2015	70	-	1M	0
TOTAL	583	12	23	5

Appendix S4. Home range overlap for pairs of captured individuals (including two male juveniles [M5 and M6]) using 95% kernel hB; values range between 0 (no overlap) and 100% (complete HR overlap).

	F1	M1	F2	M2	F3	M3	M4	M5	F4	F5	M6
F1	-	60	14	21	49	47	23	56	49	19	50
M1	65	-	11	15	38	84	12	37	41	05	81
F2	31	23	-	25	20	20	23	25	26	23	23
M2	21	14	11	-	11	12	19	32	22	22	37
F3	95	70	18	21	-	53	21	81	44	23	51
M3	48	79	09	13	28	-	05	39	32	15	92
M4	32	16	15	26	15	07	-	36	30	33	28
M5	41	26	09	24	31	29	19	-	32	24	67
F4	49	39	12	22	22	31	22	43	-	29	60
F5	24	06	13	27	15	18	30	41	36	-	43
M6	29	44	06	22	15	53	12	52	35	21	-

Appendix S5. Core area overlap for pairs of captured individuals (including two male juveniles [M5 and M6]) using 50% kernel hB; values range between 0 (no overlap) and 100% (complete HR overlap).

	F1	M1	F2	M2	F3	M3	M4	M5	F4	F5	M6
F1	-	16	06	00	12	25	10	00	13	02	00
M1	17	-	00	01	00	45	00	00	00	00	45
F2	21	00	-	00	00	00	00	00	17	00	00
M2	00	01	00	-	00	01	00	16	07	10	00
F3	45	00	00	00	-	00	18	00	00	09	00
M3	27	43	00	01	00	-	00	00	00	00	29
M4	19	00	00	00	10	00	-	00	00	10	00
M5	00	00	00	18	00	00	00	-	09	18	00
F4	17	00	06	08	00	00	00	09	-	05	03
F5	04	00	00	13	04	00	07	22	05	-	05
M6	00	28	00	00	00	19	00	00	02	02	-



Appendix S6. Home range size of Geoffroy's cat in two different seasonal partitions (spring-summer and fall-winter) using two distinct estimators (95% kernel and 100% MCP) at our study site.

Appendix S7. Home range (HR) and core area (CA) size of Geoffroy's cats at our study site, estimated for two different seasonal partitions (spring-summer and fall-winter), using two distinct estimators: 95%(HR) and 50% (CA) kernel and 100% (HR) and 50% (CA) MCP.

ID	50% MCP	100% MCP	50% kernel hB	95% kernel hB
F2_summer	12.5	97.5	32.0	163.3
F3_summer	2.1	89.9	12.6	100.5
$\bar{X}$	7.3	93.7	22.3	131.9
SD	7.3	5.4	13.7	44.4
F2_winter	15.6	195.3	31.0	183.3
F3_winter	5.4	68.3	20.0	11.2
$\bar{X}$	10.5	131.8	25.5	97.2
SD	7.2	89.8	7.8	121.7
M1_summer	43.3	206.4	105.0	447.1
M2_summer	79.0	250.5	135.7	596.3
M4_summer	70.8	455.3	146.1	674.4
$\bar{X}$	64.4	304.1	128.9	572.6
SD	18.7	132.8	21.4	115.5
M1_winter	63.2	172.5	160.2	584.2
M2_winter	73.8	279.9	261.6	1062.7
M4_winter	99.6	414.9	207.6	1005.4
$\bar{X}$	78.9	289.1	209.8	884.1
SD	18.7	121.4	50.7	261.3

Appendix S8. Overlap of home range from Geoffroy's cat individuals at our study site in the spring-summer seasonal partition. Values range between 0 (no overlap) and 100% (complete HR overlap).

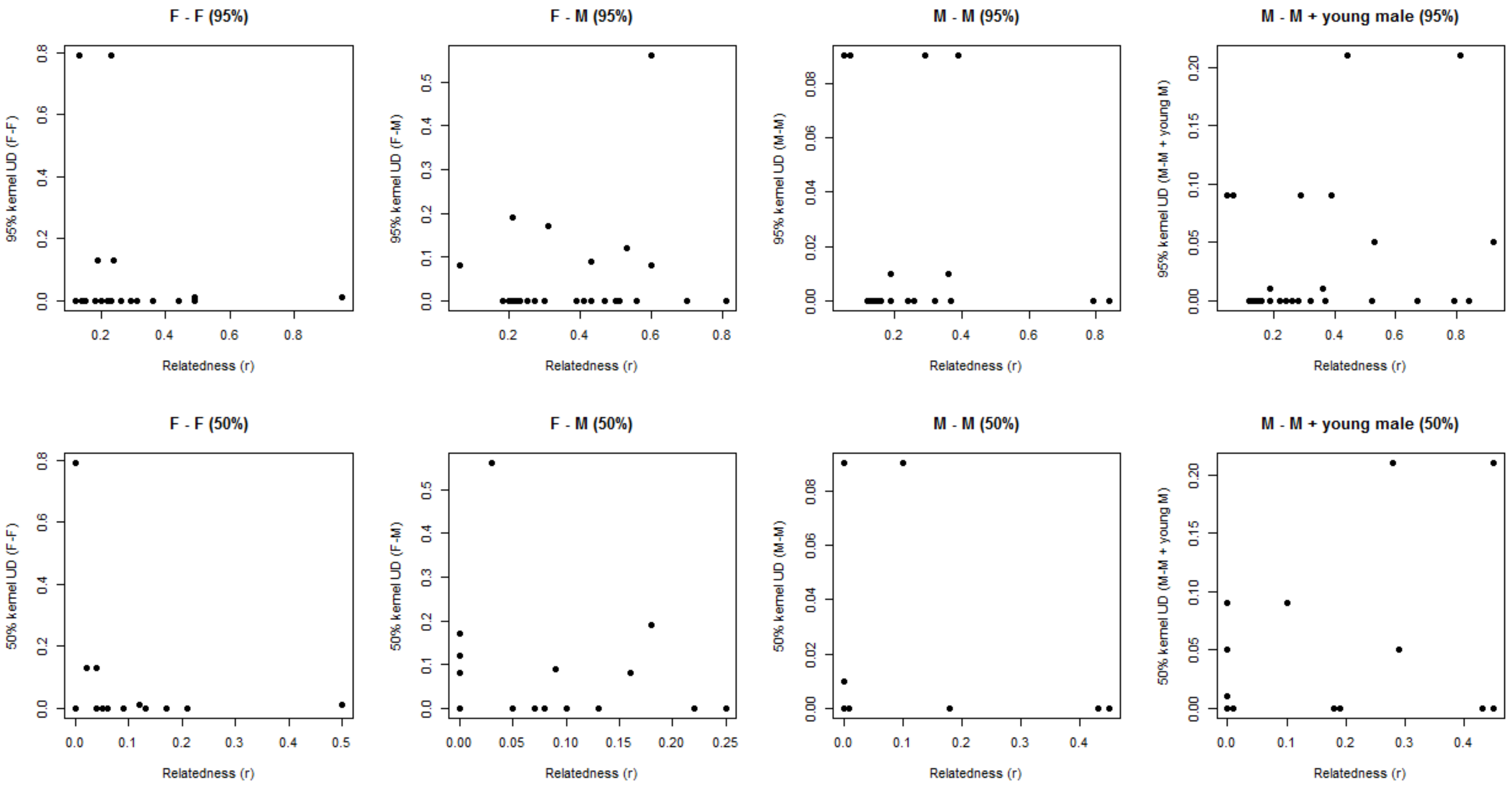
	F2	F3	F4	M1	M2	M3	M4
F2	-	04	37	28	05	30	01
F3	04	-	64	31	35	55	20
F4	11	21	-	27	22	40	16
M1	15	17	48	-	14	86	11
M2	03	19	38	13	-	26	18
M3	13	25	57	70	22	-	17
M4	01	18	43	18	29	32	-

Appendix S9. Overlap of home range from Geoffroy's cat individuals at our study site in the fall-winter seasonal partition. Values range between 0 (no overlap) and 100% (complete HR overlap).

	F2	F3	F4	M1	M2	M3	M4
F2	-	23	04	43	30	48	35
F3	17	-	13	91	28	1	68
F4	02	09	-	17	57	41	14
M1	15	45	12	-	29	76	65
M2	08	10	30	22	-	32	17
M3	13	35	21	55	31	-	38
M4	18	48	14	94	33	75	-

Appendix S10. Pairwise relatedness index (*r*) values for Geoffroy's cat individuals sampled at our study site, estimated from microsatellite data using the software ML-Relate. All results were indicative of unrelated pairs, except in the cases with $r \geq 0.19$, whose relationships were inferred to be as follows: PO: parent-offspring; FS: full-sibling; HS: half-sibling.

	F1	M1	F2	M2	F3	M3	M4	M5	F4	F5	M6
F1	1										
M1	0.08	1									
F2	0	0	1								
M2	0	0	0	1							
F3	0.01	0	0	0	1						
M3	0	0	0	0	0.12	1					
M4	0	0	0	0	0.19(HS)	0.09	1				
M5	0	0	0	0	0	0.09	0.01	1			
F4	0	0	0	0	0	0.17	0	0.09	1		
F5	0.13	0.08	0.79(FS)	0	0	0	0	0	0	1	
M6	0	0.21(HS)	0	0	0	0.05	0	0	0.56(PO)	0	1



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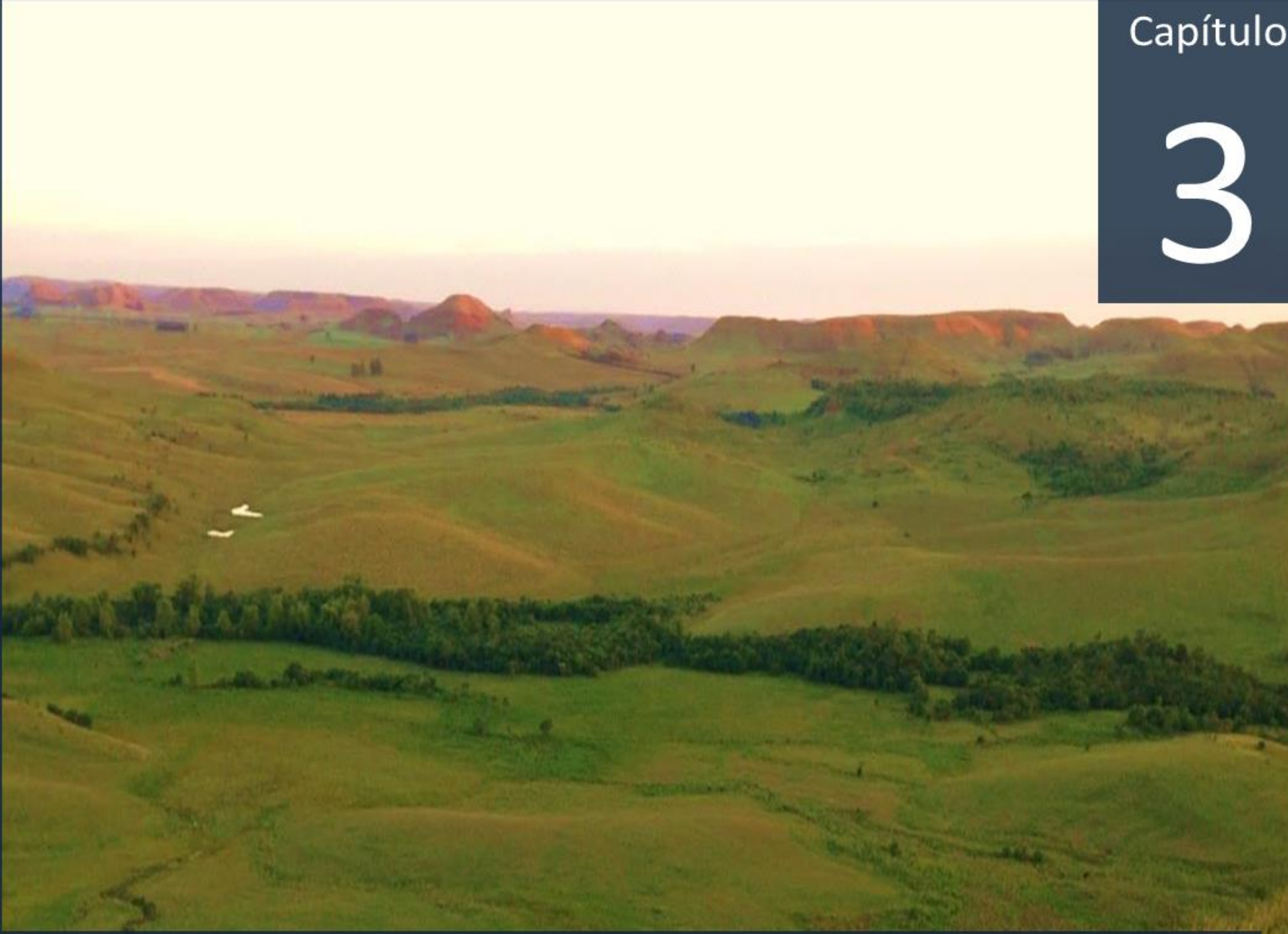

An assessment of factors underlying the persistence of Geoffroy's cat populations in human-dominated landscapes in the Brazilian pampas

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An assessment of factors underlying the persistence of Geoffroy's cat in human-dominated landscapes in the Brazilian pampas

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Abstract

Geoffroy's cat is small Neotropical felid that seems to be able to adjust its population density and behavioural patterns depending on the ecological context. We investigated which factors influence the persistence of the species in one of the most threatened biomes in Brazil, the Pampas. We recorded 516 camera-trap images of Geoffroy's cat and twelve individuals were captured using box-traps. Of those, seven adults were radio-collared (4M and 3F), and monitored through VHF telemetry for a cumulative period of 16 months (434 locations). The density estimated using SECR ranged from 34.54 [±13.51 SE] to 41.78 [±16.12 SE] individuals/100km², reasonable values in comparison to other areas. Although Geoffroy's cat is considered an open-area species, our study population significantly selected riparian vegetation (D=0.45, P<0.01 [95%Kernel]; D=0.48, P<0.01 [95%MCP]), and avoided open grassland (D=-0.59, P<0.01 [95%Kernel]; D=-0.51, P<0.01 [95%MCP]). Since the protection of riparian vegetation in private lands is required by Brazilian national law, this habitat may play an important role in the context of the long-term persistence of Geoffroy's cats in this region. With respect to activity patterns, this population was significantly nocturnal (Z=8.03, P<0.01). We observed a slight difference between seasonal partitions (Δ_4=0.79 [CI 0.71-0.85]), with the proportion of nocturnal activities increasing during spring-summer. When the activity patterns were assessed in different habitats, the use of grasslands and croplands (non-protected areas) was significantly non-uniformly distributed through the hours of the day ($U = 202.32$, $P < 0.01$; $U = 186.12$, $P < 0.05$, respectively). This significance was not found for riparian vegetation ($U = 116.04$, $0.9 > P < 0.5$), suggesting that activity in this habitat is more uniform than in the other ones. The results obtained in this first study of density and behavioural patterns of Geoffroy's cat in Brazil should contribute to the design of conservation and management actions on behalf of this species in this threatened biome.

Keywords: *Leopardus geoffroyi*, Brazilian Pampas, farmlands, density, habitat selection, activity patterns.

Introduction

Behavioural and ecological features of wild animal populations influence their response to human disturbance, which in turn has implications on their long-term persistence in the face of anthropogenic changes to their habitats (Frid & Dill, 2002; Ciuti *et al*., 2012). While some species are driven to extinction rather quickly in human-dominated landscapes, others persist, raising the issue of what biological features underlie such discrepant outcomes. At the same time, the type and level of human disturbance on wildlife can vary widely among regions, and depends on various aspects of land use that are driven by economic pressures, public policies and regulations.

Brazil is a developing country (IMF, 2015) that harbours one of the largest animal diversities in the world (Lambertini, 2000). It comprises several widely distinct terrestrial biomes, from the equatorial forests of the Amazon region to the poorly investigated Pampas in the more temperate, southernmost tip of the country. The Pampas is one of the most threatened biomes in Brazil, with ~97% of its surface located in non-protected areas (MMA, 2007) that are mainly used for cattle ranching on native grasses and/or for growing crops such as soybeans and rice (Pillar *et al*., 2009). Nonetheless, even in such private properties, stretches of riparian vegetation are protected under Brazilian law due to their relevance in preventing soil erosion, mitigating floods, protecting marshes, and sheltering native flora and fauna (Brazilian Federal Law 12.651/2012).

Seven species of wild felids have been historically recorded in the Brazilian Pampas (IUCN, 2015). Of these jaguar, *Panthera onca* is already considered extinct in this region (Sanderson *et al*., 2002), and little information is available on the current distribution and status of the other species (IUCN, 2015). The most frequently sighted felid in this region (personal observation from the authors) is Geoffroy’s cat (*Leopardus* *geoffroyi*), a solitary small cat categorized by the IUCN as "Least Concern" (Pereira *et al.*, 2015), which occurs in savannas, grasslands and scrublands from Bolivia and southernmost Brazil to the southern tip of South America (Macdonald & Loveridge, 2010; Cuyckens *et al*., 2015). Previous studies have estimated very different population densities for this species across field sites located in Bolivia, Argentina and Chile (W. Johnson, pers. comm. in Nowell & Jackson, 1996; Cuellar *et al*.2006; Pereira *et al*., 2011; Caruso *et al*., 2012). Moreover, in some areas the species is very rare (Castillo et al. 2008), and it is still unclear what factors underlie such variation, hampering a precise assessment of population trends and conservation status.

There are also open questions regarding habitat use by this species. Even though it tends to range mostly in ecoregions characterized by open areas, such as Humid and Semi-arid Pampas, Puna grassland, or Uruguayan Savannah, previous studies have reported that it can use open as well as closed habitats, with preference varying depending on the scale that was assessed (Johnson & Franklin, 1991; Manfredi *et al*., 2006, 2012; Pereira *et al*., 2006, 2012, Caruso *et al*., 2016). Regarding activity patterns, this species seems to be more crepuscular and nocturnal than diurnal (Johnson & Franklin, 1991; Cuellar *et al*., 2006; Manfredi *et al*., 2011; Pereira *et al*., 2011), although it seemed to switch to daytime activity during a period of food shortage (Pereira, 2010). Taken together, these observations suggest that this felid is able to adjust its density and behavioural patterns depending on the ecological context. These features may underlie its apparent resilience to human impacts, and characterizing them in quantitative detail, especially in disturbed habitats, is thus required to make more precise conservation assessments for this species.

To address these issues, we aimed to estimate population density and to characterize behavioural patterns of Geoffroy's cats in a human-dominated landscape of the Brazilian Pampas. Our specific objectives were: (i) to estimate population density using different data and methods, and compare it with previous estimates for other areas; (ii) to test if Geoffroy’s cats use different habitats uniformly in the study area; (iii) to measure activity patterns, testing its uniformity and its variation between seasonal partitions; and, finally, (iv) to assess the activity patterns in the different habitats of the focal area. By achieving these objectives, the present study provides novel insights into the ecology of this felid, which can contribute to the design of management actions that promote its long-term conservation in human-dominated landscapes.

Materials and Methods

Study area

The study was conducted from November 2013 to September 2015 in two contiguous, privately owned areas (30°04’S, 55°31’W), in the Pampas ecosystem of southernmost Brazil. The study area comprised the surroundings of a stream (‘Arroio Caverá’) and consisted of a mosaic of remaining natural habitats, cattle pastures and croplands (Fig. 1). The native vegetation is characterized by a diversity of grasslands (e.g., *Aristida murina* on shallow soils and *Paspalum notatum* on deeper soils) (Boldrini, 2009) and by riparian forests adjacent to the stream, represented mainly by *Sebastiania schottiana*, *Phyllantus sellowianus*, *Pouteria salicifolia* and *Syagrus romanzoffiana* (Guadagnin et al., 2015). All riparian vegetation is contained in legally protected (‘permanent preservation’) areas, and most of the cattle grazing at the site is conducted on native grasses. Livestock management practices are typical for the region, with a cattle density of ~0.01 individuals per km² (1 head of cattle per hectare [ha]), and human activity (using domestic dogs) occurring every day, during daytime and the entire year. In the areas used for agriculture (*ca.* 40% of the properties), soybean and rice are sowed through the summer and exotic grasses (oat and ryegrass) are sowed during the winter to serve as artificial pasture for cattle. These activities involve the use of tractors along with standard cattle management practices. The annual rainfall average for the study area is 1500mm, and the temperature average is 13°C in winter and 24°C in summer (Nimer, 1989).

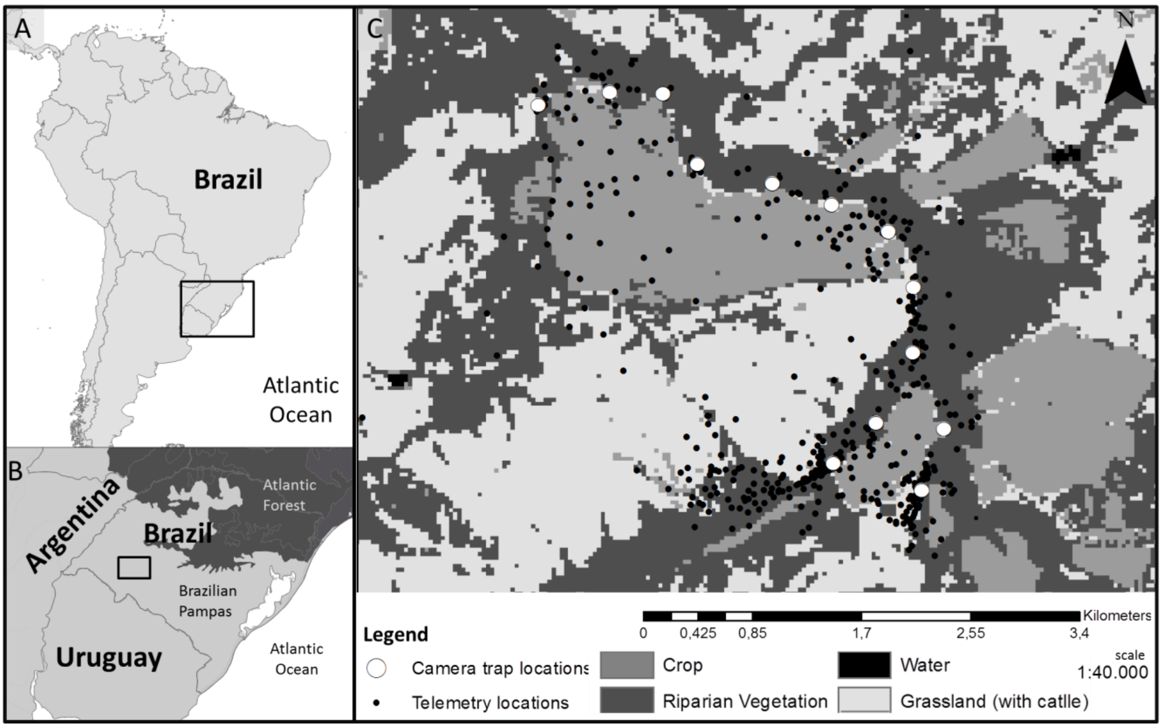


Figure 1. Location of the study site. A. Location of study area in South America. B. Location of the study site on a regional scale; light grey represents the Pampas biome and dark grey represents the Atlantic Forest biome. C. Study site habitat composition, camera trap layout and telemetry locations of radio-collared Geoffroy’s cats.

Live capture and radio telemetry

From May 2014 to August 2015, Geoffroy’s cats were live-trapped using Tomahawk box-traps baited with live domestic chickens (in a segregated compartment; see Tirelli et al., in prep. [Capítulo 2] for details). Throughout capture campaigns, traps were inspected and maintained (cleaning the enclosure and feeding the chickens) every day. Trapped Geoffroy's cat individuals were immobilized with Zoletil® (Virbac) at the intentional dose of 8-10 mg/kg (Manfredi *et al*., 2006). During handling, the eyes of the animals were moistened with Paralube® to prevent dryness of the cornea, and bands and earplugs were used to reduce visual and sound stimuli. The heart, respiratory rates and temperature were monitored every 5-10 minutes. We photographed and collected multiple external measurements of each captured individual, and also recorded its sex, age class, reproductive stage, weight and health status. All capture and animal handling procedures followed the recommendations of the Manual of Capture and Handling (Sikes *et al*., 2011), were approved by an institutional animal care and use committee (CEUA-PUCRS), and were carried out at all times under the supervision of a certified veterinarian. In addition, appropriate biosecurity techniques and personal protective equipment were employed during the procedures. The study was conducted under permit SISBIO-36803, issued by the Brazilian Ministry of the Environment.

During the period from May 2014 to February 2015 we fitted all captured adult Geoffroy's cats with VHF radio-collars equipped with activity and mortality sensors (Telonics, Mesa, Arizona; MOD-080-2). Telemetry monitoring of these individuals was carried out from May 2014 to September 2015 at different times of the day, aiming to obtain at least three data points (including location and activity) for each individual in each 1-hr period within the 24hrs cycle. For each data point, the spatial location of the individual was estimated by triangulation, using at least three different bearings (taken at most 10 min apart) per point. Activity readings relied on the activity sensor built into each radio-collar, which differentiates active (>43 beats per minute [BPM]) from non-active ($\leq$ 43 BPM) states. The threshold between the two states was defined empirically by cross-validation between the activity reading and direct observation of individuals during this study. We recorded the activity state of each individual every time the location bearing was collected, and then calculated the average activity (in BPM) for that particular data point. This average activity value indicated if the cat was active or non-active for each data point.

Photographic records

Camera trap data were collected from November 2013 to September 2015, with a gap in sampling between March and May 2014. We employed 26 digital camera traps (20 Scout Guard, 4 Bushnell and 2 Moultrie), which were positioned *ca.* 30 cm above the ground, with no lure. Cameras were active 24h per day in video mode (20s), and were checked weekly to ensure functionality. All records included date and time.

We employed two different strategies to collect camera-trap data. Surveys performed from January to August 2015 used paired cameras set up at 13 stations (Fig. 1), either inside or immediately outside the riparian vegetation, since cats could transit between both habitats. For the remaining time of the survey (2013-2014 and September 2015), cameras were placed either individually or in pairs, covering a broader portion of the study area and aiming to collect data that were used for the analysis of activity patterns (Supporting information Fig. S1). During the periods focused on activity surveys, cameras were preferentially placed in areas bearing evidence of felid activity (tracks, faeces and/or radio-telemetry data). In every case, the spacing between stations (containing one or two cameras) was maintained at *ca.* 600m.

Data analysis

Density estimates

Two standardized survey periods were delimited from within our continuous camera-trapping effort to estimate population density at our study site. One of them targeted the summer (January and February 2015) and the other the winter (end of June to the beginning of August 2015). Both periods were limited to 48 days to meet the assumption of population closure (Balme *et al.*, 2009). For this analysis, each camera-trap record was examined carefully and only those for which it was possible to discern the spotting pattern on both flanks and/or on other body parts (*e.g.* forehead markings or tail stripes) were kept in the dataset. These patterns were then drawn, producing a reference database that was used for individual identification. To maintain temporal independence between individual captures, each individual was counted only once per camera station in a 24-hr period.

Density was estimated with Spatially Explicit Capture–Recapture (SECR) models (Efford & Fewster, 2013; Royle *et al.*, 2014), using the package 'secr' (Efford, 2016a) in R 3.2.3 (R Development Core Team, 2015). Since this approach allows density to be estimated by combining different types of data (Efford, 2016a and 2016b), we complemented our camera-trapping records with animal locations determined by radio-telemetry. Since capture probabilities may be influenced by sex, age, and particular behavioural features, we used six different models including combinations of these variables to represent capture history (see Results), and tested their relative performance using the Akaike Information Criterion (Akaike, 1974). In addition, to allow direct comparisons between our density estimates and others reported previously (Cuellar *et al.*, 2006; Pereira *et al.*, 2011; Caruso *et al.*, 2012), we also estimated population density using telemetry data only (Caruso *et al.*, 2012), employing the traditional Capture-Recapture methods using MMDMOSA, MMDM, HMMDM, and home range (HR) radius to estimate the size of the sampled area (Supporting Information S1).

Home-range estimation

We used the telemetry data to estimate the HR for each of the captured individuals, employing both kernel and minimum convex polygon (MCP) approaches. The kernel estimation of utilization distribution (UD) (Worton, 1989) with a smoothing parameter (*h*) called "reference bandwidth" (Wand & Jones, 1994) incorporated 95% of the sampled points. The MCP approach (Burt, 1943) also considered 95% of the sampled points, and was performed to allow direct comparisons with previous studies reporting Geoffroy's cat HR sizes in other areas. All analyses were carried out with the 'adehabitatHR' (Calenge, 2006) package available for R software 3.2.3. Two additional R packages, 'sp' (Pebesma, 2005) and 'maptools' (Bivand, 2016) were used to work with spatial data and to create shapefiles of the home ranges.

Habitat selection

We analysed habitat selection patterns using telemetry data (fixes) and the proportion of available habitat types, considering two different spatial scales (Johnson, 1980), as implemented by Pereira *et al.* (2012): (A) a joint HR for the study area, estimated from the fixes of all sampled individuals; and (B) each individual HR assessed separately. For both scales, we estimated the HRs using both the 95% kernel and 95% MCP approaches (White and Garrott, 1990).

We downloaded a Landsat 7 TM satellite image (in rasterfile) of the study site from the National Institute of Space Research, Brazil (*Instituto Nacional de Pesquisas Espaciais* – INPE), and used ArcGIS 10.4.1 (ESRI, 2016) to generate a supervised classification of its habitats using a maximum likelihood approach (Patil *et al.*, 2012). All locations and the estimated areas of the two spatial scales were then plotted on the map, and we calculated the proportion of fixes in each habitat type and the proportion of each habitat available on each scale.

To test whether Geoffroy's cats used habitats uniformly in this area or exhibited preference for one or more habitat types, we used Jacobs's preference index (*D*) (Jacobs, 1974) for both spatial scales. This index is based on the following equation: $D = (r - p)/(r + p - 2rp)$, with r representing habitat use (*i.e.* proportion of location fixes in a given habitat) and p representing habitat availability (*i.e.* proportion of that habitat type within the areas *A* or *B*, depending on the assessed scale). Values of *D* closer to -1 indicate habitat avoidance, whereas values closer to +1 indicate preference. The distribution of *D* among individuals (spatial scale B) for each habitat type was assessed for normality with a Shapiro-Wilk test (Supporting Information Table S1), and then used to test for deviation from the null hypothesis of no habitat preference (*i.e.* $D = 0$). This was performed with a one-sample Student's *t*-test (Hayward *et al.*, 2006; Kauhala & Auttila, 2010), carried out in software R 3.2.3, using a 99% confidence interval generated from the observed data.

Activity patterns

We investigated activity patterns using both camera trap and telemetry data. For the camera traps, the total sampling effort was estimated by multiplying the number of survey days by the number of functional camera traps per day. We only included images obtained at least one hour apart from each other, which were then assumed to represent independent records. We estimated activity patterns during a 24-hr period considering both the total survey period and two separate seasonal partitions: spring-summer (October to March) and fall-winter (April to September). These partitions were defined based on their distinct patterns of sunrise and sunset at the study site during the survey period (Supporting Information Table S2), which were considered in downstream analyses (see Results).

We used two analytical approaches for both the total survey period and seasonal partitions: the Ridout and Linkie (2009) methods and Rayleigh's Uniformity test (Fisher, 1993). We initially used the Ridout and Linkie (2009) methods to fit Von Mises kernel density functions to times of observations of Geoffroy's cat in the total survey period and in the seasonal partitions and to estimate the coefficient of overlap (Δ_4) between the two seasonal partitions (the coefficient ranges from 0 [no overlap in activity patterns] to 1 [complete overlap]). These analyses were performed using the package 'overlap' (Meredith & Ridout, 2014) in R 3.2.3. In addition, we used Rayleigh's Uniformity test (implemented in the software Oriana 4.02 [Kovach, 2013]) to calculate the probability of the null hypothesis that the data were distributed uniformly, for both the total survey period and the seasonal partitions.

For the telemetry-based analyses, we considered the proportion of activity or inactivity, which was obtained by dividing the number of 'active' or 'inactive' data points by the total number of data points in each 1-hr period (see above). In this case, we only used Rayleigh's Uniformity test, since the Ridout & Linkie (2009) methods are not applicable to the proportional approach employed here.

Integrating habitat selection and activity patterns

To assess whether Geoffroy's cat activity was uniformly distributed throughout the hours of the day in the different habitat types, we carried out additional analyses using the software Oriana 4.02. Since our camera-trap layout did not sample the different habitat types equally, for these analyses we used only telemetry data. To test for uniformity throughout the hours of the day in each habitat, we used Rao's Spacing Test, and to test if activity patterns were significantly different among habitats, we employed Watson's U^2 test.

Results

Data collection

Twelve Geoffroy's cat individuals were captured during this study, seven of which (four males and three females) were radio-collared and monitored (Table 1). The four additional individuals were not monitored, since one was killed by domestic dogs two days after its capture, the other was a sub-adult, and the other two were captured in the last campaign (re-capture campaign which was focus in to collect the collars of the monitored cats). Also, one of the monitored females died two months after its capture. The overall camera trap sampling effort was 8,845 trap-days, resulting in 522 felid images recorded. Of these, 516 (98.85%) were Geoffroy's cats, two (0.38%) were margays (*Leopardus wiedii*) and four (0.77%) were feral domestic cats (*Felis catus*).

Table 1. Home range sizes (km^2) calculated though two different estimators for two spatial scales: A. Joint HR for the study area, estimated from the fixes of all sampled individuals; and B. Each individual HR assessed separately.

Spatial Scale	Specimen	N fixes	95%kernel	95%MCP
A	All	434	12.42	9.23
B	M1	59	4.66	2.24
	M2	54	2.79	1.09
	M3	23	3.42	0.75
	M4	62	8.95	3.08
	F1	34	1.56	0.5
	F2	133	1.83	1.07
	F3	69	0.66	0.3

Density

The SECR density estimate was completed using two data sets ('camera-trap only' and 'combined camera-trap + telemetry') for two different surveys (summer and winter). Each survey comprised 624 camera-trap-days. In the summer survey, we identified 11 different Geoffroy's cat individuals (four males and seven females) in 49 independent camera-trap captures. For five of them (three males and two females), we obtained telemetry data (37 total points) that were incorporated in the combined density estimates (Table 2). In the winter survey, we recognized 11 distinct individuals (five males and six females) in 33 independent camera-trap captures; nine individuals (three males and six females) overlapped with those identified in the summer. In this case, we could only include telemetry data from two females (17 total fixes), which likely biased the combined density estimates for this season (see below and Table 2).

Table 2. Density estimates (individuals per 100 km²) and standard errors (SE) derived from camera-trap data (CT only) and combined camera-trap and telemetry data (CT+TL), in two surveys (summer and winter) using six different models (see Supporting information Table S3).

Model	CT only		CT+TL	
	Summer[a] ± SE	Winter[b] ± SE	Summer[c] ± SE	Winter[d] ± SE
Bk	41.78 ± 16.12	41.44 ± 19.20	34.54 ± 13.51	63.22 ± 24.77
Bk	38.88 ± 14.14	36.45 ± 14.92	29.64 ± 11.18	55.77 ± 20.73
Sex	42.79 ± 16.08	36.98 ± 14.77	31.40 ± 12.04	52.13 ± 19.48
0	38.31 ± 13.84	37.04 ± 14.76	29.03 ± 10.91	54.89 ± 20.25
H	38.31 ± 13.84	37.04 ± 14.76	30.94 ±11.92	63.44 ± 24.73
B	45.27 ± 18.01	34.72 ± 14.78	35.16 ± 14.35	55.21 ± 21.90

[a] Derived from information on 11 individuals.
[b] Derived from information on 11 individuals, nine of which overlapped with the summer survey.
[c] Derived from camera-trap information on 11 individuals and telemetry location data from five of them (3 males and 2 females).
[d] Derived from camera-trap information on 11 individuals and telemetry location data from only two of them (both females).

The trap-specific model (bk), was the spatially explicit model that provided the best fit to both types of data in both surveys; also, the ΔAIC was >2 (in the majority of models) for the second most likely models (Supporting information Table S3). For the camera-trap data set, the capture probability (*g0* ± *SE*) was 0.03 ± 0.0132 for the summer and 0.01 ± 0.006 for the winter, while the movement parameter ($\sigma \pm SE$) was 883.4 m ± 152.8 m in the summer and 1046.5 m ± 244.5 m in the winter. When the combined data set was considered, the telemetry information yielded an average HR size ($\bar{X} \pm SD$) of 3.53 km² ± 3.2 for the summer and 1.24 km² ± 0.83 for the winter. The estimated capture probability at the home range centre ($g0 \pm SE$) was 0.02 ± 0.006 for the summer and 0.02 ± 0.009 for the winter, while the movement parameter ($\sigma \pm SE$) was 774.90 m ± 50.65 m for the summer and 595.80 m ± 64.95 m for the winter.

In addition to the SECR approach, we also estimated density using telemetry data only, as well as traditional capture-recapture methods based on camera-trap or combined data (see Supporting Information S1 for details). Most of these estimates overlapped with those obtained with the SECR method, and provided a framework for direct comparisons with results from previous studies (Table 3).

Table 3. Comparison of density estimates for Geoffroy’s cat obtained in this study with those reported previously for other areas.

	Present study	**Nowell and Jackson 1996**[d]	**Cuellar et al. 2006**	**Pereira et al. 2011**		**Caruso et al. 2012**
Location	Privately owned areas	Torres Del Paine National Park	Kaa-Iya del Gran Chaco National Park	Lihue Calel National Park	Privately owned areas	Privately owned areas
Ecoregion	Uruguayan Savanna	Magellanic subpolar forest	Dry Chaco	Low Monte-Espinal		Espinal
Country	Brazil	Chile	Bolivia	Argentina		Argentina
Human impact [a]	medium	low	low	low	high	Medium
Other felids recorded	Margay, feral cats (rare)	Puma	Jaguar, puma, jaguarundi, ocelot, margay	Pampas cat and jaguarundi (rare)	none	Pampas cat
Suitability [b]	0.25-0.50	0.25-0.50	0.25-0.50	>0.50		>0.50
Density estimates – individuals/100km²						
SECR (CT+ Telemetry)	34.54 (16.48 – 72.37) and 63.22 (30.14 – 132.58)[c]	-	-	-	-	-
SECR (CT only)	41.78 (20.14 – 86.70) and 41.44 (17.46 – 98.36)	-	-	-	-	45 (22.95-88.28)
Telemetry only	32.91	7 - 12	-	-	-	50.22
MMDMOSA	32.44 (32.44 – 36.05) and 43.25 (43.25 – 108.15)	-	-	-	-	-
HR radius	51.16 (51.16 – 56.85) and 68.22 (68.22 –170.553)	-	-	140 (94-274)	240 (189-321) and 230 (155-374)	21.94 (21.94 - 60.31)
MMDM	38.05(38.05 – 42.281) and 50.74 (50.74 – 126.85)	-	-	100 (66-191)	120 (98-167) and 160 (109-262)	16.21 (16.21-44.56)
HMMDM	87.89 (87.89 – 97.65) and 117.18 (117.18 – 292.97)	-	9.52 - 42.11	190 (122-355)	220 (170-290) and 290 (197-473)	24.18 (24.18-66.50)

[a] Definition: low (reserve areas), medium (privately owned areas with $\leq$0.01cattle per km²), high (privately owned areas with 9 to 21cattle per km²);

[b] Cuyckens *et al.* (2015);

[c] In winter we could only include telemetry data from two females which likely biased the combined density estimates.

[d] W. Johnson, pers. comm. in Nowell and Jackson (1996).

Habitat use

We classified the landscapes present at the study site into four habitat categories: riparian vegetation (RV), grassland (GS), cropland (CR) and water; only the former three categories were used in the habitat selection analysis (Table 4). The proportion of available habitats and the proportion of locations in each habitat tended to be similar for both spatial scales (Table 5). On spatial scale 'B', the proportion of habitat types in the HRs of the seven individuals was similar using both estimators (kernel and MCP), and the proportion of locations per HR in each habitat type suggested a higher use of riparian vegetation, excepting for one male. The values of Jacobs's index supported this hypothesis, indicating that riparian vegetation was favoured and grassland avoided on both scales (Table 6).

Table 4. Summary of the three main habitat types, the number of locations recorded from the joint data on all individuals (spatial scale A) and from each individual separately (spatial scale B) in each habitat type.

Habitat type	Vegetation structure	Scale A Total nº of locations	Scale B M1	M2	M3	M4	F1	F2	F3
Riparian Vegetation (RV)	Medium - dense canopy cover, with bushes and trees. Always following a river.	260	37	15	16	30	28	84	50
Grasslands (GS)	Natural grassland, short–medium grass, no trees or bushes, with livestock (~1 cattle head per ha)	68	10	4	6	5	2	32	9
Croplands (CR)	Crops of soy and rice in the summer. Exposed soil with or without cattle in winter.	108	12	35	2	27	5	17	10

Table 5. Proportion of habitat type (*p*) using both estimators (kernel and MCP) for all individuals combined (A) and in each home range separately (B), and and proportion of locations (*r*) in each habitat type on both spatial scales (A and B). On the scale B, mean and standard deviation values were calculated. RV - Riparian Vegetation, GS - Grasslands and CR – Croplands.

		p						*R*		
		Kernel 95%			MCP 95%					
Spatial scale	Individuals	RV	GS	CR	RV	GS	CR	RV	GS	CR
A	all	0.35	0.42	0.23	0.34	0.38	0.28	0.60	0.15	0.25
B	M1	0.33	0.41	0.27	0.28	0.30	0.42	0.63	0.17	0.20
	M2	0.34	0.34	0.32	0.36	0.17	0.47	0.28	0.06	0.66
	M3	0.36	0.42	0.22	0.34	0.34	0.32	0.67	0.25	0.08
	M4	0.38	0.41	0.21	0.28	0.59	0.13	0.47	0.07	0.46
	F1	0.30	0.33	0.37	0.29	0.29	0.42	0.80	0.06	0.14
	F2	0.35	0.54	0.11	0.34	0.47	0.20	0.63	0.24	0.13
	F3	0.37	0.27	0.37	0.41	0.27	0.33	0.72	0.13	0.14
	Mean (B)	0.35	0.39	0.27	0.33	0.35	0.32	0.60	0.14	0.26
	SD (B)	0.02	0.09	0.09	0.05	0.14	0.12	0.17	0.80	0.21

Table 6. Habitat selection using Jacobs's index[1] with kernel 95% and MCP 95%, on spatial scale A and B. On scale B, mean values, *t*-values and *p*-values resulting from Student *t*-test are shown. RV - Riparian Vegetation, GS - Grasslands and CR - Croplands.

		Kernel 95%			MCP95%		
Spatial scale	Individuals	RV	GS	CR	RV	GS	CR
A	All	0.48	-0.60	0.04	0.49	-0.53	-0.10
B	M1	0.55	-0.54	-0.17	0.63	-0.36	-0.47
	M2	-0.14	-0.79	0.61	-0.17	-0.56	0.37
	M3	0.56	-0.37	-0.51	0.59	-0.22	-0.67
	M4	0.18	-0.80	0.52	0.38	-0.86	0.68
	F1	0.80	-0.78	-0.55	0.81	-0.75	-0.62
	F2	0.52	-0.58	0.09	0.54	-0.47	-0.25
	F3	0.64	-0.42	-0.55	0.59	-0.41	-0.48
	Mean (B)	0.45	-0.59	-0.08	0.48	-0.51	-0.21
	t(B)	3.71	-8.84	-0.43	4.08	-6.16	-1.04
	P (B)	0.01	0.00	0.68	0.00	0.00	0.34

[1] The values of the selection index range from -1 (complete avoidance) to +1 (complete preference).

Activity patterns

The camera-trap data set used to estimate activity patterns comprised 513 records. The data for the total survey indicated that Geoffroy's cats were mostly active during the night, with 71.6% of the observations occurring between 19:00 and 07:00 (Fig. 2). When the seasonal partitions were assessed (240 records in summer-spring and 273 in fall-winter), the pattern remained consistent: cats were mostly active during the night in summer-spring (78.8% of observations between 20:00 and 7:00) and during nocturnal/crepuscular hours in fall-winter (72.5% of observations between 18:00 and 7:00) (Supporting Information Table S2). The coefficient of overlap in activity patterns between the two partitions was Δ_4=0.79 (*CI* 0.71-0.85) (Fig. 3).

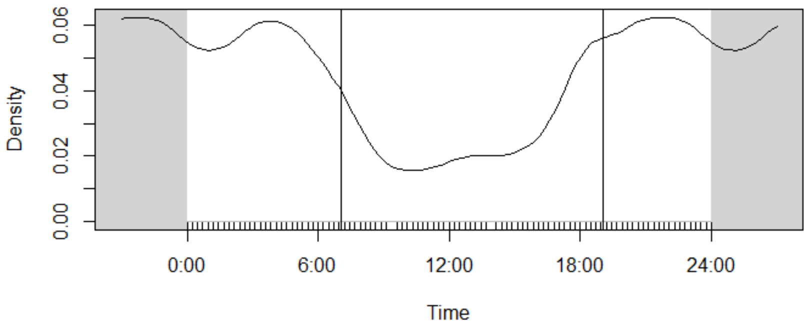


Figure 2. Kernel-density estimates of the daily activity patterns of Geoffroy's cat in the Brazilian Pampas. The short vertical lines above the x-axis indicate the times of individual photographs (n=513) and the black solid vertical lines indicate the mean time of sunrise (7:02) and sunset (19:04) for the whole year (Supporting Information Table S2).

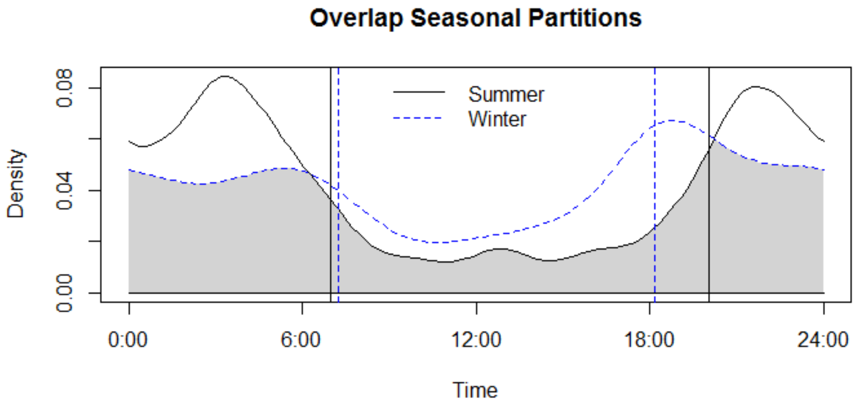
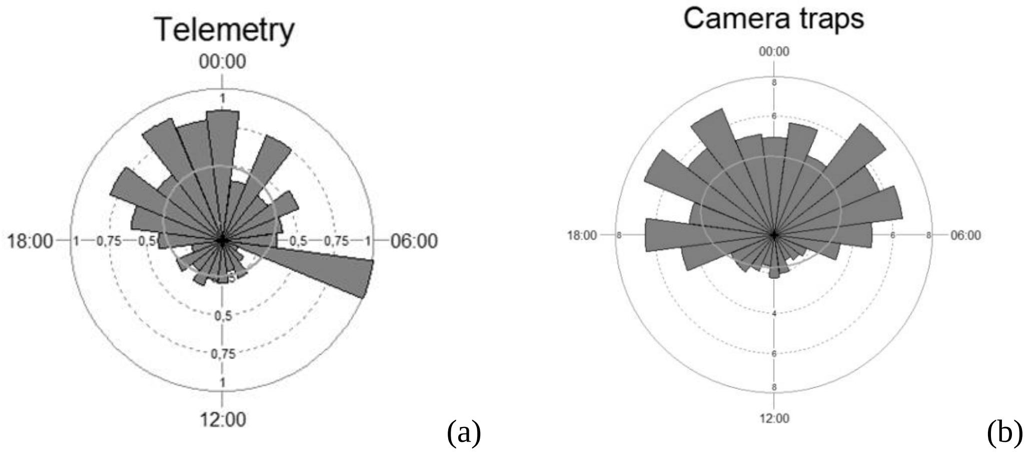


Figure 3. Kernel-density estimates for activity patterns of Geoffroy's cat in summer (n=240) and winter (n=273). The black solid and blue dashed vertical lines indicate the

mean time of sunrise and sunset in summer (6:57 am and 8:03 pm) and winter (7:15 and 6:10 pm), respectively (Supporting Information Table S2).

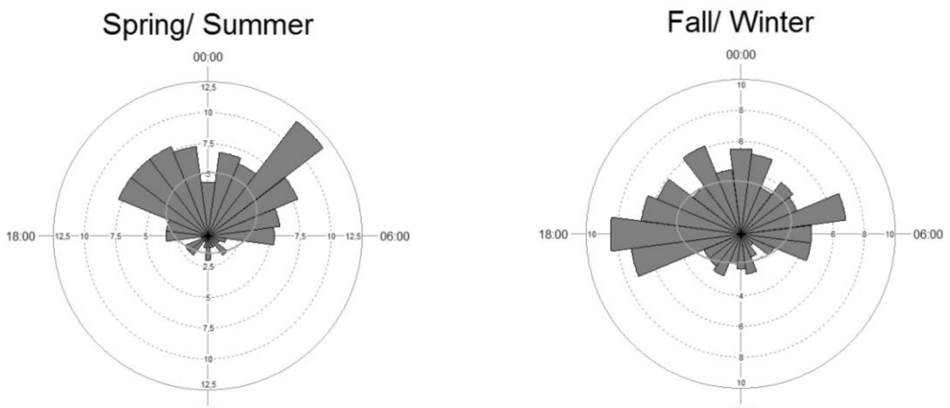
Using Rayleigh's Uniformity test, the mean activity time for the total survey period was $\bar{X}$ = 23:36 (± 06:04 *SD*) (Fig. 4); for spring-summer, it was 00:37 (± 04:44 *SD*) and for fall-winter it was $\bar{X}$ = 22:03 (± 06:56 *SD*) (Fig.5). For all data sets, our results show that the activity patterns were not uniformly distributed throughout the hours of the day (Rayleigh's Uniformity test: $Z = 8.03$, $P < 0.01$ for the total survey period; $Z = 20.97$ $P < 0.01$ for spring-summer; $Z = 3.72$, $P = 0.02$ for fall-winter).

For the telemetry-based analysis, we used 751 fixes (263 active and 488 inactive) obtained from six of the seven collared cats (the activity sensor of one of the collars failed). Since only the active fixes are informative for this analysis (which limited the available sample size), we only performed this estimate for the total survey period. The mean activity time for the telemetry data was 23:19 (± 03:47 *SD*) and activity was not uniformity distributed (Rayleigh's Uniformity test: $Z = 3.35$, $P = 0.03$) (Fig. 4).



(a) (b)

Figure 4. Circadian activity patterns of Geoffroy's cat. Bars indicate the proportion of records taken at that hour of the day. The grey line is the Standard deviation ellipse. (a) Telemetry (n=751 fixes). (b) Camera trapping (n=513 photos).



(a) (b)

Figure 5. Seasonal activity patterns of Geoffroy's cats in two different seasonal partitions, based on the camera-trap data. The grey line is the standard deviation ellipse. (a) Spring-summer (n=240). (b) Fall-winter (n=273).

Habitat selection and activity

A higher proportion of activity fixes was present in riparian vegetation (0.55) than in croplands (0.27) or grasslands (0.18). Mean activity time in the riparian vegetation was 21:00 (± 02:16 *SD*), while in the grassland it was 04:00 (± 5:39 *SD*) and in the cropland it was 22:00 (± 01:39 *SD*). Given the strong trend for predominantly nocturnal activity in all three habitat types, results of Watson's pairwise test were non-significant (riparian vegetation *vs.* grassland: $U^2 = 0.078$, $P > 0.2$; riparian vegetation *vs.* cropland: $U^2 = 0.024$, $P > 0.5$; cropland *vs.* grassland: $U^2 = 0.059$, $P > 0.5$). However, when each habitat type was assessed separately, contrasting patterns emerged: the activity was significantly non-uniformly distributed throughout the hours of the day in grassland (Rao's Spacing Test; $U = 202.32$, $P < 0.01$) and cropland ($U = 186.12$, $P < 0.05$), whereas it was uniformly distributed in the riparian vegetation ($U = 116.04$, $P > 0.5$).

Discussion

In this study of the ecology and behaviour of Geoffroy’s cat in Brazil, we used a variety of methods to characterize its density, habitat use and activity patterns in a human-dominated landscape. By employing a diverse array of approaches, we were able to discern some underlying factors that may help this population persist in this region. We observed the importance of riparian vegetation (protected areas) for this population in this threatened biome, and the significant nocturnal patterns of Geoffroy’s cat in the region, which reduces contact with humans.

With respect to density estimations, in our study the use of SECR analysis was improved when we combined data from camera traps and radio-telemetry. The results of this analysis were similar to the telemetry-based density result, supporting the reliability of this density estimator (Table 3). However, it was important to include telemetry data from males and females, because the mean HR size influences the density estimation (*e.g.* small HRs increase the density, as happened in our winter survey [only females were analysed]). In the present study, the capture-recapture traditional methods were used to compare our estimates with those reported previously for Geoffroy’s cats in other areas. However, in agreement with the conclusions of several studies on felids (*e.g.*, Trolle & Kery, 2005; Di Bitetti *et al.*, 2008; Maffei & Noss, 2008; Caruso *et al.*, 2012) one of these approaches, HMMDM, did not demonstrate reliable results in our study. The area effectively sampled (*AES*, see Supporting information S1) became considerably smaller than in the other estimations, which consequently led to an overestimate of population density. Capture-recapture methods have been frequently used for density studies over the years. However, the number of papers that criticize these conventional procedures is constantly growing, since, in contrast to SECR analyses, their estimates do not model the spatial relationships between animals and detectors (Foster & Harmsen, 2012; Efford & Fewster, 2013). Additionally, they could bias analyses when individuals differ in their exposure to traps (Efford & Fewster, 2013), which typically happens for the majority of felid species (Karanth & Nichols, 2002).

Our comparisons with all the available estimates of population density for Geoffroy’s cat showed that the species exhibits different densities across different geographic areas (Table 3). Population densities can range from seven (W. Johnson, *pers. comm.* in Nowell & Jackson [1996]) to 290 (Pereira *et al.*, 2011) individuals per 100 km². The values from our study in Brazil are similar to those observed in other countries. Caruso *et al*. (2012) provided the most meaningful comparison for our results, since that study was carried out in non-protected areas from the Argentinian Espinal, and is the only one that estimated the density using SECR methods. The similar densities we observed could be related to the similarity of these non-protected areas, whose landscapes were largely modified by humans (*e.g.* savannah regions with cattle ranches and croplands). Nevertheless, the overall resemblance of areas is not the only relevant variable when we compare areas. Pereira *et al*. (2011) carried out their study in low Monte-Espinal ecosystems from Argentina, whose characteristics are comparable to our study area in the Uruguayan Savannah ecoregion, and to Caruso *et al.*’s (2012) site in the Espinal ecoregion. However, the density estimates reported by Pereira *et al*. (2011) were considerable higher, likely due to the presence of a large number of transient individuals in that study. Another important result in Pereira *et al.*’s (2011) study was the higher density of Geoffroy’s cat in the non-protected areas than in protected ones. Geoffroy’s cat population density in southern Brazil appears to be higher than in other sites (W. Johnson, *pers. comm.* in Nowell & Jackson, 1996; Cuellar, *et al.* 2006) with the same range of habitat suitability values for Geoffroy’s cat

(0.25 – 0.50; Cuyckens, *et al.* 2015). This may be connected to some characteristics of these areas, which influence Geoffroy's cat populations. First, the presence of mid-sized felid species, which do not occur in our area; and second, these two studies were carried out in protected areas and our study was carried out in human-dominant landscapes.

Cuellar *et al*. (2006) found that Geoffroy's cat abundance was lower in the presence of other mid- or large-sized felid species (*e.g.* pumas or ocelots). This could be explained by the 'ocelot effect', which would occur when the dominant mid-sized felid impacts the dynamics of the mesopredator community (Oliveira *et al.,* 2010). In the present study, of the seven felid species originally distributed in the area (IUCN, 2015; Espinosa *et al. in prep*.), only two were recorded, with the margay presenting a substantially lower abundance relative to the Geoffroy's cat. In human-dominated landscapes, Geoffroy's cat appears to have the highest density among wild felids, as it seems to tolerate those areas better than the other species (Castillo *et al*., 2008; Caruso *et al*. 2016). According to Cuyckens, *et al.* (2015) the species is taking advantage of human-modified land and is probably expanding its geographical distribution. Caruso *et al*. (2016) observed a positive response of Geoffroy's cats to the gradients of anthropization and fragmentation; according to those authors, it may favour the species if the human persecution level is low. They also suggested that the increased presence of modified habitat in an area could intensify Geoffroy's cat use of shrublands available at a given site.

Geoffroy's cat is traditionally considered a species occurring in open areas (Sunquist & Sunquist, 2002). At our study site, we found that these felids clearly selected riparian vegetation and avoided grasslands with cattle in the Brazilian Pampas, agreeing with the suggestion of Caruso *et al.* (2016). Our results showed the same preferred and avoided habitats for almost all individuals, using different HR estimators and on distinct spatial scales, suggesting a strong pattern of selectivity in this area. The use of non-grassland habitats by this species had already been described by previous studies (Johnson & Franklin, 1991; Manfredi *et al*., 2006, 2012; Pereira *et al.*, 2006, 2012). Some studies also observed preference for covered areas, although the patterns of selectivity observed were not always constant in terms of the spatial scale (Manfredi *et al*., 2006, 2012; Pereira *et al.*, 2006, 2012). Therefore, within open area landscapes – most characteristic feature of the Geoffroy’s cat distribution – covered habitats seem to be important for the species.

In addition to the results on habitat selectivity, we recorded videos with males(Supporting information video S1) and/or females urinating and sniffing in riparian vegetation, suggesting that this type of vegetation could be used as a ‘communication centre’, as hypothesized by Manfredi *et al.* (2006) and Soler *et al.* (2009). We also recorded females hunting, resting and grooming themselves and with kittens in these areas, and even staying in these areas during a flooding event (Supporting information videos S2- S4). The results are indicative that Geoffroy’s cats seems to be very connected to riparian vegetation, a habitat that may work as a shelter for this species in this non-protected, otherwise open area. Our findings of preference for forested habitats relative to other habitats are similar to the results from another relatively altered area in central Argentina (Manfredi *et al*., 2012); however, in that Geoffroy’s cat preferred exotic woodlands. It is also interesting that this “open-area species” avoided grassland at our study site. This may occur due to a need of avoiding human activities associated to cattle husbandry occurring daily in these habitats and always employing herding dogs. Because dogs can easily kill wild cats in the open field (as occurred with one of the individuals we monitored), riparian vegetation may provide an important shelter for these felids, as they are not directly used in the intensive management of cattle and its trees likely serve as refuge against attacks. These results demonstrate the extreme importance of these preserved areas, highlighting that riparian vegetation needs to be maintained as a protected area.

During both seasons, Geoffroy’s cats exhibited significantly nocturnal habits in our study area, as observed previously in other ecoregions: Humid Pampas (Manfredi *et al*., 2011; Castillo *et al*., 2008), Low Monte-Espinal (Pereira *et al*., 2011), Dry Chaco (Cuellar *et al*., 2006), and Magellanic subpolar forest (Johnson & Franklin, 1991). Different sources of data (camera trap and telemetry) consistently located the mean of activity at 23:00, which supports data reliability. Our results indicated some variation from nocturnal patterns in spring-summer to a nocturnal-crepuscular activity in fall-winter in southern Brazil. A study carried out in the Monte-Espinal of central Argentina demonstrated that this species was significantly more active during daytime than night (Pereira, 2010) during a period of food shortage, whereas the opposite was true when prey was abundant (Pereira *et al*., 2011). We conclude that all the available information on Geoffroy’s cat activity shows that it is a nocturnal species with seasonal cathemeral abilities.

By integrating habitat use and activity patterns, it was possible to observe that the averages of activity in each habitat type were different (albeit not significantly so); this indicated once more nocturnal patterns of the species in the region. When we focused on each habitat, the activity throughout the hours of the day was significantly not uniformly distributed on grasslands and croplands (non-protected areas); the results differed for riparian vegetation, which demonstrated a more uniform activity.

Conclusion

A comparative review of the data available on this felid leads to the conclusion that populations of Geoffroy's cat seem to be able to adjust their density and behavioural patterns depending on the ecological context. The medium-low population density, essentially nocturnal habits, preference for riparian forest and avoidance of grasslands in the Brazilian pampas are likely to be behavioural adaptations that enable this small cat to occur with a reasonable population density in a human-dominated landscape. In spite of this ecological flexibility, there is a potential threat to the persistence of this species in this region. The Brazilian pampas is currently undergoing an expansion of crop monocultures and pastures based on exotic grasses (MMA, 2007). In Brazil, economic interests have put pressure on policy and regulations, and a new law called "New Forest Code" is already in effect (Brazilian Federal Law 12.651/2012); this allows increased deforestation in some areas (Brazilian Federal Law 4.771/ 1965), including a decrease in the width of protected riparian vegetation. In addition to that, the culture of "gaúchos" (farm workers of the Brazilian Pampas) is profoundly connected to domestic dogs, for their help with cattle management. The incorrect training of dogs by humans (or altogether lack of training), help increase the conflicts with native fauna, since dogs reportedly hunt multiple species. Also, some dogs are abandoned or run way to other farms, thus becoming feral and producing serious impacts on native fauna (Dimond, 1989; Galetti & Sazima, 2006).

As the results of this study demonstrate that riparian vegetation is very important for Geoffroy's cat in these human-dominated landscapes, it serves as an example to highlight the importance of these habitats for local fauna in general. The survival of Geoffroy's cats populations on farmlands in the Brazilian pampas is likely possible with sustainable land-use management, including strict conservation of remaining riparian vegetation, improved training and supervision of herding dogs, and eradication of feral dogs. Adequately tackling these complex issues will be crucial to ensure the long-term persistence of these felids in the Brazilian pampas.

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Figure Legends

Figure 1. Location of the study site. A. Location of study area in South America. B. Location of the study site on a regional scale; light grey represents the Pampas biome and dark grey represents the Atlantic Forest biome. C. Study site habitat composition, camera trap layout and telemetry locations of radio-collared Geoffroy’s cats.

Figure 2. Kernel-density estimates of the daily activity patterns of Geoffroy’s cat in the Brazilian Pampas. The short vertical lines above the x-axis indicate the times of individual photographs (n=513) and the black solid vertical lines indicate the mean time of sunrise (7:02) and sunset (19:04) for the whole year (Supporting Information Table S2).

Figure 3. Kernel-density estimates for activity patterns of Geoffroy’s cat in summer (n=240) and winter (n=273). The black solid and blue dashed vertical lines indicate the mean time of sunrise and sunset in summer (6:57 am and 8:03 pm) and winter (7:15 and 6:10 pm), respectively (Supporting Information Table S2).

Figure 4. Circadian activity patterns of Geoffroy’s cat. Bars indicate the proportion of records taken at that hour of the day. The grey line is the Standard deviation ellipse. (a) Telemetry (n=751 fixes). (b) Camera trapping (n=513 photos).

Figure 5. Seasonal activity patterns of Geoffroy’s cats in two different seasonal partitions, based on the camera-trap data. The grey line is the standard deviation ellipse. (a) Spring-summer (n=240). (b) Fall-winter (n=273).

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supporting information S1. Capture-Recapture methods to estimate density.
Table S1. The Shapiro–Wilk normality test (95%) for Jacobs's index values.
Table S2. Mean and standard deviation from data of sunrise, sunset and time of the day collected during the entire period of study field and divided between two seasons, in Alegrete, RS, Brazil.
Table S3. Density estimation models ('secr' package (Efford 2016a) in R) and their corresponding AIC values to two surveys for analyses only with camera traps data (summer and winter) and combined with telemetry data (summer combined and winter combined).
Figure S1 - Camera-traps installed for at least three months in all the study area. The filled circles are the camera-trap locations with occurrence of Geoffroy's cat, the empty circles are the camera-trap locations with no occurrence of the species. The dashed line is the area we actually worked on density and telemetry monitoring (see Figure 1).
Video S1 – S4. Videos of Geoffroy's cat behaviour in the riparian vegetation.

Supporting information S1. Capture-Recapture methods to estimate density

Material and Methods – Traditional Capture-Recapture methods were used here to compare with other density studies. First, we estimated abundance (*N*) model results for the both survey periods (summer and winter); this was carried out in the software CAPTURE (Rexstad & Burnham, 1991), and we selected the best model using the model selection criteria (based in a godness-of-fit test) in same software (Otis, 1978). Second, we calculated the area effectively sampled (*AES*) applying four different approaches of buffers width estimations: 1) mean maximum distance moved by individuals outside the area delineated by the outer traps (MMDMOSA); 2) mean maximum distance moved (MMDM); 3) the half mean maximum distance moved (HMMDM) (Balme *et al*., 2009); and 4) the radius of the mean adult home-range size of the studied population (HR radius) (Pereira *et al.* 2011). The estimations were measured from individuals of Geoffroy's cats of the area. Third, we calculated density by dividing

N of each survey period by the values of *AES* generated from each method. Density was also estimated by telemetry data only; to do so it was assessed the HR of radio-collared individuals with 95% kernel, the overlaps of home ranges were then corrected and the total area occupied by monitored cats was calculated (Caruso *et al*., 2012). Finally, the total number of individuals monitored was divided by the *AES* estimated. All *AES* analyses were calculated using ArcGIS 10.4.1 (ESRI, 2016).

Results - The best models to explain the capture and recapture of the species were M(0) = 0.95 and M(h) = 1.00 in the summer and M(0) = 1.00 and M(h) = 0.91 in the winter. White *et al*. (1982) suggested precaution in use of null model, M(0), especially in situation with small sample number or possibility of individual heterogeneity. Then here we used the M(h) model, which assumes heterogeneity among individuals in their capture probabilities (White et al. 1982). Our sampling structure was not validated for the closure test provided by CAPTURE; as our surveys were performed in 48 days we assumed no change in the population due these periods (Pereira *et al.* 2011; Caruso *et al*. 2012). The capture probability using CAPTURE was 0.09 for summer and 0.05 for winter and the abundance was 09 (± 1.98 *SE*) for summer (*CI* 9-9) and 12 (± 3.02 *SE*) for winter (*CI* 12-30). By using MMDMOSA (1.73km ± 0.83) the *AES* was 27.74km²; with MMDM (1.52 km ± 0.88 *SD*) the *AES* was 23.65 km²; by using HMMDM (0.76 km ± 0.44 *SD*) it was 10.24 km²; and with HR radius the total sample area was 17.59 km². Finally, from seven radiocollared Geoffroy's cat HRs the total sampled area with telemetry was 21.27 km². All density estimations were than calculated (Table 3).

Table S1. Shapiro–Wilk normality test (95%) for Jacobs's index values.

Jacobs' index results	Kernel 95%		MCP 95%	
	W	*p*-value	*W*	*p*-value
Cropland	0.85	0.13	0.83	0.09
Grassland	0.87	0.18	0.96	0.09
Riparian Vegetation	0.88	0.23	0.81	0.06

Table S2. Mean and standard deviation from data of sunrise, sunset and time of the day collected during the entire period of study field and divided between two seasons, in Alegrete, RS, Brazil.

	Sunrise		Sunset		Time of the day	
	Mean	SD	Mean	SD	Mean	SD
All seasons	07:02	0.013	19:04	0.045	12:01	0.056
Summer-spring	06:57	0.011	20:03	0.030	13:16	0.054
Fall-winter	07:15	0.014	18:10	0.011	10:54	0.024

*Data base collected from © 2011-2016 sunrise-and-sunset.com

Table S3. Density estimation models ('secr' package (Efford 2016a) in R) and their corresponding AIC values to two surveys for analyses only with camera traps data (summer and winter) and combined with telemetry data (summer combined and winter combined). Parameters include: predictor variables[a], g0 = baseline detection, sigma = scale parameter. Additionally, P= Parameters; logLik = strength of evidence of each model; AIC$_c$ = AIC with small sample bias adjustment, ΔAIC$_c$ = difference between AIC$_c$ model and the best model; AIC$_{cwt}$ = weight attributed to each model.

Models (detection function= half normal)		P	logLik	AIC	AIC$_c$	ΔAIC$_c$	AIC$_{cwt}$
Summer							
bk	g0~bk sigma~1	3	-231	469	472.021	0	0.75
Bk	g0~Bk sigma~1	3	-233	472	475.294	3.273	0.15
sex	g0~1 sigma~1	2	-236	476	477.411	5.39	0.05
0	g0~b sigma~1	3	-235	476	479.468	7.447	0.02
h	g0~Sex sigma~1	3	-236	478	480.944	8.923	0.01
b	g0~h2 sigma~1 pmix~h2	4	-236	480	486.578	14.56	0
Winter							
bk	g0~bk sigma~1	3	-152	309	313.204	0	0.85
Bk	g0~Bk sigma~1	3	-154	313	317.222	4.018	0.11
sex	g0~1 sigma~1	2	-157	318	319.777	6.573	0.03
0	g0~b sigma~1	3	-157	320	323.915	10.71	0
h	g0~Sex sigma~1	3	-157	320	324.059	10.86	0
b	g0~h2 sigma~1pmix~h2	4	-157	322	330.063	16.86	0
Summer combined							
bk	lambda0~bk sigma~1 hazard	3	-541	1089	1092.21	0	1
Bk	lambda0~Bk sigma~1 hazard	3	-549	1104	1107.3	15.09	0
sex	lambda0~1 sigma~1 hazard	2	-553	1111	1112.09	19.88	0
0	lambda0~b sigma~1 hazard	3	-551	1109	1112.12	19.91	0
h	lambda0~Sex sigma~1 hazard	3	-552	1110	1113.12	20.91	0
b	lambda0~h2 sigma~1 pmix~h2 hazard	4	-550	1109	1115.41	23.2	0
Winter combined							
bk	lambda0~bk sigma~1 hazard	3	-230	465	469.184	0	0.43
Bk	lambda0~Bk sigma~1 hazard	3	-230	466	470.104	0.92	0.27
sex	lambda0~1 sigma~1hazard	2	-233	470	471.285	2.101	0.15
0	lambda0~bsigma~1hazard	3	-233	472	475.569	6.385	0.02
h	lambda0~Sexsigma~1hazard	3	-232	470	473.934	4.75	0.04
b	lambda0~h2 sigma~1pmix~h2hazard	4	-228	464	472.325	3.141	0.09

[a] Predictor variables used in the construction of density models in "secr" package (Efford 2016a): bk=Animal x site learned response; Bk= Animal x site transient response; sex= Capture probabilities differ between sexes; 0= Capture probabilities are homogeneous among individuals; h= Individual heterogeneity; and b= Learned response.

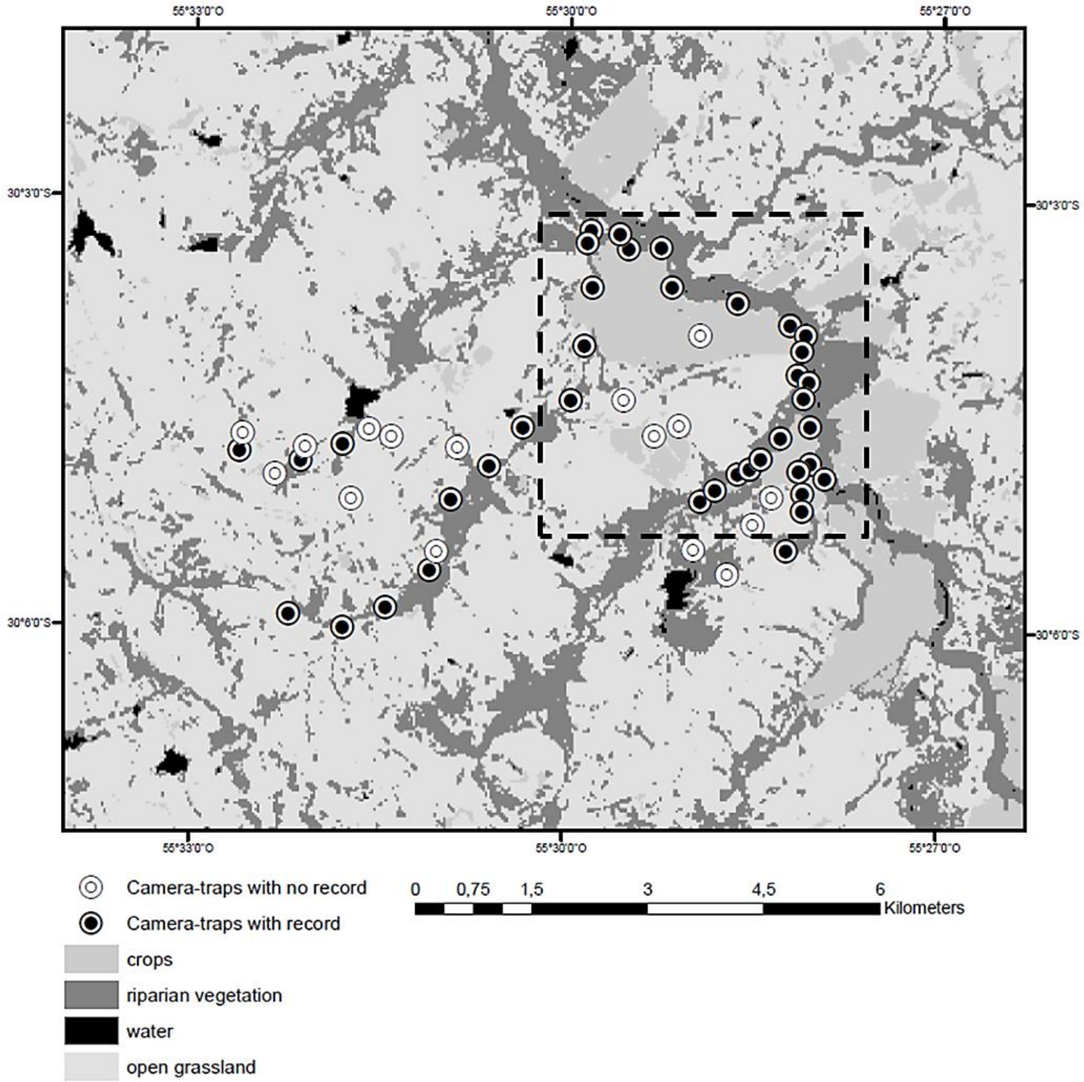

Figure S1 – Camera-traps installed for at least three months in the study area. Circles with a black core represent the camera-trap locations which captured the occurrence of Geoffroy's cats; empty circles represent camera-trap locations with no recorded occurrence of the species. The dashed line delimits the area in which we performed camera-trap based density surveys as well as telemetry monitoring (see Figure 1).

Capítulo

4

Distribution modelling and conservation assessment of the pampas cat (*Leopardus colocolo*) in the Uruguayan Savannah

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Distribution modelling and conservation assessment of the Pampas cat (*Leopardus colocolo*) in the Uruguayan Savannah ecoregion

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Abstract

The Pampas cat is a poorly known small felid that occurs in open habitats of South America. Previous studies have suggested that Pampas cat populations occurring in the endangered Uruguayan Savannah ecoregion (comprising grasslands of southern Brazil and Uruguay) comprise a distinct ‘Evolutionarily Significant Unit’ (ESU), and as such should be the target of separate conservation assessment. However, there is very scarce data on the ecology of this population, as Pampas cats are rarely sighted in the region, posing challenges to assessments of their spatial distribution, population size and conservation status. To address these issues, we developed spatial distribution models (SDM) for this Pampas cat ESU, and used them to generate estimates of population size for the Uruguayan Savannah. We then assessed the conservation status of this ESU based on IUCN criteria, and identified priority areas for its conservation. We constructed two models (with and without anthropogenic variables), each of them built separately with two different algorithms (Maxent and Maxlike). All models were very similar, with strongly correlated results ($r_s \geq 0.88$; $P<0.01$), indicating higher suitability (or probability of occurrence [PO]) in grassland areas from sea level to 400m of altitude. The two models incorporating anthropogenic variables provided the best fit to the data, and yielded a more restricted distribution, supporting the conclusion that this Pampas cat population is affected by human disturbance. In these two best-fit models, the areas with the highest suitabilities and PO were located mainly in central and southwestern Rio Grande do Sul state (Brazil), and in central and southern Uruguay. Those regions are naturally composed of tall grasslands, but include strongly altered areas, which may affect the persistence of Pampas cat populations. Using available density estimates, we calculated the joint population size of high-suitability areas to be $\leq$250 individuals, suggesting that this ESU may be critically endangered (equivalent results from the Maxlike model yielded ~1,800–9,000 individuals, leading to an ‘endangered’ categorization). Very little of the high-suitability landscape is presently protected, highlighting the urgent need to expand the few existing protected areas in this region, as well as to create new ones to ensure the long-term survival of this elusive felid.

Keywords: *Leopardus colocolo*, Felidae, niche modelling, South America, Neotropics, grassland, conservation status.

Introduction

The increasing loss of natural habitats is leading to severe declines in geographic range and population size for a large number of wild species on a global scale (Butchart et al., 2010; Li et al., 2016). Another negative consequence of this process is habitat fragmentation, which creates a matrix of human-transformed land cover isolating patches of remaining natural ecosystems (Rands et al., 2010). By reducing the amount and connectivity of suitable habitat, human disturbances tend to have negative impacts on wildlife, decreasing effective population sizes and even inducing local extirpation (Fahring, 2003). This is especially problematic in species with discontinuous ranges and/or strong population structure, because an important portion of their genetic diversity is preserved in different populations (evolutionary units) present in distinct regions. As genetic diversity is the result of several dynamic processes, and provides the basis of future adaptations (Mergeay & Santamaria, 2012), it is very important to conserve such differentiated populations.

Therefore, for species with strong population structure, conservation actions at the species level may be insufficient. In such cases, the evaluation of threats and the design of management plans should instead focus on infra-specific units. The term ‘Evolutionarily Significant Unit’ (ESU) was coined to delimit evolutionarily differentiated populations whose conservation is important to preserve the full complement of genetic diversity that characterizes the species as a whole (Ryder, 1986; Moritz, 1994). This term is relevant in the context of conservation planning for distinct population segments, regardless of on-going taxonomic debates (*e.g.* if this unit should be recognized as a subspecies, or whether it would be defined as a distinct species based on a given conceptual framework).

The Pampas cat *Leopardus colocolo* is a Neotropical felid that presents a broad and apparently discontinuous geographic distribution in South America (Lucherini *et al.*, 2016; Kitchener et al., in press). The species appears to be declining in many regions due to several threats, such as reduction in extent and/or quality of its habitats, road kills, predation by dogs, and hunting (Johnson et al., 1999; Napolitano et al., 2008; Cossíos et al., 2009; Lucherini et al., 2016). As it is one of the least known felid species worldwide (Brodie, 2009; Macdonald et al., 2010), its conservation status is ‘data deficient’ in several regions of its distribution (Cossíos et al., 2009). Although its current species-wide status in the IUCN Red List is “Near Threatened”, the IUCN assessment recommended that it should not be evaluated as a single evolutionary unit (Lucherini et al., 2016), given its strong population structure (Johnson et al., 1999; Napolitano et al., 2008; Cossíos et al., 2009; Santos, 2012). It also highlighted the urgent need for research into Pampas cat ecology, distribution, threats and taxonomy (Lucherini et al., 2016).

The taxonomic classification of Pampas cat has been discussed over the past two decades, with distinct propositions of subspecies and species. Still, all studies converged on a common conclusion: there are considerable differences among populations living in distinct portions of the species’ range. While molecular data have so far supported a recent origin, indicative of a single species with strong population structure (Johnson et al., 1999; Napolitano et al., 2008; Cossíos et al., 2009; Santos, 2012; Sartor, 2016), morphological data suggested that the group comprises three distinct species (*L. colocolo, L. pajeros,* and *L. braccatus*) and eleven subspecies (Garcia-Perea, 1994; Barstow & Leslie, 2012). Within one of the proposed species, *L. braccatus*, morphological studies have suggested two subspecies, *L. b. braccatus* for the central-western region of Brazil (Cerrado and Pantanal biomes) and *L. b. munoai* for Brazilian-Uruguayan Pampas (Ximenez, 1961; Garcia-Perea, 1994). The latter subspecies was first described by Ximenez (1961) as *Felis colocola* *munoai*, with a proposed range that encompassed Uruguay and southernmost Brazil (Rio Grande do Sul [RS] state), exactly matching the presently recognized Uruguayan Savannah ecoregion (Olson et al., 2001). Nascimento (2010), using phenotypic features, suggested raising the subspecies *L. b. braccatus* and *L. b. munoai* to species level (*L. braccatus* and *L. munoai*, respectively). Recent molecular studies, based on mitochondrial DNA and microsatellite markers, also observed significant distinctions between these populations (Santos, 2012; Sartor, 2016), and proposed that they should be recognized as distinct ESUs.

The genetic isolation of Pampas cats from Uruguay and Brazil from nearby populations due to the action of geographic barriers such as the La Plata and Paraguay rivers had been hypothesized by Johnson et al. (1999). This view was corroborated by Santos (2012) and Sartor (2016), whose analyses indicate that rivers La Plata, Uruguay and Parana/Paraguay effectively isolate this ESU on its southern and western boundaries, respectively, while the Atlantic Forest has been a barrier to gene flow with the central Brazilian populations (which we refer to as *L. colocolo braccatus*). Depending on the exact position of the western boundary, this ESU may include (in addition to Uruguay and RS state in Brazil) populations from the north-eastern Argentinean provinces of Entre Ríos, Corrientes and Misiones. This would be the case if the ESU were bounded on the west by the Parana/Paraguay river, instead of the Uruguay river (Fig. 1). Since these Argentinean populations have so far not been assessed genetically, we conservatively assume for this study that this ESU is restricted to the Uruguayan Savannah ecoregion, and focus most of our analyses on this well-delimited area (Fig. 1).

The Uruguayan Savannah is a sub-tropical grassland ecoregion (Olson et al., 2001) that comprises a mosaic of different habitats, mainly characterized by open grasslands interspersed with riparian vegetation (MMA, 2007). Due to its weather and soil features, as well as its natural phyto-physiognomy, the region is highly exploited by human activities such as cattle ranching, agriculture and forest plantations (Martino, 2004; MMA, 2007). These economic activities have led to extensive alteration of the original landscape, resulting in scarce, isolated fragments of natural habitats and the conclusion that this ecoregion should be considered 'Critical/Endangered' (Loyola et al., 2009; WWF, 2016). Additionally, based on terrestrial vertebrate distribution patterns, the Uruguayan Savannah is regarded as one of the highest conservation priorities in the Neotropics (Loyola et al., 2009).

The few surveys of Pampas cats performed so far in this ecoregion indicate that it occurs at very low densities (0.01 to 0.05 individuals/km^2) (Oliveira, *pers. comm.* in Queirolo et al., 2013). The Brazilian National Action Plan for the Conservation of Small Cats (CENAP/ICMBio) lists as a priority the goal of obtaining ecological data on *L. colocolo* in Rio Grande do Sul state, illustrating the present scarcity of information on this species in this region. In Uruguay, a study of the potential distribution of *L. colocolo* using bioclimatic variables estimated a potential range spanning almost the entire country (Bou, 2012). However, high-suitability habitats were found only in the southern and eastern portions of the country, which are undergoing considerable human alteration. That study emphasized the need for potential distribution studies of these populations incorporating anthropogenic variables in the estimated models (Bou, 2012).

In the present study, we aimed to assess the current spatial distribution and the conservation status of the Pampas cat ESU present in the Uruguayan Savannah. To accomplish this, we developed spatial distribution models (SDM) using physical, bioclimatic and human-related variables, and characterized habitat suitability (or probability of occurrence) for the species throughout this ecoregion and adjacent areas. We used these estimates to project the number of individuals contained in this ESU under different scenarios (levels of suitability and probability of occurrence), and to assess its conservation status based on IUCN criteria. Finally, we identify priority areas for Pampas cat conservation in the Uruguayan Savannah, and discuss challenges for their effective protection and long-term management.

Methods

Species occurrence samples

We collected geographic coordinates on all recorded *L. colocolo* occurrences throughout the Uruguayan Savannah ecoregion, encompassing southernmost Brazil and Uruguay (Fig. 1). We also included two additional records, obtained in northeastern Argentina, to construct alternative niche models assuming the Paraná river as the isolating barrier for this ESU (Fig. 1; Supporting information S1). Records were obtained from road-killed individuals, records of predation by dogs, photographic records (including camera-trap images), museum specimens, personal observations from trustworthy field biologists, and published scientific articles (Supporting information Table S1). The locations of all records were converted into decimal degree coordinates using the WGS84 reference system.

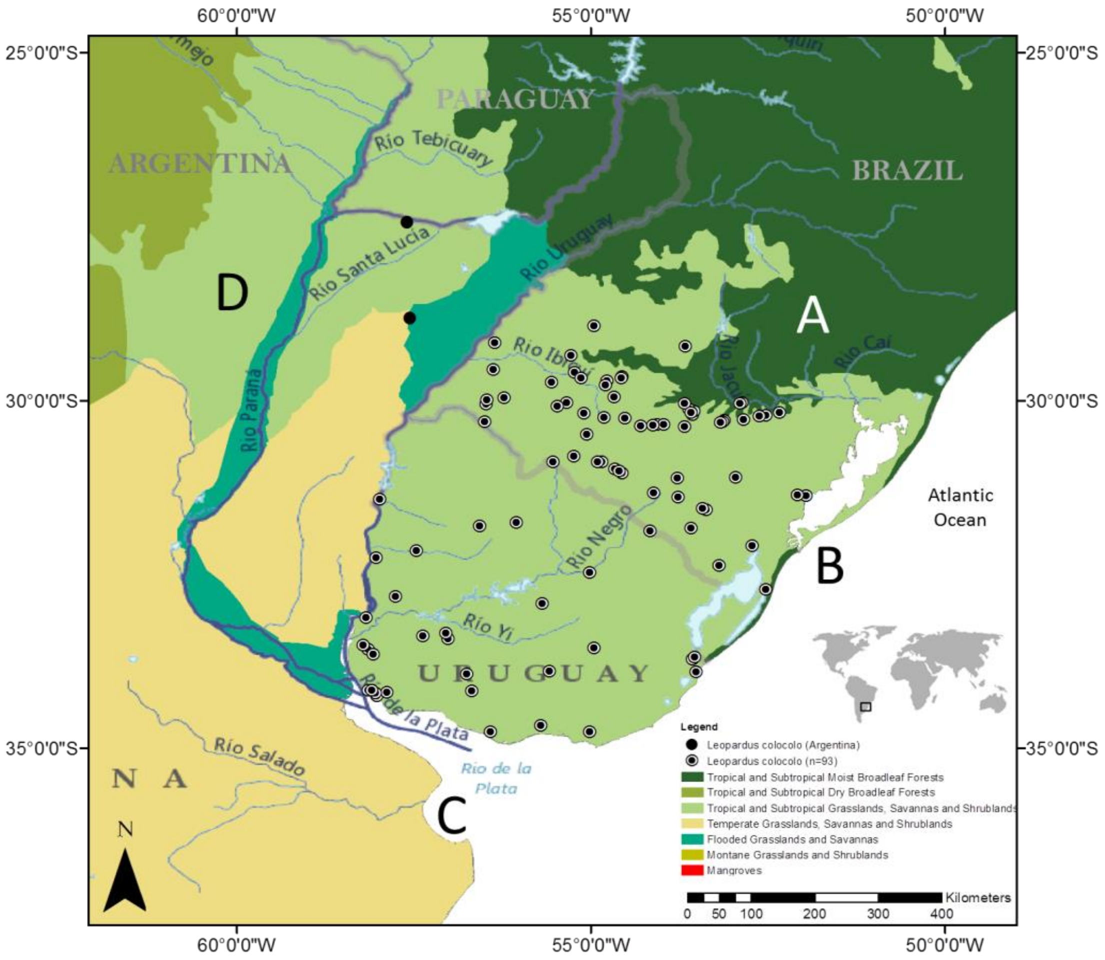

FIG. 1 Records of *Leopardus colocolo* (n=93) in the Uruguayan Savannah (white circles with black cores), plus two records from Argentina (black circles). The map also depicts the ecoregions (Olson et al., 2001) and natural geographic barriers that seem to bound this evolutionary unit: A) Atlantic Forest. B) Atlantic Ocean. C) La Plata River and D) Uruguay and/or Paraná Rivers.

To reduce the spatial correlation among the records, a sampling bias file was created using the ‘dismo’ package (Hijmans et al., 2017) in R 3.2.3 (R Development Core Team, 2015). Only presence points located > 5 km apart from each other were used in our analyses, since this is the radius of a circle encompassing 19.63 km², a conservative assumption given the published data on home range sizes for this species (19.47 km² [Silveira et al., 2005] and 14.90 km² [Tellaeche, 2015]).

Selection of Variables

We constructed two different models of the potential distribution of the Pampas cat in this region. In ‘model 1’, we initially included 35 bioclimatic variables (WorldClim database [http://www.worldclim.org] and Climond [http://www.climond.org]) and altitude (Shuttle Radar Topographic Mission [http://www2.jpl.nasa.gov/srtm]). In ‘model 2’, we included the same variables and three additional ones: the Normalized Difference Vegetation Index (MODIS/NDVI) (Time Series Database from the Global Agriculture Monitoring Project; GLAM – data from July 2016, http://pekko.geog.umd.edu/usda/beta/), Global Cattle Density (FAO 2005, http://www.fao.org/geonetwork) and Landscape information, including anthropogenic land use (ESA GlobCover Project 2009, http://due.esrin.esa.int/page_globcover.php) (Supplementary material Table S2). We selected these variables because they likely represent the impact of the most important economic human activities in the region. We modified the global environmental layers in ArcMap from ArcGIS 10.4.1 (ESRI, 2016) to equalize their geographic boundaries, cell size and coordinate system. To do so, we extracted them with a South American mask and resampled each of them to the same resolution (Young, 2011).

To avoid collinearity in the modelling process, we generated 500 random points from the known geographic distribution layer of the Pampas cat (obtained from the IUCN Red list data base), using packages ‘raster’, ‘dismo’ and ‘sdm’ (Hijmans et al., 2016; Hijmans et al. 2017; Naimi & Araujo, 2016) in R 3.2.3. We combined these random points with known Pampas cat occurrence points into a single ‘data.frame’, and then extracted the values for all variables from each point using the ‘dismo’ package. These extracted values were used to remove collinear variables prior to model fitting, applying the variance inflation factor (VIF) (Marquardt 1970) with Pearson's (*r*). The VIF method is considered more precise than Pearson's (*r*) or Spearman's (r_s) correlation coefficients alone, because it can measure how much of the variance of an estimated regression coefficient is inflated because of collinearity (Naimi & Araújo, 2016). Here we first calculated correlation coefficients between variables to identify strongly correlated pairs with the highest coefficients (>0.7); then we excluded from each of these pairs the variable with the highest VIF (>10) (Chatterjee & Hadi, 2012; Naimi & Araújo, 2016). These analyses were performed with the package 'sdm' (Naimi & Araújo, 2016) in R 3.2.3. We also assessed the likely biological significance of each variable within each strongly correlated pair, and used this criterion to ascertain if the decision based on the VIF results was the most plausible (Fitzpatrick et al., 2013). In every case, we found no reason to alter the VIF-based decision. Finally, the 'Landscape information' layer, included in Model 2, was not tested with respect to its collinearity, since it is a categorical variable.

Building the predictive models

We used presence-only data to model the distribution of this Pampas cat ESU using Maxent (Phillips et al., 2006) and Maxlike (Royle et al., 2012). The loglinear model used in Maxent estimates habitat suitability indices. Although this is one of the most popular approaches for SDM, it may be incorrect to interpret its output as a direct estimator of the probability of species occurrence (Royle et al., 2012). As an alternative, Maxlike (using a logit-linear model) can estimate the absolute probability of species occurrence (PO*;* Royle et al., 2012; Merow & Silander Jr., 2014). Therefore, to estimate this parameter of interest and to verify congruence with the models generated with Maxent, we also employed this approach. Furthermore, we used Maxlike because it allows the use of presence-only data, differently, for instance, from GLM methods, which present consistent performance with binary data (*e.g.* presence–absence) (Merow & Silander Jr., 2014).

In Maxent, the occurrences were randomly divided into training and testing data sets to allow *post-hoc* validation of the results. We used 75% of the included points for training and 25% for testing the models. The data were resampled by bootstrapping, with all runs including 10,000 hidden background points, a random seed and a convergence threshold of 0.00001, with 500 iterations (Young et al. 2011). Degrees of habitat suitability are represented as 0 for unsuitable to 0.99 for the highest suitability (Pearson, 2007). Maxent estimates the relative contribution of each environmental variable to the model via jackknife analyses, with the output being a table with the percent contribution of each variable. We also examined the response curves of these variables to understand how they affected the generated model.

Maxlike models were constructed with the ‘maxlike’ package (Chandler & Royle, 2013) using a maximum of 10,000 iterations maximizing the log-likelihood function and using the “BFGS” algorithm. Duplicate observations within a grid cell were removed from the models (Merow & Silander Jr., 2014). Maxlike output generates a table with estimates of Wald statistic (Z) and P-values of every variable implemented in each model. We then observed the Z values and examined how these variables affected the PO (Sarre et al., 2012) of Pampas cat in the Uruguayan Savannah. The probability of occurrence ranges between 0 (no occurrence) and 1 (highest PO). These analyses were carried out in R 3.2.3.

To also consider the scenario that this ESU is bounded on the west the Parana/Paraguay rivers, we constructed two additional models (models 3 and 4), including the same variables and the same data set, but adding two records from the Entre Ríos province in Argentina (Supplementary Material Fig. S2 and Fig. S3).

All final models were plotted onto the layer of Pampas records in South America and in the Uruguayan Savannah and adjacent areas, using R. These models have continuous values of suitability or PO, and allow the inference of the most important areas for this ESU. We also converted models 1 and 2 from each algorithm to ASCII format files to examine them visually in ArcGIS 10.4.1. We plotted the Pampas cat records onto each final model and extracted the minimum value of suitability (Maxent) and PO (Maxlike), delimiting threshold values below which an area was deemed to be no longer suitable/probable for the species. We classified the continuous values of each model in six different categorized levels, in each case considering the upper category (the highest values) as the suitable or high-PO areas.

Performance, similarity and comparison of models

For Maxent, the performance of each independent model was evaluated by calculating the area under the receiver operating characteristic (*ROC*) curve (ranging from 0.5 = random to 1 = perfect discrimination), abbreviated to Area Under the Curve (*AUC*) (Pearson, 2007; Jimenez, 2012). To measure similarity between models without assuming that the values should be proportional to probabilities, we calculated the Spearman rank correlation (r_s) between them (Phillips et al., 2009). To assess which model provided the best fit to the data, we calculated the *Log Likelihood* of Maxent models and used Akaike Information Criterion (*AIC*) with the ‘Model Selection’ option of the software ENMTools (Warren & Seifert, 2011). We also used the *AIC* to compare Maxlike models using the functions included in the package ‘maxlike’.

Status assessment of Uruguayan Savannah Pampas cats

Using the best-fit model of each method (Maxent and Maxlike), and progressively more stringent thresholds of habitat suitability (or PO), we calculated the area available to Pampas cats in the Uruguayan Savannah. We then estimated the number of individuals that likely exist in the ecoregion by multiplying the available area by the density estimates available for this species in the Brazilian Pampas (Oliveira *pers. comm.* in Queirolo et al., 2013). Finally, we used the estimated number of individuals to assess this ESU based on IUCN Red List criteria, so as to assign it to one of the established threat categories (Critically Endangered [CR], Endangered [EN] or Vulnerable [VU]).

Pampas cat SDMs and Protected areas

To assess the degree of protection of habitats in which Pampas cats are expected to occur in this region, we downloaded the World Database on Protected Areas (http://www.wdpa.org/), which includes nationally protected areas, areas designated under regional and international conventions, privately protected areas, and areas of indigenous peoples and communities. We overlaid the protected areas onto our models and measured the areas with highest suitability/PO of Pampas cat that are officially protected. Lastly, we calculated the percentage of these areas compared to the total area of high suitability/PO generated in each model. We also performed a visual assessment to suggest priorities for expanding existing protected areas, as well as creating new ones to enhance the probability of long-term persistence and continued connectivity of Pampas cat populations remaining in this ecoregion.

Results

We collected 107 location records of Pampas cats in the Uruguayan Savannah ecoregion. As we subsampled the data to reduce spatial autocorrelation, the dataset was reduced to 93 records (Fig. 1). To construct the SDMs, we removed variables with high collinearity as follows: in ‘model 1’, of 36 initially selected variables, seven remained (BIO2, BIO8, BIO9, BIO 13, BIO15, BIO31 and altitude); in ‘model 2’, of 39 initial variables, ten remained (BIO2, BIO6, BIO8, BIO13, BIO15, BIO25, altitude, NDVI, cattle density and Globe cover).

The environmental variable with the highest positive contribution was BIO8 (Mean Temperature of Wettest Quarter), and the one with the highest negative contribution was BIO15 (Precipitation seasonality) for ‘Maxent model 1’ (Table 1) and for ‘Maxlike model 1’ (Table 2). In ‘Maxent model 2’, the environmental variable with the highest positive contribution was BIO6 (Min Temperature of Coldest Month) and the one with the highest negative contribution for suitability was BIO15 (Table 1). For ‘Maxlike model 2’ the most important variable for predicting Pampas cat occurrence was BIO08 and the one most negatively related to Pampas cat occurrence was BIO15 (Table 2). The third variable that most contributed to three of the models (‘Maxent model 1’, ‘Maxent model 2’ and ‘Maxlike model 2’) was “altitude”; it had a negative influence in all of them. For ‘Maxlike model 1’, the third most important variable was BIO31 (Moisture index seasonality), with a positive influence. For the Maxent models, the BIO8 and BIO6 output plots were similar to a simple quadratic response, while BIO15 and “altitude” fit a simple negative linear relationship (Supplementary Material Fig. S1)

TABLE 1. Average percent contribution of each variable to the Maxent models.

	'Maxent Model 1'		'Maxent Model 2'	
	% Contribution	SD	% Contribution	SD
BIO2	1.7	1	0.48	0.49
BIO6	-	-	15.16	3
BIO8	14.12	1.94	1.12	0.86
BIO9	1.34	1.38	-	-
BIO13	6.58	0.99	4.4	1.28
BIO15	61.6	3.27	56.07	3.53
BIO25	-	-	0.72	0.48
BIO31	2.59	1.27	-	-
Altitude	12.08	2.55	9.11	2.33
NDVI	-	-	0.53	0.8
Cattle den.	-	-	7.37	4.69
Land use	-	-	5.05	3.13

TABLE 2. Maxlike variables describing the best logistical models for Pampas cat (coefficients [Est, Z], standard error [SE] and *P*- values).

	Maxlike model 1				Maxlike model 2			
	Est	SE	*z*	*P*(>|*z*|)	Est	SE	*Z*	*P*(>|*z*|)
(Intercept)	7.24	3.6	2.01	0.04	2.11	3.33	0.63	0.53
BIO2	-0.06	0.03	-1.92	0.05	-0.02	0.02	-0.71	0.48
BIO6	-	-	-	-	-0.03	0.02	-1.59	0.11
BIO8	0.05	0.01	3.46	0	0.04	0.02	2.26	0.02
BIO9	0	0.01	0.26	0.8	-	-	-	-
BIO13	-0.02	0.01	-2.45	0.01	-0.02	0.01	-1.9	0.06
BIO15	-0.89	0.22	-3.98	0	-0.47	0.12	-3.74	0
BIO25	-	-	-	-	0.02	0.02	1.45	0.15
BIO31	56.05	19.56	2.87	0	-	-	-	-
Altitude	-0.01	0	-2.64	0.01	-0.01	0	-3.33	0
NDVI	-	-	-	-	0.01	0.01	1.04	0.3
Cattle den.	-	-	-	-	0.02	0.01	2.15	0.03
Land use	-	-	-	-	-0.01	0.01	-1.83	0.07

Maxent models

In 'Maxent model 1', the area covered by the total model (South America mask) was 446,163.2 km² (Supplementary Material Fig. S2). Concentrating on the of Uruguayan

Savannah, it comprised 362,553.6 km² (Fig .2). The results showed that only 6.06 % (22,006.4 km²) of the Uruguayan Savannah region (363.000 km²) (Hasenack *et al.,* 2010) emerged as highly suitable areas (> 0.7) for Pampas cat. The pixels with the highest suitability values were concentrated around the region of the Central Depression and central-western RS state, in Brazil, as well as southern and southeastern Uruguay. Considering 'Maxent model 2', the estimated area of suitable habitat was 377,200 km² for the South America mask (Supplementary Material Fig. S2) and in Uruguayan Savannah it was 343,822.4 km². Only 1.38% (5,004.8 km²) emerged as highly suitable areas for Pampas cat (0.71 – 0.83) in the Uruguayan Savannah; this model was 17,001.6 km² more restricted than same level of suitability in 'Maxent model 1'. The areas of highest suitability were again concentrated on the Central Depression and western regions of RS state, in Brazil, and in southern and southeastern Uruguay. In this model including anthropic variables, 53.3% of all high-suitability areas were included in the territories comprised by five Brazilian municipalities: São Gabriel, São Sepé, Cachoeira do Sul, Restinga Seca and Dom Pedrito. In Uruguay, the departments with the largest areas of high suitability were Colonia, Canelones, San José, Montevideo and Rocha (still, they covered only to 5.5% of the high-suitability area of Uruguayan Savannah in the 'Maxent model 2').

Maxlike models

'Maxlike model 1' estimated a total PO area of 786,563.2 km² (Supplementary Material Fig. S2), whereas the extent of occurrence of Pampas cat in the Uruguayan Savannah was 360,180 km² (Fig. 2). An area of 224,222.4 km² (61.77% of the Uruguayan Savannah) emerged as high-PO areas (>0.8) for the Pampas cat. The pixels with the highest probabilities (>0.8) were also concentrated around the region of Central Depression and western RS state, in Brazil, along with the entire Uruguay except for its northwestern portion. Considering 'Maxlike model 2' the estimated extent of Pampas cat PO in the entire model was 832,268.8 km² and in Uruguayan Savannah it was 385,130.4 km². High-PO areas for Pampas cat (0.80 – 1.00) emerged as 49.82% of the Uruguayan Savannah (180,835.2 km²); it was 43,387.2 km² more restricted than same level of PO in 'Maxlike model 1'. These areas were concentrated in the Central Depression, southwestern and southern regions of RS state, as well as eastern, central, southern Uruguay. In this model, the five municipalities in Brazil with the largest areas presenting high probability of occurrence were Cacequi, Dilacermo Aguiar, São Gabriel, São Sepé, and Rosário do Sul; and in Uruguay the departments were: Florida, Canelones, Flores, San José and Lavalleja.

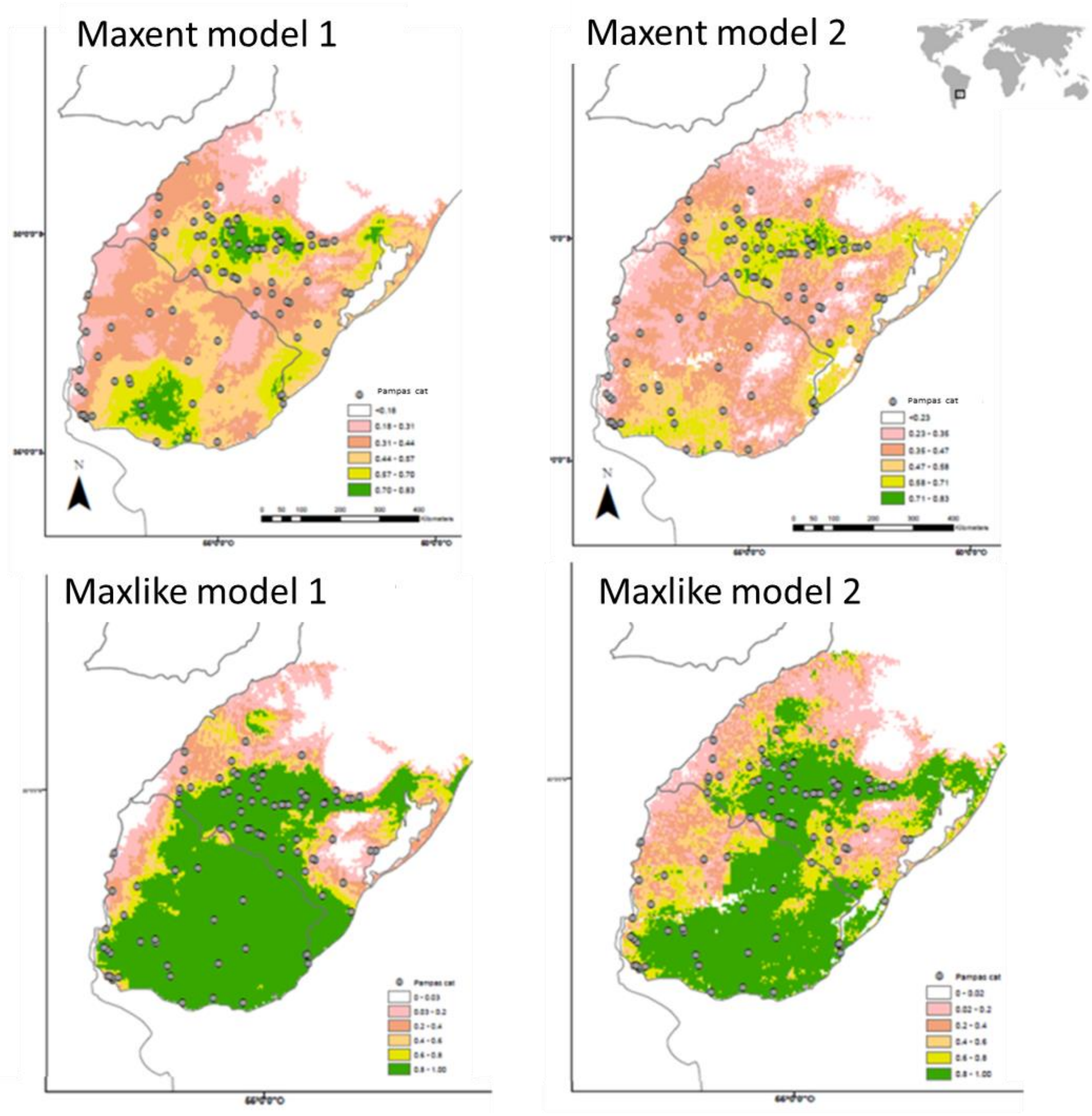

FIG. 2. Spatial distribution models of Pampas cats in the Uruguayan Savannah ecoregion, with categorized levels of suitability (Maxent) or Probability of Occurrence (Maxlike); dots represent the records of Pampas cat (n=93). For each method, ‘Model 1’ is based on bioclimatic variables and altitude, while ‘Model 2’ is based on bioclimatic variables, altitude, NDVI, cattle density and land use.

Comparison of models

Based on the AUC analysis, ‘Maxent model 2’ presented a slightly better performance (*AUC* = 0.991 ± 0.001 *SD*; test= 0.988 ±0.002 *SD*) than ‘Maxent model 1’ (*AUC* = 0.989 ± 0.001 *SD*; test= 0.988 ±0.002 *SD*). The pairwise correlations between all models were positive and statistically significant (Table 3). However, when we compared all models (*AIC*) we observed stronger support for ‘Maxent model 2’ and ‘Maxlike model 2’, the latter one being the best-fit model overall (Table 4).

TABLE 3. Spearman rank correlation (r_s) between the different models of Pampas cat spatial distribution and *P*-values.

Model	Maxent M2	Maxlike M1	Maxlike M2
Maxent M1	r_s= 0.96, P<0.01	r_s= 0.93, P <0.01	r_s= 0.95, P <0.01
Maxent M2		r_s= 0.88, P <0.01	r_s= 0.92, P <0.01
Maxlike M1			r_s= 0.98, P <0.01

TABLE 4. Comparison of Pampas cat spatial distribution models. Values of *Log Likelihood* and Akaike Information Criterion (*AIC*) for each independent model.

	Maxent M1	Maxent M2	Maxlike M1	Maxlike M2
Log Likelihood	-914.02	-918.64	-927.06	-880.67
AIC score	1862.04	1851.28	1870.129	1783.32

Assessment of IUCN threat categories

To estimate the total population size for this ESU, and consequently its conservation status, we used the two best-supported models. The conservation status of this ESU varied according to the estimated extent of suitable habitat, as well as the assumed density (Table 5). We observed that 70% of all results were estimated as <10,000 individuals for the population, indicating that the majority of results would lead to its inclusion in some threatened category. If only the areas with the highest suitability and PO were considered likely to sustain this population in the long term, and thus its range restricted to them, this ESU would be assigned to the “Critically Endangered” or “Endangered” category, depending on the model (see Table 5).

TABLE 5. Population size estimations and corresponding conservation status of the Uruguayan Savannah Pampas cat ESU in the two best-fit models (Maxent 'model 2' and Maxlike 'model 2'), based on increasingly stringent thresholds of Suitability and probability of occurrence (PO). ‘N ind’ is the estimated number of individuals for a given area.

	Maxent 'model 2'				Maxlike 'model 2'			
ind/km^2*	Suitability	Area (km^2)	N ind	Status**	PO	Area (km^2)	N ind	Status**
0.05	0.23-0.83	343822	17191	NT	0.02-1.00	385130	19257	NT
0.01	0.23-0.83	343822	3438	VU	0.02-1.00	385130	3851	VU
0.05	0.35-0.83	244278	12214	NT	0.20-1.00	325606	16280	NT
0.01	0.35-0.83	244278	2443	EN	0.20-1.00	325606	3256	VU
0.05	0.47-0.83	133124	6656	VU	0.40-1.00	277546	13877	NT
0.01	0.47-0.83	133124	1331	EN	0.40-1.00	277546	2775	VU
0.05	0.59-0.83	46754	2338	EN	0.60-1.00	233257	11663	NT
0.01	0.59-0.83	46754	468	EN	0.60-1.00	233257	2333	EN
0.05	0.71-0.83	5004.8	250	CR	0.80-1.00	180835	9042	EN
0.01	0.71-0.83	5004.8	50	CR	0.80-1.00	180835	1808	EN

*Oliveira pers. comm. in Queirolo *et al.* 2013 and Lucherini *et al.* 2016.
**Criterion C of threatened categories of the IUCN Red List (CR<250/ EN<2,500/VU<10,000 individuals) and other categories (NT<20,000 individuals).

SDMs and Protected areas

The results from ‘Maxent model 2’ indicated that only 0.7% of high-suitability areas are contained in protected areas (Fig. 3a). The overlap of the ‘Maxlike model 2’ with the protected area layer showed that 9.8% of high-PO areas are restricted to five protected areas (Fig. 3b). All other regions of the Uruguayan Savannah ecoregion, especially in central and southwestern RS state (Brazil) and southern and southwestern Uruguay, were areas estimated as high-suitability and high-PO which do not include any protected areas, or whose protected areas are too small and isolated. We suggest considering as priority areas for Pampas cat conservation those presented in the best-fit model 'Maxlike model 2'. Based on this model, we propose increasing three existing protected areas (2. Indigenous territory Irapurá; 4. Rocky area of Chamangá; and 5. Protected Area of Humedales del Santa Lucia) and the creation of two new ones (x and y) connecting Brazilian protected areas and Uruguayan ones (Fig. 3c).

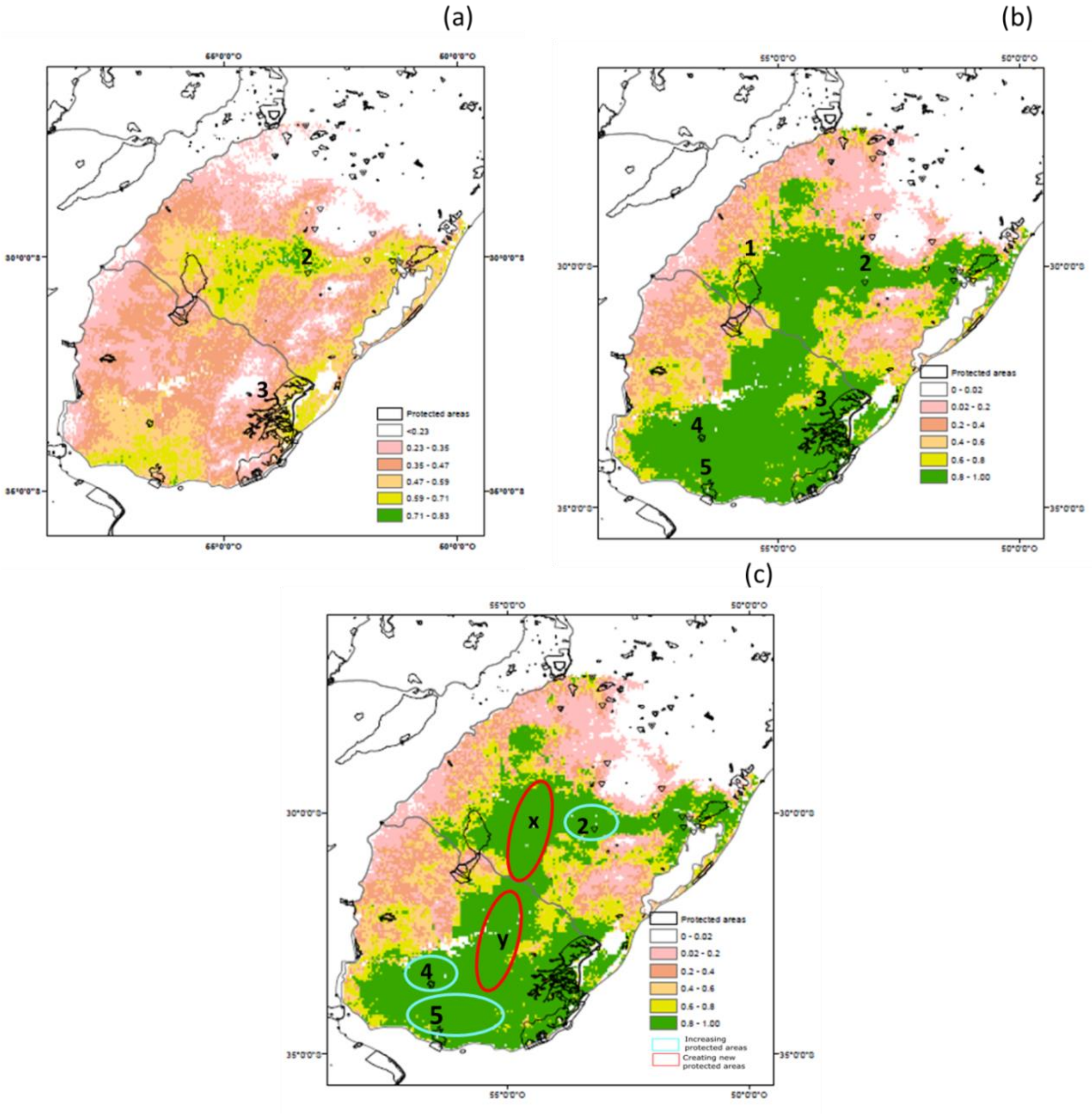

FIG. 3 Best-fit models for *Leopardus colocolo* in the Uruguayan Savannah ecoregion, overlaid on the World Database on Protected Areas layer. (a) Maxent Model 2; (b) Maxlike Model 2; and (c) Maxlike Model 2 with the suggestion of protected areas that should be expanded (blue lines), and new ones that should be created (red lines – see text for details). The protected areas in which high-suitability and high-PO areas were observed are: 1. Protected Area of Rio Ibirapuitã; 2. Indigenous territory Irapurá; 3. Protected Area

of Bañados del Estey Franja Costera - Biosphere Reserve Bañados del Este; 4. Rocky area of Chamangá; and 5. Protected Area of Humedales del Santa Lucia.

Discussion

Our results provide novel information that should be useful in the context of conservation planning on behalf of this genetically distinct unit of Pampas cat, restricted to this threatened ecoregion. We considerably increased the number of location points available for the species in the Uruguayan Savannah and, by testing different spatial distribution models, we made available the first comparison between models exclusively based on bioclimatic variables (‘model 1’) and models including anthropogenic variables (‘model 2’) for this felid.

The broad distribution of the Pampas cat in South America leads to extreme differences in many variables, such temperature or altitude. The species tolerates temperatures spanning from the temperate ones of southernmost Patagonia (Argentina and Chile) or the Andes to those found in tropical areas such as the Pantanal or Cerrado biomes in Brazil. The Uruguayan Savannah ESU is positively influenced by temperature, with the highest suitability being observed between 10ºC and 20ºC degrees (BIO6 [Mean Temperature of Wettest Quarter] and BIO8 [Min Temperature of Coldest Month], Supplementary Material Fig.S1).

Altitude and Precipitation seasonality were strongly and negatively correlated with the suitability and the PO for the species in the Uruguayan Savannah. In this ecoregion, the altitudes range from sea level to *ca*. 1000 m (Hasenack et al., 2010), and the ESU showed better suitability and PO in areas with altitudes between sea level and 400m. Although the species has been recorded at over 5,000 m in the high Andes (Nowell & Jackson, 1996), its PO also seems to decrease with altitude in the highest portion of its distribution (Napolitano et al., 2008). In northern Chile, Napolitano et al. (2008) justified the negative correlation with altitude as a strategy in order to avoid competition with the Andean cat, however this species does not occur in Uruguayan Savannah, where Pampas cats do not seem to have a competitor at higher altitudes. Therefore, other factors may also influence this felid’s distribution in this region. According to previous studies, seasonal precipitation combined with other variables (climatic fluctuations, soil fertility, drainage, fire regime) impact on the growth of the different vegetative stages within the range of Pampas cats (Trolle, 2003; Bagno et al., 2004; Barstow & Leslie, 2012). Usually, seasonal precipitation is positively correlated with the three growth stages of forested habitats (Brienen & Zuidema, 2005), and here this variable is negatively correlated with Pampas cat distribution. In all the models we tested, the high-suitability and high-PO areas were distributed on regions covered with grasslands. Conversely, the areas with no or very low suitability and PO were forested. Since the Pampas cat is mainly found in open areas, on a macrogeographic scale (Silveira, 1995; Bagno et al., 2004; Lucherini et al., 2016), our modelling results seem to agree with the available general biological information on this species.

Our correlation analyses indicated that all models produced similar results. However, ‘model 2’ was more strongly supported than ‘model 1’ with both algorithms. These results indicated that the models incorporating land use and other human-related variables were most similar to the real distribution of Pampas cat in the ecoregion. Because the two best-fit models were more restricted than models ‘1’, these results suggest that the Pampas cat distribution on this ecoregion is negatively influenced by human disturbance.

Some areas of high suitability and high PO for Pampas cat overlapped with areas with intermediate level of anthropogenic activity, which may indicate that this felid could tolerated a certain level of human disturbance, as already mentioned in previous studies (Bagno et al. 2004; Bou, 2012). However, it could also suggest that the same areas that were suitable for Pampas cats could be important for human activities and consequently Pampas cat populations could be negatively affected by ongoing and future alterations of these areas.

The system designed by IUCN was created to determine the relative risk of extinction of species (or other taxonomic levels such as subspecies, varieties, and subpopulations). The organisms facing a certain risk of extinction are included in one of three threatened categories: “Critically Endangered”, “Endangered” and “Vulnerable” (IUCN 2001). With regard to the conservation status of the Pampas cat ESU present in Uruguayan Savannah, we observed that the majority of estimates generated a population number that would make it fall into one of the threatened categories. When we only included in this assessment the highest suitability areas of ‘Maxent model 2’, this population would be assigned to the “Critically Endangered” category, since the number of individuals would be estimated at $\leq$250. Even based on the ‘Maxlike model 2’, which predicts a broader extent of occurrence than ‘Maxent model 2’, if only the areas with the highest probability of occurrence were considered, the status of this population would be “Endangered”, since we would estimate the existence of ~1,800 – 9,000 individuals in this ESU. Regardless the exact threat category that is assigned to this Pampas cat ESU, these results strongly suggest that it is facing the risk of extinction.

In this study, we concluded that there is a threatened Pampas cat ESU in the Uruguayan Savannah, an ecoregion that has only ~4% of its range included in protected areas. These findings highlight a critical concern in terms of conservation. If we are to conserve this ESU, it is crucial to increase the existing protected areas and create new ones with the objective of connecting them, through the creation of corridors characterized by a mosaic of natural open grasslands and other native vegetation. The expansion of three existing protected areas and the creation of two new ones could ensure appropriate corridors connecting the majority of areas currently under protection. Pampas cats possibly tolerate some level of human disturbance (Bagno et al., 2004; Bou, 2012), although this level may need to be combined with sustainable practices. The continuing monoculture-based agriculture expansion, along with other human disturbances, could lead the extinction of this ESU. Here we suggest that these new protected areas could include sustainable farming systems. Nevertheless, it is import also to create strict nature reserve (IUCN category Ia) or wilderness area (IUCN category Ib) (Dudley, 2013) within these protected areas. Overall, the future of this Pampas cat ESU seems to depend on conservation plans that successfully combine biodiversity conservation with sustainable agriculture.

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Author contributions

F.P.T. collected data, analysed data and wrote the manuscript, L.G.S. discussed about analysed data, D.Q. collected data and discussed about analysed data, T.C.T. discussed about analysed data, C.B.K. collected data, F.P. collected data, C.E. collected data. F.M. collected data, D.W.M. discussed about analysed data and wrote the manuscript, M.L. discuss about analysed data and wrote the manuscript, and E.E. discussed about analysed data and wrote the manuscript.

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Biographical sketches

FLÁVIA P. TIRELLI’s research focuses on wildlife conservation, especially on ecology and conservation of felids. LUCAS GONÇALVES DA SILVA’s studies focuses on spatial distribution of mammals using Maxent software. DIEGO QUEIROLO’s research interests include taxonomy, ecology and conservation of mammals from Uruguayan Savannah, TATIANE C. TRIGO’S research focuses on genetic, ecology and conservation of felids. CARLOS BENHUR KASPER’S studies focuses on ecology of mammals from southern Brazil, FELIPE PETERS’S studies focuses on ecology of mammals from southern Brazil, CAROLINE ESPINOSA’s interests include ecology and conservation of felids from southern Brazil. FÁBIO D. MAZIM’s studies focuses on ecology of mammals, especially felids. DAVID W. MACDONALD’s research focuses on wildlife conservation and environmental management, with emphasis on carnivores. MAURO LUCHERINI’S research focuses on ecology and conservation of carnivores, especially Neotropical felids, EDUARDO EIZIRIK’S studies focuses on evolution, genomic, molecular ecology and conservation genetics of carnivores, especially felids.

Supporting information S1

Assuming that Parana River is the unique west geographic barrier, we developed others SDMs of Pampas cat. These models included two occurrences from the region between Uruguay and Paraná Rivers in Argentina (Fig. 1). This analyses were performed with same methods of others SDMs (see Material and Methods), however the names of each models were: ‘Maxent model 3’, ‘Maxent model 4’, ‘Maxlike model 3’, ‘Maxlike model 4’.

We collected 109 locations of Pampas cat in the Uruguayan Savanna ecoregion. As we subsampled the data to reduce spatial autocorrelation, the dataset was reduced to 95 occurrences. The most negative influent variable in Maxent models were BIO 15 (61.3%, 55.6% respectively) and altitude (11.2%, 10% respectively), for the ‘Maxent model 3’ the variable BIO8 influenced positively the model with 11% of percentage of importance, while for ‘Maxent model 4’ the variable BIO6 influenced in 15.8%. For Maxlike models, ‘Maxlike model 3’ and ‘Maxlike model 4’, the most negative influent variables also were BIO 15 (z = -3.99 [$P<0.01$] and z = -4.57 [$P<0.01$] respectively) and altitude (z= -3.36 [$P<0.01$] and z= -3.47 [$P<0.01$] respectively), and the most positive influence was BIO8 for both models. In the models ‘Maxent model 3’and ‘Maxent model 4’, the estimated area for total model was 559341.6 km^2 and 583132.8 km^2, respectively.

Concerning Maxlike models the ‘Maxlike model 3’ estimated a total PO area of 1212063.2 km^2 and the ‘Maxlike model 4’ estimated a total PO area of 1367322.4 km^2 (Supplementary Material Fig. S2 and Fig. S3). The suitability and probability of occurrence was low in the areas were the two samples of Argentina were distributed for all models. This may happened due the number of records, very low in these part of Argentina compared with number of records in Brazil and Uruguay, which results in distribution models effected from the most samples area (in this case Brazil and Uruguay).

Supporting information Table S1 Database of geographic occurrence of Pampas cat used in the present study for modelling.

Coordinate ID number	**Sample ID (museum)**	**Dec.Deg, WGS84**		**Sample**	**Year**	**Locality**	state	Contry	**Source**
		Lat	**Long**						
Lcolocolo_SU_01	MCP1813	-30.2456	-54.8154	road kill	2012	BR-290. São Gabriel and Rosário	RS	Brazil	Col: Caio Wahl, Marcio Reppening, E. Chiarani/MCT-PUCRS
Lcolocolo_SU_02	MCR1799	-31.1129	-53.7810	road kill	2009	BR156. Bagé	RS	Brazil	Col: Fábio Mazim/ Genoma Database/MCT-PUCRS
Lcolocolo_SU_03	MCT1796/bLco_039	-29.6523	-54.5611	road kill	2012	Mata	RS	Brazil	Col: Giuliano Brusco & Alan Bolzan/ Genoma Database/MCT-PUCRS
Lcolocolo_SU_04	MCT1957/#198/FT159	-30.7992	-55.2403	road kill	2014	Santana do Livramento	RS	Brazil	Col: Flávia Tirelli & Henrique Sitjá/MCT-PUCRS
Lcolocolo_SU_05	#222/FT170	-31.0377	-54.5581	road kill	2014	Dom Pedrito	RS	Brazil	Col: Ana Paula Albano & Paola Faria/MCT-PUCRS
Lcolocolo_SU_06	MCT1899/#139	-29.3502	-55.2844	road kill	2013	Manoel Viana	RS	Brazil	Col: Caroline Espinosa/MCT-PUCRS
Lcolocolo_SU_07	MCT1793	-29.1621	-56.3615	road kill	2010	BR 472. Close to city entrance of Maçambará, Itaqui	RS	Brazil	Col: Márcio Reppening/MCT-PUCRS
Lcolocolo_SU_08	/	-29.7177	-54.7705	road kill	2012	Manoel Viana	RS	Brazil	Col: Caroline Espinosa
Lcolocolo_SU_09	/	-29.7402	-55.5606	road kill	2010	São Vicente do Sul	RS	Brazil	Col: Caroline Espinosa
Lcolocolo_SU_10	/	-29.7742	-54.7964	camera-trap	2008	São Vicente do Sul	RS	Brazil	Espinosa, C. 2009
Lcolocolo_SU_11	/	-29.5920	-55.2317	report	2015	São Francisco de Assis	RS	Brazil	C. Espinosa pers. com.
Lcolocolo_SU_12	/	-30.2123	-53.5613	road kill	2014	São Sepé	RS	Brazil	Col: Felipe Peters
Lcolocolo_SU_13	bLco_018	-30.0228	-55.3412	road kill	2002	Alegrete	RS	Brazil	Michalski & Hasenack 2002/ Weber et al. 2013/ Genoma Database
Lcolocolo_SU_14	/	-30.2505	-54.5172	scientific article		Banhado Inhatium. São Gabriel	RS	Brazil	Indrusiak & Eizirik 2003/ Weber et al. 2013
Lcolocolo_SU_15	/	-30.2838	-53.1172	report	no data	BR-290. Barro Vermelho	RS	Brazil	Weber et al. 2013
Lcolocolo_SU_16	/	-30.0338	-52.8839	scientific article	no data	Cachoeira do Sul	RS	Brazil	Weber et al. 2013
Lcolocolo_SU_17	bLco_016	-31.3838	-53.7672	road kill	2000	Candiota	RS	Brazil	Weber et al. 2013/ Genoma Database
Lcolocolo_SU_18	MCNU1023/ bLco_027	-30.9838	-54.6672	report	2009	Dom Pedrito	RS	Brazil	Weber et al. 2013/Museum ULBLA/ Genoma Database
Lcolocolo_SU_19	/	-30.8838	-54.8506	scientific article	2009	BR-293, km272. Dom Pedrito	RS	Brazil	Weber et al. 2013
Lcolocolo_SU_20	MCNU1068	-30.0338	-52.8672	road kill	no data	Encruzilhada do Sul	RS	Brazil	Weber et al. 2013/ Museum ULBRA
Lcolocolo_SU_21	MNRJ 42100/bLco_001	-30.0505	-56.4839	skin	no data	BR-290. Between Alegrete and Uruguaiana	RS	Brazil	Weber et al. 2013/ Genoma Database
Lcolocolo_SU_22	/	-32.7172	-52.5339	scientific article		Estação Ecológica do Taim (reserve)	RS	Brazil	Indrusiak & Eizirik 2003/ Weber et al. 2013
Lcolocolo_SU_23	/	-30.3005	-56.5006	scientific article		Santa Virgínia Farm, Quaraí	RS	Brazil	Indrusiak & Eizirik 2003/ Weber et al. 2013

Lcolocolo_SU_24	/	-29.2172	-53.6672	scientific article		Júlio de Castilhos	RS	Brazil	Silveira 1995/ Weber et al. 2013
Lcolocolo_SU_25	MCT725	-30.1672	-52.3339	road kill		Pantano Grande	RS	Brazil	Weber et al. 2013/ MCT-PUCRS
Lcolocolo_SU_26	/	-31.5672	-53.3672	report	no data	Pedras Altas, Pinheiro Machado	RS	Brazil	Weber et al. 2013
Lcolocolo_SU_27	/	-30.8838	-55.5339	scientific article		Santana do Livramento	RS	Brazil	Indrusiak & Eizirik 2003/ Weber et al. 2013
Lcolocolo_SU_28	/	-31.3504	-51.9672	scientific article	no data	São Lourenço do Sul	RS	Brazil	Ihering 1892/ Silveira 1995/ Weber et al. 2013
Lcolocolo_SU_29	MCNU1694/ bLco_026	-30.1505	-53.5505	scientific article	2009	São Sepé	RS	Brazil	Weber et al. 2013/ Museum ULBRA/ Genoma Database
Lcolocolo_SU_30	bLco_021/ MCN	-30.2671	-52.8505	scientific article	2008	BR-209, km277	RS	Brazil	Weber et al. 2013/Genoma Database/ FZB
Lcolocolo_SU_31	/	-31.8344	-53.5883	direct visualization	2000	Pedras Altas	RS	Brazil	F. D. Mazim pers. com.
Lcolocolo_SU_32	/	-32.3680	-53.1866	direct visualization	2000	Arroio Grande	RS	Brazil	F. D. Mazim pers. com.
Lcolocolo_SU_33	/	-31.1010	-52.9598	direct visualization	2000	Piratini	RS	Brazil	F. D. Mazim pers. com.
Lcolocolo_SU_34	/	-31.5492	-53.4251	direct visualization	2000	Pinheiro Machado	RS	Brazil	F. D. Mazim pers. com.
Lcolocolo_SU_35	/	-31.3647	-52.0849	direct visualization	2000	São Lourenço do Sul	RS	Brazil	F. D. Mazim pers. com.
Lcolocolo_SU_36	/	-29.9464	-54.6739	direct visualization	2000	Cacequi	RS	Brazil	F. D. Mazim pers. com.
Lcolocolo_SU_37	/	-29.6778	-55.1425	skin	2000	São Francisco de Assis	RS	Brazil	Col: Fábio D. Mazim
Lcolocolo_SU_38	MCNU880/ bLco_020	-31.0158	-54.6043	road kill	2007	Dom Pedrito	RS	Brazil	Col: Felipe Peters
Lcolocolo_SU_39	MCN2992/bLco_002	-30.2111	-52.5249	road kill	no data	Cachoeira do Sul	RS	Brazil	Genoma Database/ FZB
Lcolocolo_SU_40	bLco-011	-30.8850	-54.9094	Zôo Sapucaia	no data	Dom Pedrito	RS	Brazil	Genoma Database
Lcolocolo_SU_41	MCN3271/NE234/ bLco_017	-30.3025	-53.1360	road kill	2001	BR 290 35Km. Close to entrance of Cachoeirinha	RS	Brazil	Jan Mähler Jr./ FZB/ Genoma Database
Lcolocolo_SU_42	MCT1783/ bLco_038	-31.3523	-52.0847	road kill		BR116. Divisa Pelotas - Camaquã	RS	Brazil	Cols: Flávia P. Peter e Virgiane Knorr
Lcolocolo_SU_43	CMU26	-32.0810	-52.7183	road kill	2000	Rio Grande	RS	Brazil	Col: Fernando Quintela/ UFRGS Mammal Collection
Lcolocolo_SU_44	/	-30.1222	-53.6170	road kill	2008	BR-392, km 299. São Sepé	RS	Brazil	Silva et al. 2011
Lcolocolo_SU_45	/	-30.1955	-53.5630	road kill	2011	BR-392, km 290. São Sepé	RS	Brazil	Silva et al. 2011
Lcolocolo_SU_46	/	-31.3667	-51.9667	scientific article	no data	São Lourenço do Sul	RS	Brazil	García-Perea 1994
Lcolocolo_SU_47	/	-30.0392	-52.8939	no data	no data	Capão Veado	RS	Brazil	Indrusiak e Eizirik 2003
Lcolocolo_SU_48	Tati A36	-30.3622	-54.2954	road kill	2015	BR 290. São Gabriel	RS	Brazil	Col: Mariano Cordeiro Pairet Jr.

Lcolocolo_SU_49	FT174	-30.4812	-55.0621	road kill	2015	BR158. Between Rosário do Sul e Santana do Livramento	RS	Brazil	Col: Flávia Tirelli
Lcolocolo_SU_50	Gramado Zôo-F	-31.3219	-54.1157	alive- raised as a domestic	2014	Bagé	RS	Brazil	Gramado Zôo- female
Lcolocolo_SU_51	Gramado Zôo-M	-30.0428	-53.6775	alive- ran over road	2014	BR392. between São Sepé e Santa Maria	RS	Brazil	Gramado Zôo- male (road)
Lcolocolo_SU_52	/	-29.6722	-54.5736	video	2015	São Vicente	RS	Brazil	Col: Mr. Augusto SOBRENOME
Lcolocolo_SU_53	/	-28.9240	-54.9596	photo/report	2015	RS168. close to Bossoroca	RS	Brazil	Col: Fábio D. Mazim
Lcolocolo_SU_54	MZPAMPA M0001	-30.3391	-54.0634	road kill	2013	Santa Margarida do Sul	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_55	MZPAMPA M0055	-30.3712	-53.6742	road kill	2014	São Sepé	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_56	MZPAMPA M0166	-30.3624	-54.2956	road kill	2015	São Gabriel	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_57	MZPAMPA M0245	-30.3084	-53.1695	road kill	2015	Cachoeira do Sul	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_58	MZPAMPA M0250	-30.3445	-53.9737	road kill	2015	Santa Margarida do Sul/Vila Nova	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_59	MZPAMPA M0274	-30.2219	-52.622	road kill	2015	Cachoeira do Sul/Rio Pardo	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_60	MZPAMPA M0487	-30.3483	-54.1251	road kill	2016	Santa Margarida do Sul	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_61	MZPAMPA M0489	-30.1674	-53.5814	road kill	2011	São Sepé	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_62	MZPAMPA M0446	-29.9838	-56.476	road kill	2016	Uruguaina	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_63	MZPAMPA M0447	-29.9567	-56.2223	road kill	2016	Alegrete	RS	Brazil	Col: Carlos B. Kasper/MZPAMPA
Lcolocolo_SU_64	/	-30.0750	-55.473	report	2011	Cerro dos Porongos Farm, Rosário do Sul	RS	Brazil	C. B. Kasper pers. com.
Lcolocolo_SU_65	/	-30.1830	-55.099	report	2006	BR290. Rosário do Sul	RS	Brazil	F. P. Tirelli pers. com.
Lcolocolo_SU_66	/	-29.5571	-56.377	report, photo, video	2016	Alegrete	RS	Brazil	Col: Henrique Farret
Lcolocolo_SU_67	MNHN884	-33.3860	-57.3780	scientific article	1959	A. Perdido	Soriano	Uruguay	Ximénez 1961 / Olazarri et al. 1970 / Garcia-Perea 1994 / Bou 2012/ Queirolo 2009
Lcolocolo_SU_68	MNHN971	-33.5527	-54.9550	scientific article, skin, skull	1960	Bella Vista Farm, Zapicán	Lavalleja	Uruguay	Ximénez 1961 / Olazarri et al. 1970 / Ximénez et al. 1972 / Garcia-Perea 1994 / Bou 2012/ Queirolo 2009
Lcolocolo_SU_69	MNHN875	-32.4666	-55.0166	scientific article	1959	Juan Escoto Farm, Tarariras	Cerro Largo	Uruguay	Ximénez 1961 / Olazarri et al. 1970 / Ximénez et al. 1972 / Garcia-Perea 1994 / Bou 2012/ Queirolo 2009
Lcolocolo_SU_70	/	-33.7128	-53.5694	scientific article	no data		Rocha	Uruguay	Queirolo 2009
Lcolocolo_SU_71	ZVC-M1492	-33.5800	-58.1460	scientific article, skin, skull	no data	River San Salvador, close to Paso de Ramos, Dolores	Soriano	Uruguay	Bou 2012/ Queirolo 2009
Lcolocolo_SU_72	/	-33.5190	-58.2160	scientific article, captured	2005	Dolores	Soriano	Uruguay	Bou 2012/ Queirolo 2009
Lcolocolo_SU_73	MNHN1315	-34.1650	-58.0970	scientific article, skin, skull	1968	A. Limetas, San Jorge Farm	Colonia	Uruguay	Ximénez et al. 1972 / Bou 2012/ Queirolo 2009

Lcolocolo_SU_74	MNHN1400/ bLco_037	-34.2430	-58.0270	scientific article	1969	Los Cerros de San Juan Farm, Paraje Punta Francesa	Colonia	Uruguay	Bou 2012/ Queirolo 2009/ Genoma Database
Lcolocolo_SU_75	MNHN2926/ bLco_036	-34.1899	-57.8862	scientific article, skin, skull, skeleton	1986	Ayo. Miguelete, Ruta 21, Paso del Pelado	Colonia	Uruguay	Bou 2012/ Queirolo 2009/ Genoma Database
Lcolocolo_SU_76	MNHN2433	-34.2050	-58.0460	scientific article, skin, skull, skeleton	1971	3km N from Punta Pereyra	Colonia	Uruguay	Bou 2012/ Queirolo 2009
Lcolocolo_SU_77	MNHN4706	-33.8857	-55.5840	scientific article, skin, skull, skeleton	2005	R.7 km 137, Artega	Florida	Uruguay	González & Sappa 2007 / González & Martínez 2010 / Bou 2012/Queirolo 2009
Lcolocolo_SU_78	MNHN4785/ bLco_033	-32.8122	-57.7610	scientific article, skin	1969	Pueblo Sanchés	Río Negro	Uruguay	Bou 2012/ Queirolo 2009/ Genoma Database
Lcolocolo_SU_79	MNHN879	-34.1670	-56.6870	scientific article	1959	Chamizo	San José	Uruguay	Ximénez 1961 / Olazarri et al. 1970 / Garcia-Perea 1994 / Bou 2012/ Queirolo 2009
Lcolocolo_SU_80	MNHN1375	-33.9310	-56.7560	scientific article, skin, skull, skeleton	1969	San Gregorio	San José	Uruguay	Garcia-Perea 1994 / Sicuro & Oliveira 2011 / Bou 2012/ Queirolo 2009
Lcolocolo_SU_81	MNHN4786/ bLco_034	-33.6502	-58.0752	scientific article, skin	1971	Close to Pueblo Cañada Nieto, Río San Salvador, Canada Nieto	Soriano	Uruguay	Bou 2012/ Queirolo 2009/ Genoma Database
Lcolocolo_SU_82	MNHN2603	-33.1150	-58.1760	scientific article, skin	1971	Bopicuá 10km NW of Fray Bentos	Río Negro	Uruguay	González 1973 / Bou 2012/ Queirolo 2009
Lcolocolo_SU_83	MNHN3374	-34.1602	-58.1437	scientific article, skull	1975	Field next to A. Tigre	Colonia	Uruguay	Bou 2012/ Queirolo 2009
Lcolocolo_SU_84	MNHN3224	-34.7637	-56.4178	scientific article, skull, skeleton	1990	Bañados de Playa Pascual	San José	Uruguay	Bou 2012/ Queirolo 2009
Lcolocolo_SU_85	MNHN	-34.6668	-55.7040	scientific article, skull	no data	A. mosquitos, Soca	Canelones	Uruguay	González & Sappa 2007/ Bou 2012/Queirolo 2009
Lcolocolo_SU_86	/	-34.7619	-55.0208	scientific article	no data		Maldonado	Uruguay	González & Martínez 2010
Lcolocolo_SU_87	MNHN	-32.9140	-55.6890	scientific article, skin, skull, skeleton	2011	A. el Chileno, 6 km SW from Blanquillo	Durazno	Uruguay	Bou 2012
Lcolocolo_SU_88	MNHN6909	-32.2550	-58.0290	scientific article, liquid	2009	R. 3 going to Paysandú	Paysandú	Uruguay	Bou 2012
Lcolocolo_SU_89	/	-31.7510	-56.0560	scientific article, report, photo	2010	R.26, Tacuarembó	Tacuarembó	Uruguay	Bou 2012
Lcolocolo_SU_90	MNHN 1385	-34.1650	-58.0970	skin,skull	1966	San Jorge-Matín Chico Farm	Colonia	Uruguay	MNHN Uruguay/ Bou 2012
Lcolocolo_SU_91	MNHN 1390	-34.1654	-58.0970	skin	1958	Campamento Farm - Conchillas	Colonia	Uruguay	MNHN Uruguay
Lcolocolo_SU_92	/	-31.8720	-54.1690	captured	2001	Aceguá	Cerro Largo	Uruguay	Col:Juan Villalba/ Bou 2012
Lcolocolo_SU_93	MNHN2780	-34.1650	-58.0970	scientific article, skin, skull, skeleton	1974	A. Limetas, Estancia San Jorge, Conchillas	Colonia	Uruguay	Bou 2012/ MNHN Uruguay
Lcolocolo_SU_94	MNHN2432	-34.1650	-58.0970	scientific article, skin, skull, skeleton	1971	A. Limetas, Estancia San Jorge, Conchillas	Colonia	Uruguay	Bou 2012/ MNHN Uruguay
Lcolocolo_SU_95	MNHN4705	-34.1650	-58.0970	scientific article, skull	no data	A. Limetas, Estancia San Jorge, Conchillas	Colonia	Uruguay	Bou 2012/ MNHN Uruguay
Lcolocolo_SU_96	MNHN2479	-34.1650	-58.0970	scientific article, skull, skeleton	no data	A. Limetas, Estancia San Jorge, Conchillas	Colonia	Uruguay	Bou 2012/ MNHN Uruguay
Lcolocolo_SU_97	/	-33.4250	-57.0200	scientific article, report	2009	km 206, R.3	Flores	Uruguay	Bou 2012
Lcolocolo_SU_98	/	-34.7880	-55.0640	scientific article, report	no data	Lahuna del Sauce	Maldonado	Uruguay	Bou 2012

Lcolocolo_SU_99	/	-32.1520	-57.4620	scientific article, report	1991	Rincón de Pérez, Queguay	Paysandú	Uruguay	Bou 2012
Lcolocolo_SU_100	MNHN3413	-33.1150	-58.1760	scientific article, skull, skeleton	1973	Bopicuá 10km NW of Fray Bentos	Río Negro	Uruguay	Bou 2012
Lcolocolo_SU_101	/	-33.8950	-53.5150	scientific article, report	no data	La Coronilla	Rocha	Uruguay	Bou 2012
Lcolocolo_SU_102	/	-33.6900	-53.5380	scientific article, camera-trap	2002	San Miguel Park	Rocha	Uruguay	Bou 2012/ González 2002
Lcolocolo_SU_103	/	-31.4120	-57.9830	scientific article, report	no data	Salto	Salto	Uruguay	Bou 2012
Lcolocolo_SU_104	/	-31.8070	-56.5740	scientific article, report	2010	Salto	Salto	Uruguay	Bou 2012
Lcolocolo_SU_105	AMNH189394	-33.9310	-56.7560	scientific article, report	1962	Estancia Herminia, San Gregorio Park	San José	Uruguay	Bou 2013/ AMNH USA
Lcolocolo_SU_106	MNHN1275/bLco035	-34.1670	-56.6870	scientific article, skin	no data	Santa Clara Farm,Chamizo	San José	Uruguay	Bou 2012/ MNHN Uruguay
Lcolocolo_SU_107	/	-33.3990	-57.0310	road kill	2016	Ruta 3, km 210,	Flores	Uruguay	Col: José M. Venzal, Luis Carvalho and Diego Queirolo
Lcolocolo_SU_108*	Nº CFA-11107	-27.4396	-57.5933	road kill	2007	National Road 12, km 1158, Puerto Yahapé-Itá Ibaté	Corrientes	Argentina	Chebez et al. 2008
Lcolocolo_SU_109*	/	-28.8171	-57.5526	road kill	2008	Mercedes	Corrientes	Argentina	Soljan et al. 2010

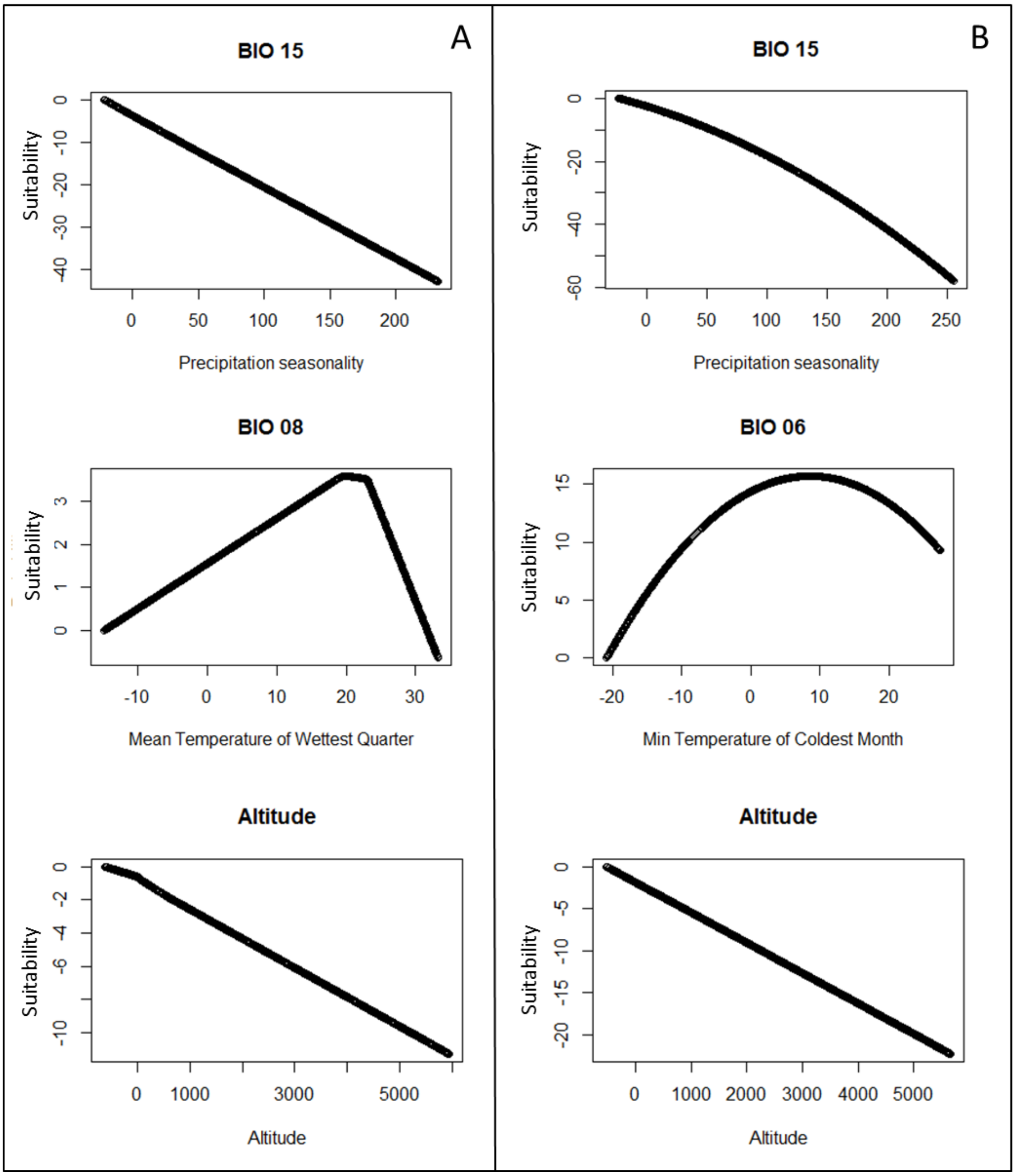
*Occurrences from a region between Uruguay River and Paraná River, in Argentina. This data is included only in additional information models, since there is no genetic confirmation that this population is included in the distinct genetic unit of Uruguayan Savannah.

TABLE S2 All environmental variables initially selected from modelling.

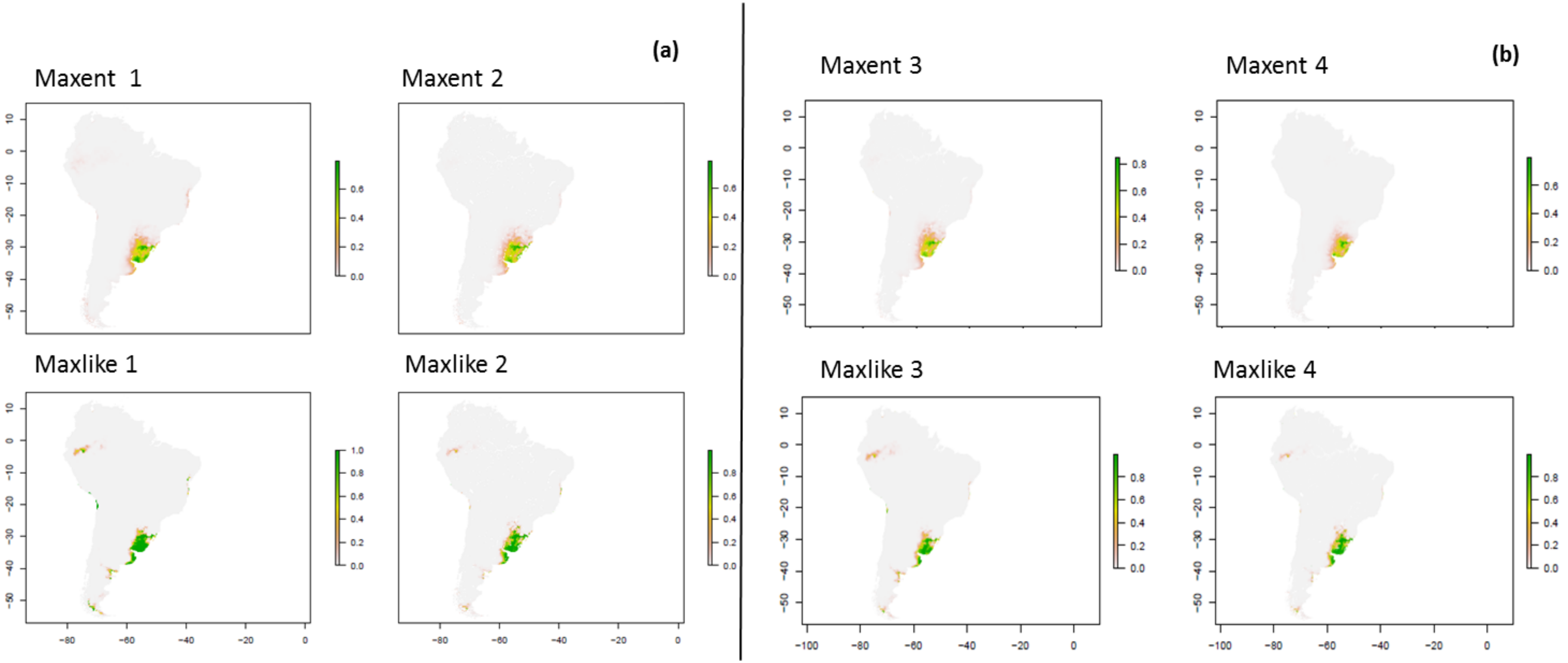
	Variable number	Variables/ Features
Bioclimatic	BIO1	Annual Mean Temperature
	BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
	BIO3	Isothermality (BIO2/BIO7) (* 100)
	BIO4	Temperature Seasonality (standard deviation *100)
	BIO5	Max Temperature of Warmest Month
	BIO6	Min Temperature of Coldest Month
	BIO7	Temperature Annual Range (BIO5-BIO6)
	BIO8	Mean Temperature of Wettest Quarter
	BIO9	Mean Temperature of Driest Quarter
	BIO10	Mean Temperature of Warmest Quarter
	BIO11	Mean Temperature of Coldest Quarter
	BIO12	Annual Precipitation
	BIO13	Precipitation of Wettest Month
	BIO14	Precipitation of Driest Month
	BIO15	Precipitation Seasonality (Coefficient of Variation)
	BIO16	Precipitation of Wettest Quarter
	BIO17	Precipitation of Driest Quarter
	BIO18	Precipitation of Warmest Quarter
	BIO19	Precipitation of Coldest Quarter
	BIO20	Annual mean radiation (W m-2)
	BIO21	Highest weekly radiation (W m-2)
	BIO22	Lowest weekly radiation (W m-2
	BIO23	Radiation seasonality (C of V)
	BIO24	Radiation of wettest quarter (W m-2)
	BIO25	Radiation of driest quarter (W m-2)
	BIO26	Radiation of warmest quarter (W m-2)
	BIO27	Radiation of coldest quarter (W m-2)
	BIO28	Annual mean moisture index
	BIO29	Highest weekly moisture index
	BIO30	Lowest weekly moisture index
	BIO31	Moisture index seasonality (C of V)
	BIO32	Mean moisture index of wettest quarter
	BIO33	Mean moisture index of driest quarter
	BIO34	Mean moisture index of warmest quarter
	BIO35	Mean moisture index of coldest quarter
Altitude		
NDVI		Normalized Difference Vegetation Index
Global Cattle Density		
Landscape information*		

14	Rainfed croplands
20	Mosaic Croplands/ Vegetation
30	Mosaic Vegetation/ Croplands
40	Closed to open broadleaved evergreen or semi-deciduous forest
50	Closed broadleaved deciduous forest
60	Open broadleaved deciduos forest
100	Closed to open mixed broadleaved and needleleaved forest
110	Mosaic Forest-Srubland/ Grassland
120	Mosaic Grassland/ Forest-Srubland
130	Closed to open shrubland
140	Closed to open grassland
150	Sparse vegetation
170	Close broadleaved forest permanently flooded (saline-brackish water)
180	Close to open vegetation regularly flooded
190	Artificial areas
200	Bare areas
210	Water bodies
220	Permanent snow and ice

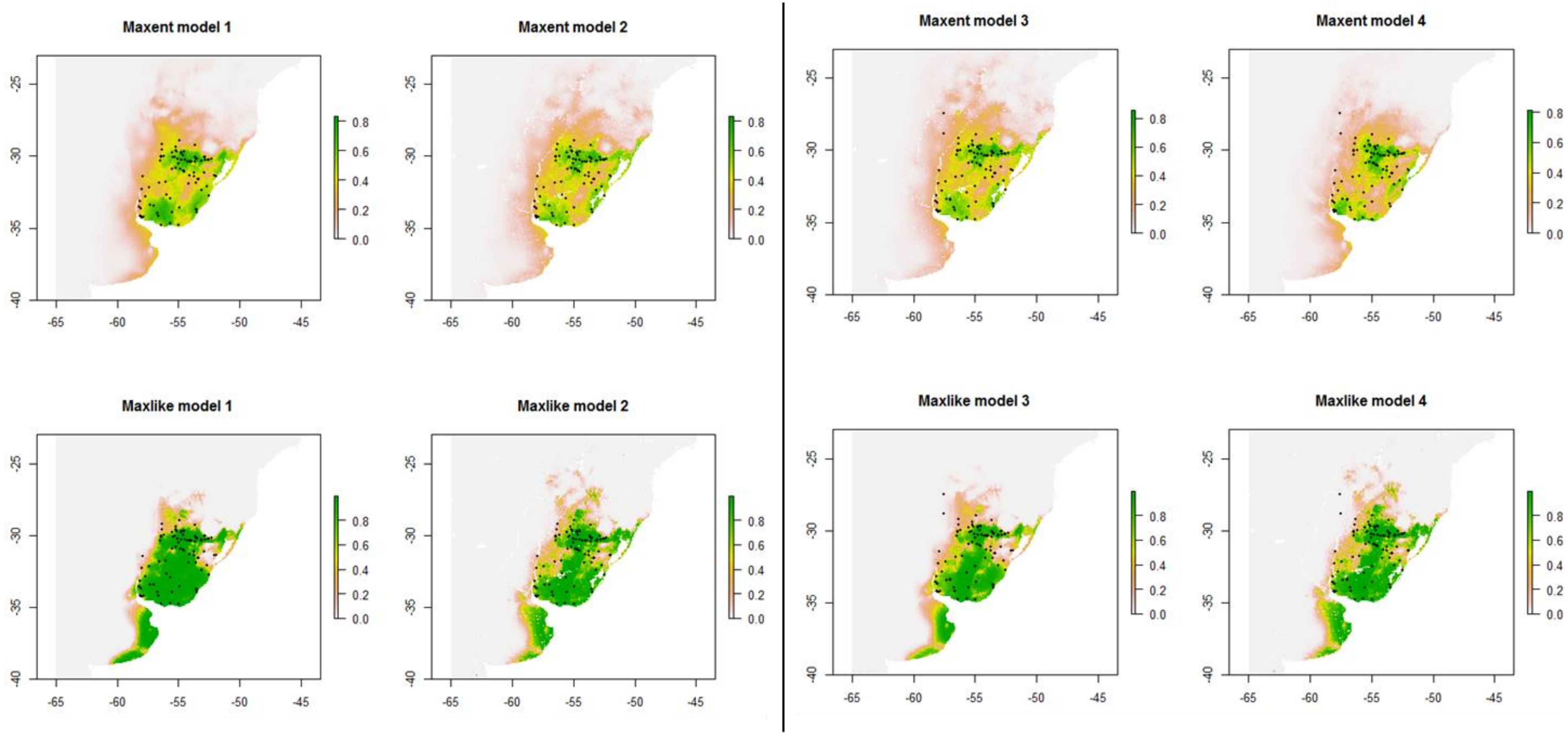
*Only classes visible in extend map of Uruguayan Savannah.



SUPPLEMENTARY MATERIAL FIG.S1 Response curves showing how each of the highest variable contribution affects Maxent prediction. (a). Model 1. (b). Model 2.



Supplementary material Fig. S2 Entire spatial distribution models of Pampas cat with continuous levels of suitability (Maxent) or Probability of occurrence (Maxlike). **(a)** SDM models with Pampas cat data collected in Uruguayan Savannah: Model 1(bioclimatic variables and altitude) performed in Maxent and in Maxlike; and Model 2 (bioclimatic variables, altitude, NDVI, cattle density and land use) performed in Maxent and in Maxlike **(b)** SDM models with Pampas cat data collected in Uruguayan Savannah including records from region of Entre Ríos, Argentina: Model 3 (bioclimatic variables and altitude) performed in Maxent and Maxlike; and Model 4 (bioclimatic variables, altitude, NDVI, cattle density and land use) performed in Maxent and Maxlike.



SUPPLEMENTARY MATERIAL FIG. S3 Partial spatial distribution models (with a zoom scale in the Uruguayan Savannah ecoregion and surrounds) of pampas cat with continuous levels of suitability (Maxent) or Probability of occurrence (Maxlike). **(a)** SDM models with pampas cat data collected in Uruguayan Savannah: Model 1(bioclimatic variables and altitude) performed in Maxent and in Maxlike; and Model 2 (bioclimatic variables, altitude, NDVI, cattle density and land use) performed in Maxent and in Maxlike **(b)** SDM models with pampas cat data collected in Uruguayan Savannah including records from region of Entre Ríos, Argentina: Model 3 (bioclimatic variables and altitude) performed in Maxent and Maxlike; and Model 4 (bioclimatic variables, altitude, NDVI, cattle density and land use) performed in Maxent and Maxlike

Capítulo 5 - Conclusões Gerais

O presente estudo descreveu e analisou informações importantes sobre a ecologia de *Leopardus geoffroyi* e *L. colocolo* na Savana Uruguaia. Os resultados da relação espécie-ambiente foram de grande valia para o entendimento da biologia das populações dessas espécies na região, assim como a compreensão dos padrões ecológicos também indicou como estas populações respondem às perturbações humanas. Esses dados podem auxiliar na categorização do *status* de conservação destas espécies e servirão de base para estratégias de manejo e conservação desses felídeos nesta ecorregião altamente ameaçada.

Foi observada uma discrepância entre as espécies, ao se investigar sua presença na área de estudo focal. A espécie *L. geoffroyi* representou aproximadamente 99% dos registros fotográficos e 100% das capturas de felídeos nativos encontrados na área, ao passo que para *L. colocolo* não foram encontrados quaisquer tipos de registro. Tais resultados indicam a escassez ou ausência completa de *L. colocolo* na área. O estudo na área focal, consequentemente, se destinou somente à espécie ali presente e de elevada ocorrência.

Na população estudada de *L. geoffroyi*, os machos apresentaram maiores áreas de vida, de uso e maior peso corporal do que as fêmeas. Entre os machos, foi observado que o peso corporal influenciou positiva e significativamente no tamanho da área de vida, resultado que não se repetiu na comparação entre as fêmeas. Quando essas análises foram estimadas entre diferentes populações da espécie (comparando os dados do presente estudo com dados gerados por estudos anteriores, em outras regiões da distribuição da espécie), não foram observadas influências significativas de uma variável sobre a outra, sugerindo que este processo ocorre localmente. Os resultados dão apoio à hipótese segundo a qual a competição entre machos seria um dos fatores que influenciaria no dimorfismo sexual da espécie. Também foi observada extensa sobreposição de áreas de vida e de uso entre os indivíduos monitorados. Tendo em vista que a maioria dos espécimes não tinha relação de parentesco entre si, inferiu-se que, nessa população, a relação de parentesco não influenciou a sobreposição de área de vida, nem de área de uso, em quase todos os casos. A alta sobreposição em todos os tipos de pares (fêmea-fêmea, fêmea-macho e macho-macho) parece indicar certo grau de sociabilidade (Macdonald, Mosser & Gittleman, 2010). Além disso, as mudanças não significativas na sobreposição de área de vida e de uso dos indivíduos nas distintas partições sazonais indicaram que a estrutura espacial, nesta população, parece estável. Os resultados moleculares apresentados no estudo focal indicaram que a população possui variabilidade genética considerada alta, inferindo-se que a mesma seja exocruzada (“outbred”), e portanto geneticamente saudável.

Além da estrutura sócio-espacial, este estudo investigou características ecológicas e comportamentais que parecem auxiliar as populações da espécie a se manterem em áreas antropizadas na região analisada. Os resultados indicaram que os indivíduos da população estudada selecionaram a vegetação ripária e evitaram os campos nativos com a presença de gado, fato que pode ser considerados curioso, visto que a espécie é tida como característica de ambientes abertos. Todavia, este resultado não surpreende, já que o uso ou mesmo a seleção por áreas com cobertura vegetal mais densa já foi observada em diversos estudos (Johnson & Franklin, 1991; Manfredi *et al.*, 2006, 2012; Pereira *et al.*, 2006, 2012). No Brasil, a vegetação ripária, mesmo em propriedades privadas, é considerada Área de Preservação Permanente (APP), que se destina à proteção do solo, dos recursos hídricos e da biodiversidade dos biomas, servindo como corredores ecológicos e como abrigo para muitas espécies da fauna (Lei

Federal Brasileira nº 12.651/2012). No bioma Pampa, essas áreas fazem parte de um mosaico de vegetação que inclui campos com criação de gado e áreas destinadas à agricultura e silvicultura. Dessa forma, esse hábitat possivelmente serve de abrigo, fonte de recursos hídricos e de presas, bem como constitui corredores ecológicos para populações de *L. geoffroyi* em áreas antropizadas do pampa brasileiro, hipóteses importantes a serem testadas em estudos futuros. Outra característica que parece auxiliar as populações da espécie a se manterem na região é o seu padrão de atividade, cujo resultado indicou ser significativamente noturno. Além das informações comportamentais, o presente estudo estimou a densidade populacional, utilizando tanto de métodos recentes como tradicionais. Os resultados indicaram que a densidade parece ser de valor intermediário, quando comparada à estimada em outras regiões da distribuição da espécie (W. Johnson, pers. comm. in Nowell & Jackson, 1996; Cuellar *et al.*2006; Pereira *et al.*, 2011; Caruso *et al.*, 2012).

Por outro lado, mesmo sendo uma espécie que apresenta comportamentos espaciais e temporais quer minimizam a sobreposição com as atividades humanas, o que possivelmente facilita a sua resiliência em ambientes antropizados, seus indivíduos continuam a sofrer com conflitos persistentes: dentre os 12 indivíduos capturados no presente estudo, dois morreram. Um macho foi morto por cães domésticos de um trabalhador rural, devido à suspeita de predação sobre aves domésticas, e uma fêmea foi encontrada envenenada, não sendo impossível compreender se propositalmente ou se ela consumiu algum animal envenenado. Além desses casos, ao longo do período do estudo cerca de 90 espécimes foram encontrados mortos em rodovias da região, indicando que o impacto humano sobre a espécie é alto, mesmo que ela pareça tolerar em certo nível a degradação do ambiente.

No mesmo período e mesmas rodovias em que foi encontrado um número considerável de *L. geoffroyi* atropelados, encontraram-se somente nove indivíduos de *L. colocolo*. Esta observação, somada à total falta de registros no estudo focal e os poucos dados disponíveis sobre a espécie na região, geraram algumas perguntas: onde estariam as populações de *L. colocolo* na ecorregião da Savana Uruguaia? Quais seus possíveis tamanhos populacionais? E, consequentemente, qual seu *status* de conservação na ecorregião? Essas perguntas tornam-se ainda mais importantes ao sabermos que população da Savana Uruguaia é evolutivamente distinta de outras, sendo considerada uma Unidade Evolutivamente Significante (‘Evolutionarily Significant Unit’ [ESU]) distinta (Santos, 2012).

Para tentar responder às perguntas anteriormente referidas, este estudo utilizou o método de distribuição potencial, valendo-se de dois modelos (“modelo 1” - somente com variáveis ambientais e relevo; e “modelo 2” - incluindo estas citadas mais algumas variáveis antrópicas) e dois algoritmos distintos (Maxent e Maxlike). Os dados utilizados para a realização das análises vieram de diferentes fontes, muitas delas registros fotográficos de animais atropelados ou de indivíduos predados por cães domésticos em fazendas (ver Tabela S1, Informação Suplementar, no Capítulo 4). Tais dados, além de servirem de base para as estimativas de distribuição, são importantes fontes que demonstram os impactos humanos sobre esta população. Todos os modelos resultaram em distribuições similares, onde as áreas de alta adequabilidade e de alta probabilidade de ocorrência (PO) da espécie eram localizadas em campos com altitude entre o nível do mar e 400m. Em ambos os algoritmos, segundo o AIC (“Akaike Information Criterion”), o “modelo 2” teve uma melhor performance em comparação ao “modelo 1”. Além disso, as áreas de alta adequabilidade e de alta PO do “modelo 2” apresentaram distribuição mais restritas. Esses resultados sugerem que a população de

L. colocolo da Savana Uruguaia parece estar sendo afetada pelas perturbações humanas. Os modelos com melhor desempenho tiveram suas áreas localizadas nas regiões centro e sudoeste do estado do Rio Grande do Sul e nas regiões central e sul do Uruguai, formadas naturalmente com campos de pasto alto e muitas vezes de solo fértil, o que inclui também áreas antrópicas voltadas à agricultura. Tais resultados indicam sobreposição no uso de áreas entre *L. colocolo* e humanos. Os cálculos de estimativa populacional resultaram em <250 indivíduos nas áreas de maior adequabilidade, utilizando o Maxent, e entre 1800–9000 indivíduos nas áreas de alta PO, mensuradas pelo Maxlike. Estes resultados, segundo o critério (C) da IUCN, indicaram que a população estaria "Criticamente Em Perigo" ou "Em Perigo". O presente estudo também observou que cerca de 4% das áreas de maior PO para a espécie estão contidas em áreas protegidas (incluindo áreas de terras indígenas, áreas de economia sustentável [ex: APAs] e áreas de reserva). A combinação dos fatos narrados acima – Unidade Evolutivamente Significativa ameaçada em uma ecorregião impactada que possui um percentual tão baixo de áreas protegidas – é crítica em termos de conservação. Dessa maneira, esta tese sugere que seja priorizado um aumento nas áreas protegidas e a criação de novas áreas, a fim de aumentar o tamanho dos fragmentos disponíveis e gerar corredores ecológicos entre áreas de campo com vegetação nativa, de forma a proteger espécies adaptadas a ambientes abertos, como é o caso do *L. colocolo*.

O presente estudo conclui que, mesmo espécies de felídeos neotropicais tipicamente de áreas abertas e que apresentam tamanho similar, possuem respostas distintas à degradação do ambiente. A espécie *L. geoffroyi* apresenta características plásticas, generalistas e parece ser relativamente abundante na região, além de parecer tolerar áreas com um certo nível de perturbação antrópica. Tais padrões corroboram os atributos da espécie observados em outras áreas de sua distribuição geográfica (Pereira *et al.*, 2015). Apesar disso, nesta ecorregião, suas populações continuam sendo afetadas pelos humanos através de conflitos como a caça por retaliação e mortes por atropelamentos, o que as tornam vulneráveis. Já a espécie *L. colocolo*, por sua vez, é rara e habita áreas de campos abertos e preferencialmente com gramíneas altas, muitas vezes em solos férteis, o que sugere uma competição com humanos sobre estes habitats. A espécie, além sofrer por outros impactos humanos, como atropelamentos e predação por cães domésticos, parece estar sendo altamente impactada pela perda de habitat. Adicionalmente, e de forma distinta em comparação a *L. geoffroyi*, a espécie não parece possuir uma plasticidade no uso de ambientes, o que a torna mais sensível. As populações de *L. colocolo*, assim, parecem sofrer mais com as perturbações antrópicas, o que a torna severamente ameaçada de extinção na ecorregião. De forma geral, espera-se que os resultados derivados deste estudo contribuam para o delineamento e implementação de estratégias eficazes para a conservação em longo prazo de ambas as espécies na Savana Uruguaia e demais áreas de sua ocorrência na região Neotropical.

Porto Alegre, 25 de novembro de 2017.

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Apêndices

Apêncide 1: Journal of Zoology

Author Guidelines

Presentation

Papers should not exceed 5000 words including references (excluding the abstract and figure and table legends). Typescripts must be typed in double spacing, and pages should be numbered consecutively, including those containing acknowledgements, references, tables and figures. Lines must be numbered, preferably within pages.

Manuscripts for review must consist of a single text file with figure and table legends included at the end of this file on separate pages and with figure files uploaded separately. Please note that separate high resolution figure files will be required upon acceptance (see below). Typescripts must be in English (both English and American English are acceptable). The Editors reserve the right to modify accepted manuscripts that do not conform to scientific, technical, stylistic or grammatical standards, and these minor alterations may not be seen by the authors until the proof stage.

Conventions

The Metric system must be used and SI units where appropriate. For further details see Baron, D.N. (1988). *Units, symbols and abbreviations*. 5th edition. London: Royal Society of Medicine Series. Whole numbers one to nine should be spelled out and number 10 onwards given in numerals. If a new taxon is described, the institution in which the type material is deposited must be given, together with details of the registration assigned to it. Full binomial names should be given on the first occasion an organism is mentioned (and abbreviated thereafter), except at the beginning of a sentence. Avoid footnotes except to add information below the body of a table. Do not use initial capitals for the common names of animals unless derived from a proper noun.

Typescript

The typescript should follow the conventional form and must include: (1) ***Title page*** giving a concise title (do not include scientific names in the title), followed by a list of authors' names and the institutions where the work was carried out. The name, address and email address of the corresponding author should also be given. A short title for page headings must be provided (maximum eight words). (2) ***Abstract*** of not more than 300 words which should list the main results and conclusions. The abstract should also explain the importance of the paper in a way that is accessible to non-specialists. Authors may submit non-English abstracts for online publication to allow the international research community greater access to published

articles. Translated abstracts should be submitted in pdf format as supplementary material. The Editors have no input into the content of supplementary material, therefore accuracy is the sole responsibility of the authors.
(3) ***Keywords***. A maximum of eight keywords may be suggested.
(4) ***Introduction***, which should not provide a review of the area of work but should introduce the reader to the aims and context for the work described.
(5) ***Materials and Methods*** should be sufficient to allow the work to be replicated, but should not repeat information described fully elsewhere.
(6) ***Results*** should be restricted to a factual account of the findings obtained and the text must not duplicate information given in Tables and Figures.
(7) ***Discussion***. This should point out the significance of the results in relation to the reasons for undertaking the research.

Please note that appendices are no longer published in the printed version of the journal. Supplementary material may be published in electronic form. Please click here for our supplementary material guidelines.

References

Accuracy of references is the responsibility of the author(s). References must be checked against the text to ensure (a) that the spelling of authors' names and the dates given are consistent and (b) that all authors quoted in the text (in date order if more than one) are given in the reference list and vice versa. The full title of the paper must be given together with the first and last pages.

Journal titles should be abbreviated in accordance with the Zoological Record Serial Sources, published annually by BIOSIS.

Book titles should be followed by the place of publication and the publisher. Please give the name of the editor(s) if different from the author cited.

In the text, references must be arranged chronologically with the surname(s) of the author(s) followed by the date.

Use *a, b*, etc. after the year to distinguish papers published by the same author(s) in the same year. Reference should not be made to unpublished data.
(i) *Two authors*:use both names and the year. Do not use et al.
(ii) *Three authors*: on first citation use all authors' names and the year. Thereafter it is usually sufficient to give the name of the first author followed by et al. and the date.
(iii) *More than three authors*: on first citation and thereafter give the name of the first author followed by et al. and the date.

In the list, references must be arranged first alphabetically under author(s) name(s) and then in chronological order if several papers by the same author(s) are cited.

Examples

Lemelin, P. (1996a). Relationships between hand morphology and feeding strategies in small-bodied prosimians. *Am. J. phys. Anthrop* ` (Suppl.) 22, 148.
Lemelin, P. (1996*b*). *The evolution of manual prehensility in primates: a comparative study of prosimians and didelphid marsupials*. PhD thesis, State University of New

York at Stony Brook.
Pianka, E. R. (1978). *Evolutionary ecology*.2nd edn. New York: Harper & Row.
Whitear, M. (1992). Solitary chemosensory cells. In *Fish chemoreception*: 103-125. Hara, T. J. (Ed.). London: Chapman & Hall.

References in Articles

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. EndNote reference styles can be searched for here: http://www.endnote.com/support/enstyles.asp

Reference Manager reference styles can be searched for here:http://www.refman.com/support/rmstyles.asp

Figures

Figures should be submitted as separate electronic files and figure legends should be included at the end of the main manuscript file. Illustrations may be line drawings or photographs and should be numbered consecutively in the text as Fig. 1, Fig. 2 etc. Component parts of figures should be labelled (a), (b), (c) etc. Captions for figures, which should be self-explanatory, must be typed, double spaced, on a separate page.

Our preferred electronic file type is vector-format encapsulated post script (EPS) because these images are scaleable and therefore do not lose quality in the online PDF. All line drawings or photographs with added labelling should be supplied in EPS format. Half tones without any labelling should be supplied in TIFF format at 300 dots per inch minimum. If line drawings cannot be supplied as EPS files then they must be in TIFF format with a minimum resolution of 800 dpi. These resolutions also apply to any images embedded into an EPS file. Please click here for our artwork guidelines. Please also see the illustration submission section in the 'author resources' section of the author services site. This page has some useful documents that explain why we use vector format images and TIFF files rather than JPEG or other formats.

Line drawings should not be larger than **twice** the final size and in no circumstances should exceed 168 x 220 mm. The axes of graphs should be carefully chosen so as to occupy the space available to the best advantage. When reduced, the drawing should fit into either one (80 mm) or two (168 mm) columns, preferably the former. Lines should be bold enough to stand reduction to about 0.25-0.35 mm. Line drawings should be as simple as possible and many computer-generated figures, such as 3-dimensional graphs, fine lines, gradations of stippling and unusual symbols, cannot be reproduced satisfactorily when reduced. Unsatisfactory line drawings will have to be redrawn at the author's expense. Preferred symbols are open and filled circles, boxes and triangles, and these should be used consistently. Lettering should be kept to a minimum and should be self-explanatory and unambiguous and of sufficiently high quality and size to be clearly visible after reduction to final size. Lettering of all figures within the manuscript should be of uniform style in a sans serif typeface (Helvetica) and capitals should be used for the initial letter of the first word only. Bold lettering should not be used. Photographs should be the same size as they will appear in the journal and should be selected to fit neatly into one column (80 mm) or two columns (168 mm).

Photographs should be labelled and numbered as for line drawings. For microscopical preparations, scale bars with appropriate units must be provided; statements of magnification are not acceptable.

Colour figures may be accepted provided that they are of a very high quality. The cost of reproduction must be met by the author(s) and a binding agreement to meet the costs will be required before the manuscript can be accepted for publication. For colour figures, the instructions for the preparation of photographs should be followed. Original illustrations should not be sent until the paper has been accepted and will only be returned on request. Any article received by Wiley Blackwell with colour work will not be published until the colour work agreement form has been returned to the following address:

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Decisions and invitations to revise

All submissions are subject to peer review and authors can expect a decision, or an explanation for the delay, within 3 months of receipt. If a revision is requested, the corresponding author should submit the revised manuscript within 2 months unless there are special reasons for a delay, agreed in advance with the Editor. Papers not received within 2 months may be treated as new submissions and sent for further evaluation by new referees.

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The corresponding author will receive an email alert containing a link to a web site from where a PDF file of the proof can be downloaded. The corresponding author must provide a reliable email address and inform the Production Editor of any extended period when the email address is not effective. Instructions for returning the proofs will be sent with the proof. Excessive alterations to the text, other than printer's errors, may be charged to the author. The Editors reserve the right to correct the proof themselves, using the accepted version of the typescript, if the author's corrections are overdue and the journal would otherwise be delayed. The *Journal of Zoology* has had a change in journal style, Latin species names are no longer given in the article title but are instead inserted in the abstract, please keep this in mind when choosing the titles for submitted articles.

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Apêncide 2: Animal Conservation

Author Guidelines

Animal Conservation provides a forum for novel, peer-reviewed research into the conservation of animal species and their habitats. The focus is on rigorous quantitative studies of an empirical or theoretical nature, which may relate to populations, species or communities and their conservation. The journal publishes single-species papers only when they have clear broader implications for conservation of other species or systems. A central theme is to publish important new ideas of broad interest and with findings that advance the scientific basis of conservation.

Instructions To Authors

Papers should be submitted online at http://mc.manuscriptcentral.com/acv Full upload instructions and support are available online from the submission site via the 'Get Help Now' button. Please submit your covering letter or comments to the editor when prompted online.

***Research papers*:** must be limited to 4000 words, excluding references, tables and figures

Presentation

Typescripts must be typed in double spacing, and pages should be numbered consecutively, including those containing acknowledgements, references, tables and figures. Lines must be numbered, preferably within pages.

Manuscripts for review must consist of no more than two files and should, ideally be a single file with figures embedded in the text (please note that separate high resolution figure files will be required upon acceptance - please see below). Typescripts must be in English (both English and American English are acceptable).

The Editors reserve the right to modify accepted manuscripts that do not conform to scientific, technical, stylistic or grammatical standards, and these minor alterations may not be seen by the authors until the proof stage.

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(1) Title page giving a concise title (do not include scientific names in the title), followed by a list of authors' names and the institutions where the work was carried out. The name, address and email address of the corresponding author should also be given. A short title for page headings must be provided (maximum 8 words).
(2) Abstract of not more than 300 words which should list the main results and conclusions. The abstract should also explain the importance of the paper in a way that is accessible to non-specialists and should describe the novel aspects of the research and highlight the relevance of the findings to other taxa or general principles in conservation biology. Authors may submit non-English abstracts for online publication to allow the international research community greater access to published articles. Translated abstracts should be submitted in pdf format as supplementary material. The Editors have no input into the content of supplementary material, therefore accuracy is the sole responsibility of the authors.
(3) Keywords. A maximum of eight keywords may be suggested.
(4) Introduction, which should not provide a review of the area of work but should introduce the reader to the aims and context for the work described.
(5) Materials and Methods should be sufficient to allow the work to be replicated, but should not repeat information described fully elsewhere.
(6) Results should be restricted to a factual account of the findings obtained and the text must not duplicate information given in Tables and Figures. (7) Discussion. This should point out the significance of the results in relation to the reasons for undertaking the research, and describe the novel aspects of the research and the relevance of the findings to a range of taxa or general principles in conservation biology.

Please note that appendices are no longer published in the printed version of the journal. Supplementary material may be published online only. References Accuracy of references is the responsibility of the author(s).

References

must be checked against the text to ensure (a) that the spelling of authors' names and the dates given are consistent and (b) that all authors quoted in the text (in date order if more than one) are given in the reference list and vice versa. The full title of the paper must be given together with the first and last pages.

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(iii) More than three authors: on first citation and thereafter give the name of the first author followed by *et al*. and the date.

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Examples
Lemelin, P. (1996a). Relationships between hand morphology and feeding strategies in small-bodied prosimians. *Am. J. phys. Anthrop*. (Suppl.) 22, 148.
Lemelin, P. (1996b). *The evolution of manual prehensility in primates: a comparative study of prosimians and didelphid marsupials*. PhD thesis, State University of New York at Stony Brook.
Pianka, E. R. (1978). *Evolutionary ecology*. 2nd edn. New York: Harper & Row.
Whitear, M. (1992). Solitary chemosensory cells. In *Fish chemoreception*: 103-125. Hara, T. J. (Ed.). London: Chapman & Hall.

References in Articles

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting.

EndNote reference styles can be searched for here: http://www.endnote.com/support/enstyles.asp

Reference Manager reference styles can be searched for here:http://www.refman.com/support/rmstyles.asp

Figures
Illustrations may be line drawings or photographs and should be numbered consecutively in the text as Fig. 1, Fig. 2 etc. Component parts of figures should be labelled (a), (b), (c) etc. Captions for figures, which should be self-explanatory, must be typed, double spaced, on a separate page and must not contain details of results.

Our preferred electronic file type is vector-format encapsulated post script (EPS) because these images are scaleable and therefore do not lose quality in the online PDF. All line drawings or photographs with added labelling should be supplied in EPS format. Half tones without any labelling should be supplied in TIFF format at 300 dots per inch minimum. If line drawings cannot be supplied as EPS files then they must be in TIFF format with a minimum resolution of 800 dpi. These resolutions also apply to any images embedded into an EPS file.

Line drawings should not be larger than twice the final size and in no circumstances should exceed 168 x 220 mm. The axes of graphs should be carefully chosen so as to occupy the space available to the best advantage. When reduced, the drawing should fit into either one (80 mm) or two (168 mm) columns, preferably the former.

Lines should be bold enough to stand reduction to about 0.25-0.35 mm. Line drawings should be as simple as possible and many computer-generated figures, such as 3-dimensional graphs, fine lines, gradations of stippling and unusual symbols, cannot be reproduced satisfactorily when reduced. Unsatisfactory line drawings will have to be redrawn at the author's expense. Preferred symbols are open and filled circles, boxes and triangles, and these should be used consistently. Lettering should be kept to a minimum and should be self-explanatory and unambiguous and of sufficiently high quality and size to be clearly visible after reduction to final size. Lettering of all figures within the manuscript should be of uniform style in a sans serif typeface (Helvetica) and capitals should be used for the initial letter of the first word only. Bold lettering should not be used.

Photographs should be the same size as they will appear in the journal and should be selected to fit neatly into one column (80 mm) or two columns (168 mm). Photographs should be labelled and numbered as for line drawings. For microscopical preparations, scale bars with appropriate units must be provided; statements of magnification are not acceptable.

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Tables

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Decisions and invitations to revise

All submissions are subject to peer review and authors can expect a decision, or an explanation for the delay, within 3 months of receipt. If a revision is requested, the corresponding author should submit the revised manuscript within 2 months unless there are special reasons for a delay, agreed in advance with the Editor. Papers not received within 2 months may be treated as new submissions and sent for further evaluation by new referees.

Pre-submission English-language editing

Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Apêncide 3: Oryx – The International Journal of Conservation

General instructions

Articles (2,000–7,000 words), *Reviews* (<7,000 words), *Forum* papers (<4,000 words) and *Short Communications* (<2,000 words) are invited on topics relevant to the journal’s focus, including:

- The nature and results of conservation initiatives, including case studies
- Research on the sustainable use of wild species
- Research on the status of threatened species, taxonomic groups or habitats
- Reviews of conservation policy, strategy and legislation in practice
- Planning for species and biodiversity conservation
- The working of international conservation conventions
- The history of conservation, including the role of key people, organizations and initiatives
- The causes and consequences of biodiversity loss
- Case studies of conservation projects and programmes
- The social, economic and political dimensions of conservation

Note that word counts are all inclusive except for Tables, and that you should ensure your manuscripts are as concise as possible. The maximum word counts indicated above should not be taken to imply that a submission can be of the maximum length regardless of content. If we feel that a manuscript is overly long for the subject matter (even if it is within the word count limit) we may request that it be shortened prior to peer review.

Please read all of these Instructions for Contributors carefully. If a submission does not comply appropriately it will be returned for amendment prior to peer review.

Reviews provide comprehensive overviews of a particular conservation topic. *Forum* papers promote debate about current and controversial issues, usually with critical responses commissioned from one or more authors. *Articles* on the status or conservation of threatened species, taxonomic groups or habitats should generally be <4,500 words and have, where relevant, a context beyond that of the subject studied.

Submission Online at *Oryx* on ScholarOne Manuscripts. Upon submission the Corresponding Author must confirm that: (1) the submitted manuscript has not been published or submitted for publication elsewhere, (2) all authors have read the submitted manuscript and agreed to its submission, and (3) all authors have abided by the Code of conduct for authors contributing articles. In a covering letter/e-mail to the Editor please indicate how the research and submitted manuscript complies with points 2 and 4 of the *Code of conduct* and, in the Methods and/or Acknowledgements sections of the manuscript, as appropriate, indicate how the research complies with points 1, 3 and 5–8 of the *Code of conduct*, as required. If there is overlap with other articles, reports or similar material, including any in press or in preparation, this should be stated and the articles in question sent to the Editor. Both quantitative and qualitative methodology used in the reported work should be appropriate to the research question and must be clearly described. Management recommendations should be made where appropriate, and details of any implementation provided.

Age of data Where relevant, dates of any fieldwork must be provided. *Oryx* publishes research with the potential to have an impact on conservation outcomes. This requires conclusions to be based upon the best available and up-to-date information and analysis and we therefore look carefully at all manuscripts in which conclusions are based on field data collected some time ago (generally >4 years). We recognize, however, the value of historical field or collection records but require evidence that these studies are directly relevant to effective conservation. Bear this policy in mind when submitting your manuscript and, if the data and/or associated analyses are not recent, justify, both in your cover letter and in the manuscript, the use of these data in terms of the conservation relevance of your findings.

Preparation of manuscripts Consult a recent issue of *Oryx* for general style. Contributions should be in English, with British English spelling and terminology, double-spaced, without footnotes, and with line numbers. Submissions, which can be a single file, with all Tables, Figures, Plates and Appendices at the end, or with text and other elements in separate files, must be in DOC format (not PDF). For submission, graphics can be in low to medium resolution as long as they are clearly legible, with publication-quality graphics to be provided upon any final acceptance. Cover page should contain title, word count (all inclusive except for Tables and Figure and Plate captions), and full mailing address, e-mail, and address at the time the research was carried out, if different, of the corresponding author and all additional authors. Pages to be numbered, and order of sections to be: cover page, title, abstract, keywords, main text, acknowledgements, author contributions, references, biographical sketches, tables, figures, plates, appendices/supplementary material (the latter four items can be in separate files). Short Communications must not have any subheadings.

Title A succinct description of the work (≤20 words) preferably embodying either the aim or the overall conclusion.

Abstract In <250 words, the aims, methods, major findings and conclusions; should be informative without reference to the text and should not contain any references or undefined abbreviations.

Keywords Up to eight pertinent words or phrases, in alphabetical order (these can repeat words in the title and should be optimized for online search engines).

Structure Articles should generally follow the Introduction, Study Area (where appropriate), Methods, Results and Discussion format. Minimize use of other subheadings.

Acknowledgements A brief statement should be included that thanks, in non-effusive terms, those who have provided assistance (following peer review this should normally include acknowledgement of reviewers).

Author contributions A brief statement should be included that specifies the contributions of each author (specified by their initials).

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>
> Bowler, M. (1991) *Implications of large predator management on commercial ranchland in Zimbabwe*. MSc thesis. University of Zimbabwe, Harare, Zimbabwe.
>
> Brady, L.D., Huston, K., Jenkins, R.K.B., Kauffmann, J.L.D., Rabearivony, J., Raveloson, G. & Rowcliffe, M. (1996) *UEA Madagascar Expedition '93*. Unpublished Report. University of East Anglia, Norwich, UK.
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> IUCN (2011) *The IUCN Red List of Threatened Species v. 2011.1*. Http://www.iucnredlist.org [accessed 25 August 2011].
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> LeBoeuf, B.J., Kenyon, K.W. & Villa-Ramirez, B. (1986) The Caribbean monk seal is extinct. *Marine Mammal Science,* 2, 70–72.
>
> MacArthur, R.H. & Wilson, E.O. (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, USA.
>
> Sutherland, W.J. (ed.) (1998) *Conservation Science and Action*. Blackwell Science, Oxford, UK.

Biographical sketches The research interests of all authors (< 150 words total), over and above what is obvious from the subject of the manuscript and the authors’ affiliations.

Tables Each on a separate page, with captions that are comprehensible without reference to the text.

Figures & Plates Guidelines for the preparation of Figures and Plates are provided below. Captions should be comprehensible without reference to the text.

Appendices/Supplementary material Online platforms give authors the opportunity to include data that it would be impractical to include in the printed article. Such data may substantially enhance the importance of the research and be of benefit to readers. Authors may include tables, figures, videos and audio files. All supplementary material must be submitted with the original manuscript as separate files, cited in the text with the prefix 'S' (e.g. Supplementary Table S1, Supplementary Fig. S1, Supplementary Material 1) and listed under a Supplementary Material heading at the end of the manuscript. Supplementary files will be peer reviewed but not copy-edited or typeset, and will be published as supplied. Where authors wish to deposit data in a third-party archive (mandatory for files of 60 MB and over) instead of publishing as supplementary material in the journal, any necessary supplementary material files should be uploaded to the third-party archive and the DOI that the archive assigns to their supplemental files should be included under the Supplementary Material heading.

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Abbreviations Minimize use, and provide full expansion of any at first mention in the text.

Symbols, units & currencies Use SI units (International System of Units) and ISO 4217 currency codes.

Nomenclature Where necessary, provide the basis used for nomenclature of taxa. The first time a species is mentioned its scientific name should follow without intervening punctuation, thus African elephant *Loxodonta africana*. English names should be in lower case throughout except when they incorporate a proper name.

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Guidelines for preparation of figures and plates

For detailed guidance on designing maps and data plots, advice on the wise use of graphics formats, and screencast demonstrations to help with drafting beautiful figures, please see the journal's online Graphics for Conservationmanual.

Figures are printed at widths of 79, 107.5 and 165 mm (3.1, 4.2 and 6.5 inches, respectively); wherever possible design for the narrower widths. See recent issues of the journal and Graphics for Consevation for examples of suitably designed Figures. Plates (i.e. photographs) are usually printed at 79 mm width. For accepted articles we require Figures and Plates at publication quality (see below). We welcome colour Figures and Plates but they can be printed only at the author’s expense; contact the Editorial Office if you require a costing. Figures and Plates can, however, be published in colour online and greyscale in print at no cost to the author; in such cases authors need to ensure that any colour figure or plate will also reproduce well in greyscale.

We accept graphics for publication only in postscript (EPS/PS), scalar vector graphic (SVG) or tagged image file format (TIFF). Line artwork is best prepared in vector-based drawing software (e.g. Inkscape). Export maps and graphs thus prepared in EPS/PS or SVG format. When exporting, embed fonts if possible (or convert to outlines), do not use lines of hairline width but rather use a minimum width of 0.3 points (0.1 mm), and do not downsample the quality of any embedded images. Design specifically for one of the journal's three figure widths (see above). Submit Plates in TIFF format. For a photograph to be printed at 79 mm (3.1 inches) we require a minimum size along the width of 1,860 pixels (600 pixels x 3.1 inches).

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In general any manuscript that involves a study site or specific location will require a map Figure. Such Figures must: (1) be surrounded by a thin-line box, (2) be geographically referenced with inward-pointing latitude and longitude tic marks and/or with a small inset map indicating the location of the main figure, (3) have a scale bar in km, and (4) have all lines such as country boundaries, roads and rivers, wherever appropriate, extending to the bounding box. For detailed advice, see Graphics for Consevation.

For all Figures use line thicknesses in the range of 0.3–1.0 points (0.1–0.35 mm) and font sizes in the range of 6–12 points (2–4 mm). All fonts used in Figures should be sans serif (e.g. Arial) rather than serif (such as Times Roman). If shading is required in a greyscale figure, use only a limited range of grey shades, generally not more than four.

In general, Plates will only be included in an article if they form evidence that is integral to the subject studied (e.g. a camera-trap photograph of a rare species) and if they are of good quality.

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