Genetic diversity and chromosome complement of *Galictis cuja* (Molina, 1782) (Carnivora: Mustelidae) with comments about its role as parasite hosts

Diversidade genética e complemento cromossômico de *Galictis cuja* (Mustelidae), com comentários sobre seu papel como hospedeiro de parasitas

Júlia Bontempo[I] | Cecília Bueno[II] | Paulo Sérgio D'Andrea[III] | Cibele Rodrigues Bonvicino[IV]

[I]Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro. Rio de Janeiro, Rio de Janeiro, Brasil

[II]Universidade Veiga de Almeida. Rio de Janeiro, Rio de Janeiro, Brasil

[III]Instituto Oswaldo Cruz. Fundação Oswaldo. Rio de Janeiro, Rio de Janeiro, Brasil

[IV]Instituto Nacional de Câncer. Rio de Janeiro, Rio de Janeiro, Brasil

Abstract: The distribution of *Galictis cuja* encompasses several countries of South America, including Brazil, where it inhabits the Atlantic Forest, part of Caatinga and part of Cerrado biomes. Herein we analyzed *G. cuja* specimens from localities in the Brazilian states of Rio de Janeiro, Minas Gerais, and Bahia, and the Distrito Federal, mainly roadkilled animals. The genetic diversity was estimated based on DNA sequence data of the mitochondrial gene cytochrome b (*mt-cyb*). Analysis of *mt-cyb* identified high haplotypic diversity, albeit with low nucleotide diversity, suggesting that this population is in expansion and confirming the presence of gene flow. The karyotypes of two *Galictis cuja* specimens were described as 2n = 38 and FNa = 66. Our data showed that *G. cuja* is frequent in the investigated areas of Atlantic Forest biome, being a common roadkill mammal. Our data suggest that *G. cuja* may play a role as a spreader of zoonotic parasites.

Keywords: Lesser grison. Cytochrome b. Karyotype. Geographic distribution. Helminths.

Resumo: A distribuição de *Galictis cuja* abrange vários países da América do Sul, incluindo o Brasil, onde habita a Mata Atlântica, parte da Caatinga e parte do Cerrado. Aqui, analisamos espécimes de *G. cuja* de localidades nos estados brasileiros do Rio de Janeiro, Minas Gerais, Bahia e no Distrito Federal, a maioria deles encontrada atropelada em rodovias. A diversidade genética foi estimada com base em sequências de DNA do gene mitocondrial citocromo b (*mt-cyb*). A análise do *mt-cyb* identificou alta diversidade haplotípica, embora com baixa diversidade de nucleotídeos, sugerindo que a população está em expansão e confirmando a presença de fluxo gênico. O cariótipo de dois espécimes de *Galictis cuja* mostrou que 2n = 38 e FNa = 66. Nossos dados mostraram que *G. cuja* é frequente nas áreas de Mata Atlântica investigadas, sendo um mamífero comumente atropelado nas rodovias. Eles sugerem que *G. cuja* tem um papel como disseminador de parasitas zoonóticos.

Palavras-chave: Furão. Citocromo b. Cariótipo. Distribuição geográfica. Helmintos.

BONTEMPO, J., C. BUENO, P. S. D'ANDREA & C. R. BONVICINO, 2020. Genetic diversity and chromosome complement of *Galictis cuja* (Molina, 1782) (Carnivora: Mustelidae) with comments about its role as parasite hosts. **Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais** 15(3): 717-726. DOI: http://doi.org/10.46357/bcnaturais.v15i3.199.

Autora para correspondência: Cibele Rodrigues Bonvicino. Instituto Nacional de Câncer. Divisão de Genética. CPQ. Rio de Janeiro, RJ, Brasil. CEP 20231-050 (cibele.bonvicino@gmail.com).

Recebido em 16/10/2019
Aprovado em 21/09/2020
Responsabilidade editorial: Alexandra Maria Ramos Bezerra

INTRODUCTION

Galictis Bell, 1826 is a genus of the order Carnivora belonging to the family Mustelidae including two extant recognized species, the greater grison, *Galictis vittata* (Schreber, 1776) and the lesser grison, *Galictis cuja* (Molina, 1782). The distribution of *G. cuja* encompasses several countries of South America, including Brazil, where it inhabits the Atlantic Forest, part of Caatinga and part of Cerrado biomes (Bornholdt *et al*., 2013).

Phylogenetic reconstructions within the Carnivora were extensively carried out (*e.g*., Flynn & Nedbal, 1998; Flynn *et al*., 2005; Eizirik *et al*., 2010; Koepfli *et al*., 2007) as well as within the mustelids (*e.g*., Koepfli & Wayne, 2003; Koepfli *et al*., 2008; Sato *et al*., 2012), showing the monophyly of *Galictis* (Bornholdt *et al*., 2013). In contrast, phylogeographic studies in *Galictis* are less common (Bornholdt *et al*., 2013). Despite extensive studies of the chromosomal complement of species of the order Carnivora (Franco-de-Sá *et al*., 2007; Freitas *et al*., 1982; Kurose *et al*., 2000), there is a single karyotype description of *Galictis cuja* only published in a PhD thesis (Barbosa, 2013). Furthermore, this widespread genus has been observed to be infected by several etiological agents of zoonoses as: trypanosomiasis (Ferriolli & Barretto, 1969; Tremori, 2018), leishmaniasis (Melo, 2008), *Toxoplasma gondii* (Nicolle & Manceaux, 1908) (Torres-Castro *et al*., 2019), zoonotic giant kidney worm *Dioctophyme renale* (Goeze, 1782) (Barros *et al*., 1990; Zabott *et al*., 2012), nematodeosis (Vieira, F. *et al*., 2012), and a domestic dog strain of Canine Distemper virus (CDV, genus *Morbilivirus*), that can be transmitted by free-ranging dogs (Megid *et al*., 2013). The aims of this study are describing the karyotype of *Galictis cuja* from Southeastern Brazil, investigating the genetic diversity using the mitochondrial gene cytochrome b (*mt-cyb*) as a marker, and discuss its role as a spreader of zoonotic parasites.

MATERIAL AND METHODS

We collected 55 specimens of *G. cuja*; mainly roadkilled animals. Thirty-nine specimens were roadkilled in the BR-040 between 2007 and 2017, three in the RJ-122 in 2018, and 11 were live trapped in rural peridomicile and small fragment borders in Atlantic Forest of Rio de Janeiro and Minas Gerais state (Figure 1). In the Cerrado biome one roadkilled specimen was collected in Distrito Federal. For understanding the extant and ancient distribution of *G. cuja*, we added the locality of a Pleistocene fossil from Aurora do Tocantins, in Tocantins state (Rodrigues *et al*., 2015) in the map of Figure 1. *Voucher* specimens were deposited in the mammal collections of Museu Nacional, UFRJ (MN) and *Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres* (LBCE), *Instituto Oswaldo Cruz/ Fundação Oswaldo Cruz* (IOC/FIOCRUZ), Rio de Janeiro state, and in *Universidade Federal da Paraíba* (UFPB), João Pessoa, Paraíba state, Brazil (Appendix 1). The sample were stored at scientific tissue collection of the *Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres*, IOC/FIOCRUZ, and in the Ecology Laboratory, *Universidade Veiga de Almeida*, Rio de Janeiro state, Brazil (Appendix 1). Samples of CB and CRB 3283 in Appendix 1 refer to field numbers of Cecília Bueno (CB) and Cibele R. Bonvicino (CRB) and are only tissue samples, without a *voucher*.

One specimen of *Galictis cuja* from Sumidouro (LBCE6437), and one from Teresópolis (LBCE7963), Rio de Janeiro state, were karyotyped. Chromosome preparations were obtained following short cultures of bone marrow tissue (Andrade & Bonvicino, 2003). Only Giemsa staining coloration was carried out. Chromosomes were ordered according to morphology and decreasing size, with fundamental numbers (FNa) referring to the autosome complement (Levan *et al*., 1964).

DNA was isolated from liver and muscle samples preserved in ethanol following a phenol-chloroform protocol (Sambrook *et al.*, 1989). The *mt-cytb* sequence was amplified in 28 samples (Appendix 1) with primers "Carnivora *Forward*" and "Carnivora *Reverse*" (Ledje & Arnason, 1996), following a pre-denaturation step at 94 °C for 2 min following by 35 subsequent cycles with denaturation at 94 °C for 30 sec and annealing at

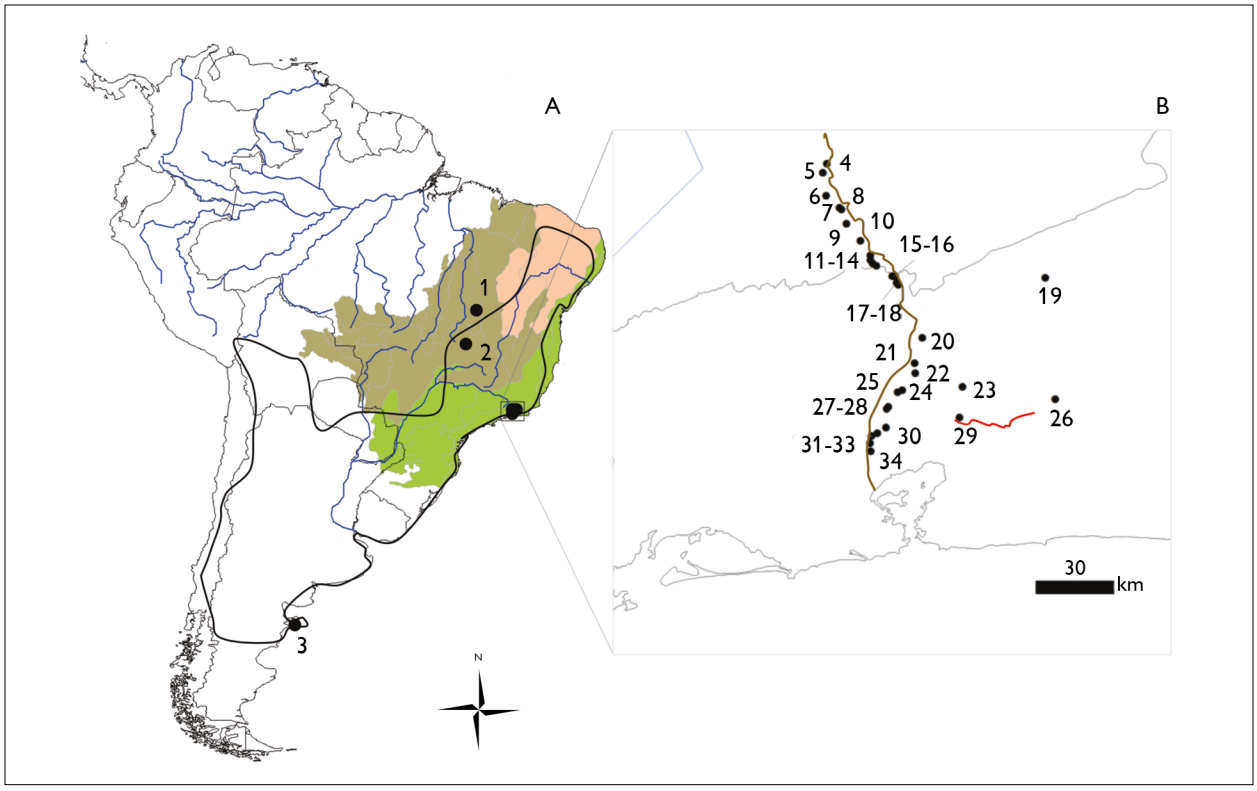

Figure 1. A) South America map showing Atlantic forest (green), Caatinga (salmon), and Cerrado (olive), the current geographic distribution of *Galictis cuja* (black line), the localities of analyzed specimens (as referred in Table 1 and Appendix 1) and locality 1 of the fossil (Rodrigues *et al*., 2015); B) the region of BR-040 (in brown) and RJ-122 (line red) highways was amplified for better visualization of collection points.

54 °C for 30 sec, and extension at 72 °C for 45 sec, and final extension of 72 °C for 5 min. Amplified products were purified with Gel Band Purification Kit (GE Healthcare), and labelling was carried out using the same primers in addition to the internal primers SOT - In1 (5'-TTRTTRGATCCTGTTTCTTG-3' - Cassens *et al*., 2000) and SOT - In2 (5'-TGAGGACAAATATCATTTTGAG-3' - Cassens *et al*., 2000). Reactions were run in an ABI3130xl (Applied Biosystems™) platform. Electropherograms were manually checked using CHROMAS PRO 1.41 (Technelysium Pty Ltd). The complete *mt-cyb* was amplified for all samples, but the different sequenced size was obtained (Table 1). Each sequence was aligned manually with MEGA 7 (Tamura *et al.*, 2013) and a concatenated matrix was manually constructed. The most appropriate nucleotide substitution model for phylogenetic reconstructions was selected using MEGA 7.0. Maximum likelihood (ML) reconstructions were carried with MEGA 7.0, and branch support was calculated using bootstrap. We also used sequences available in GenBank for *G. cuja* (KT626650, AB564025, EF987754) and *G. vittata* (AF498155). We used as outgroup two species of genera considered as closely related to *Galictis* (Koepfli *et al*., 2008; Wolsan & Sato, 2010), *Ictonyx striatus* (Perry, 1810) (AF498156.1) and *Poecilogale albinucha* (Gray, 1864) (EF472349.1). Kimura two-parameters genetic distance estimates were calculated with Mega 7.0.

Network v.4.5.1.6 (Fluxus, s. d.) was used for reconstructing a Median-Joining (MJ) network (Bandelt *et al*., 1999) based on variable sites and excluding sites containing missing *mt-cytb* data to evaluate population structure and geographic distribution patterns.

Table 1. Samples of *Galictis cuja* sequenced in this study, with GenBank accession numbers (GenBank), field or scientific collection numbers (*voucher*), localities (Loc), haplotype (H), number of sequenced base pairs (pb), and source. See Appendix 1 for localities (Loc) of collecting specimens. The number after the GenBank accession number refers to the year the sample was collected.

GenBank/year	*Voucher*	Loc	H	pb	Source
MT537199/unknown	CB118	10	H1	1.122	Present study
MT537200/2011	MN79404	10	H1	1.140	Present study
MT537201/2010	MN79247	16	H2	1.133	Present study
MT537202/2003	LBCE5276	19	H2	1.140	Present study
MT537203/2004	LBCE6587	19	H2	1.140	Present study
MT537204/2004	LBCE6619	19	H2	1.140	Present study
MT537205/2010	MN79294	16	H3	1.118	Present study
MT537206/2004	LBCE6437	19	H3	1.140	Present study
MT537207/unknown	LBCE7241	19	H3	1.084	Present study
MT537208/2011	MN79358	6	H4	1.140	Present study
MT537209/2011	MN79407	11	H4	1.140	Present study
MT537210/2015	CB991	9	H4	1.140	Present study
MT537211/2012	MN79445	8	H5	1.085	Present study
MT537213/2012	MN79501	30	H6	1.140	Present study
MT537214/2013	MN79550	30	H6	1.126	Present study
MT537212/2016	CB1089	12	H6	1.140	Present study
MT537215/2014	MN83564	13	H7	1.126	Present study
MT537218/2011	MN79363	9	H8	1.067	Present study
MT537227/2015	MN83597	5	H8	601	Present study
MT537216/2016	CB1012	7	H8	1.140	Present study
MT537217/2016	CB1185	21	H9	1.118	Present study
MT537220/2005	LBCE7780	19	H9	1.126	Present study
MT537221/2006	LBCE7949	23	H9	1.140	Present study
MT537222/2006	LBCE7956	23	H9	1.140	Present study
MT537223/2006	LBCE7963	23	H9	1.140	Present study
MT537224/2006	LBCE7969	23	H9	1.140	Present study
KT626650/unknown	Unknown	-	H9	1.140	GenBank, unpublished
MT537225/unknown	CRB3283	2	H10	1.140	Present study
MT537226/2004	MN74461	19	H11	1.140	Present study
EF987754/unknown	Unknown	-	H12	1.140	Koepfli *et al*. (2008)
AB564025/unknown	MC795	3	H12	1.140	Sato e*t al*. (2012)

RESULTS

Karyotypic analysis of two females (LBCE6437 and LBCE7963) captured in Sumidouro and Teresópolis municipalities at Rio de Janeiro Atlantic Forest, showed 2n = 38 and FN = 66, with a metacentric medium-sized X chromosome, and a secondary constriction in the largest acrocentric pair (Figure 2). The autosome complement was composed by 15 pairs of biarmed chromosome varying in size from large to small, and three pair of acrocentric chromosomes (Figure 2).

The *mt-cytb* gene sequences of 32 *G. cuja* samples, 28 herein sequenced showed 13 variable sites and 13 haplotypes. Maximum likelihood analysis showed two well defined lineage, one with *G. vittata* and another with *G. cuja* (Figure 3), with genetic distance estimates between than more than 12 %. In contrast, intraspecific genetic distance estimates within *G. cuja* vary from 0 to 0.44 %. MJ network showed a central haplotype (H9) directly connected with all other haplotypes, except H4 (Figure 3).

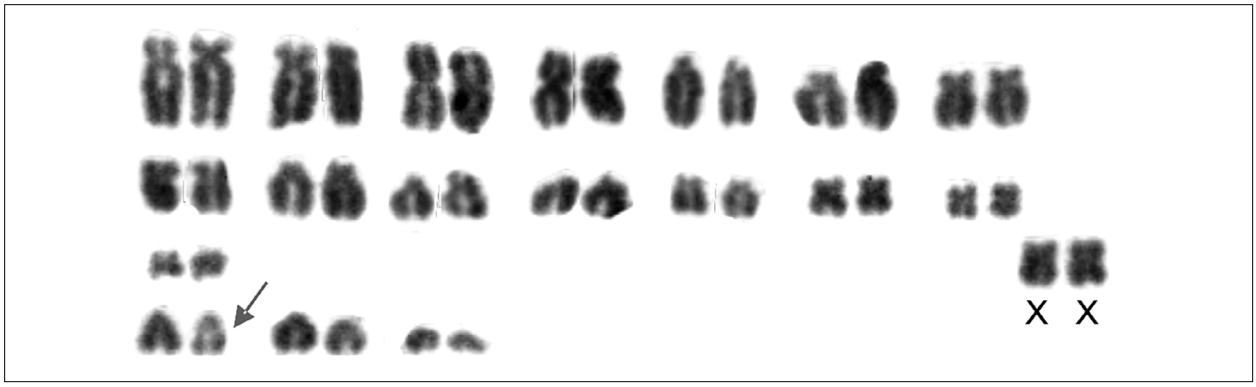

Figure 2. Karyotype with Giemsa staining of a female of *Galictis cuja* (LBCE7963) from Teresópolis, Rio de Janeiro state, Brazil, with 2n = 38 and FNa = 66. X = sexual X chromosome. The arrow indicates the secondary constriction.

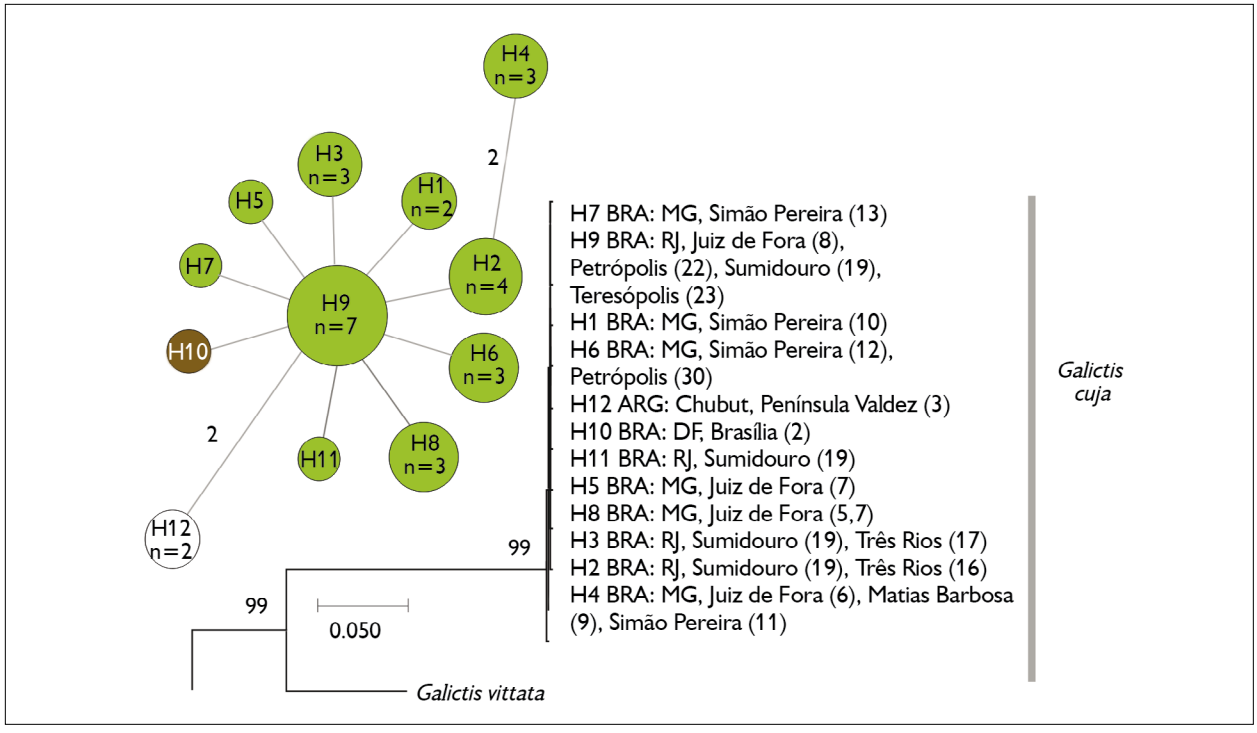

Figure 3. Maximum likelihood (ML, right) topology and median-joining (MJ, left) of *Galictis* based on *mt-Cytb*. In ML number near nodes are bootstrap values. In MJ circle size corresponds to number of individuals with a given haplotype, light gray are haplotypes from Atlantic Forest, dark gray from Cerrado, and white from Argentina. Numbers connecting branches denote more than one nucleotide substitutions; connecting branches without a number indicate one nucleotide substitution. Numbers between parentheses refer to localities in the Figure 1.

DISCUSSION

GENETIC CONSIDERATION

The karyotype of specimens from Rio de Janeiro state, with 2n = 38 and FNa = 66, recorded in this study, is similar to the single chromosome complement already reported for one male *Galictis cuja* (2n = 38 and FN = 68) from Massaranduba in Santa Catarina state (Barbosa, 2013), both karyotypes showing a median sized biarmed X chromosome. The difference in fundamental autosome number is due to a small-sized chromosome. It can be due to different interpretation in relation to the centromere position, or a pericentric inversion affecting this pair. The karyotype herein describes for *G. cuja* confirm the diploid number of 38 as widespread in both genera and species of the order Carnivora, as in *Lontra longicaudis* (Olfers, 1818) (Freitas *et al*., 1982), *Martes itatsi* Temminck, 1844 (Kurose *et al*., 2000), and *Ptenoruna brasiliensis* (Gmelin, 1788) (Franco-de-Sá *et al*., 2007), but differing in the morphology of autosome and sexual chromosomes.

The phylogenetic analysis confirms previous publication in showing *Galictis* with two lineages. *Galictis cuja* showed high haplotypic diversity (0.9021) and a low nucleotidic diversity (only 13 variable sites) suggesting that this population expanded after a period of small population size (Su *et al*., 2015). Even sample from the same locality showed high haplotypic diversity (*i.e*., Juiz de Fora with 3 haplotypes in 6 sequences; Sumidouro, with 4 haplotypes in 6 sequences). However, specimens collected in the same fragments and different years shared the same haplotype (*e.g*., LBCE7949, LBCE7956, LBCE7963, LBCE7969 from Teresópolis, haplotype H9; LBCE6587 and LBCE6619 from Sumidouro, haplotype H19; Table 1, Figure 3). The network did not show population structure, suggesting connectivity between populations as previously shown for this species using another marker (Bornholdt *et al*., 2013).

Our data confirm *G. cuja* as a common roadkilled mammal in Atlantic Forest, similar to the Cerrado, where *G. cuja* was considered the fifth more roadkill mammalian species, corresponding to 6.1% of roadkilled mammals (Vieira, E., 1996). This high frequency of roadkill *G. cuja* was well documented (Rosa & Mauhs, 2004; Casella *et al*., 2006; Cherem *et al*., 2007; Coelho *et al*., 2008; Sousa & Miranda, 2010; Bueno *et al.*, 2015). Impressive, the roadkill specimens in BR-040 were captured near urban areas with forest patches or in forested areas, but not in crop fields (Figure 1).

Traditionally *Galictis vittata* is considered as an inhabitant of humid forests from northern South America to Central America, whereas *Galictis cuja* is considered as an inhabitant of open areas in southern South America (Rodrigues *et al*., 2015). However, our data showed that *G. cuja* could be common in the areas of Atlantic Forest. The fossil records showed that these two species already occurred in Lagoa Santa in the Brazilian Cerrado biome (Rodrigues *et al*., 2015). The most recent map with the geographic distribution of *Galictis cuja* places its northmost localities in northeastern Brazil, in the Caatinga biome, and in the Federal District, in the Cerrado of central Brazil (Bornholdt *et al*., 2013). However, in the Pleistocene deposits, this species also was recorded for the north Cerrado, at Aurora do Tocantins municipality, Tocantins state (Rodrigues *et al*., 2015; Figure 1). These data showed that the extant southern South America distribution of *G. cuja*, and the northern South America distribution of *G. vittata* could have been moulded modulated by climate change (Rodrigues *et al*., 2015), together with the current anthropogenic induced range contraction.

ZOONOTIC IMPLICATIONS

Part of the *G. cuja* roadkill specimens herein analyzed (Appendix 1) was previously investigated for helminth parasitism by Corrêa *et al*. (2016), and was detected nematodes [*Molineus elegans* (Travassos, 1921), *Dioctophyme renale* (Goeze, 1782)], acanthocephalan [*Pachysentis gethi* (Machado-Filho, 1950)], and digenetic [*Platynosomum illiciens* (Braum, 1901)], showing the possible role of these animals as a spreader of parasites with zoonotic potential, particularly

to domestic carnivores, as dogs and cats. Other populations of *G. cuja* from South and Southeastern Brazil were also reported with parasites (Pesenti *et al*., 2012; Vieira, F. *et al*., 2017). Furthermore, a Canine Distemper virus has also been reported in a free-ranging *G. cuja* (Megid *et al*., 2013). These authors argue that the CDV found in *G. cuja* and other wild carnivores have resulted from a spillover transmission from domestic dogs, potential reservoirs of this pathogen. The area where the majority of *Galictis* species were collected is intensively populated, and the contact between *G. cuja* specimens and dogs in the rural area should be intense. This scenario, plus the connectivity between *G. cuja* populations suggested this mustelid can play a role in the maintenance or even the amplification of the transmission cycle of this pathogen, and that dogs non vaccinated with distemper vaccine can be a threat for *G. cuja* conservation.

CONCLUSION

Our data confirm that *G. cuja* is a common inhabitant of Atlantic Forest of southeastern Brazil, being a frequent roadkill mammal, similar to the Cerrado region. The karyotype of specimens from Rio de Janeiro state, with 2n = 38 and FNa = 66, is similar to the single chromosome complement already reported for one male *Galictis cuja* (2 n =38 and FNa = 68) from Massaranduba in Santa Catarina state (Barbosa, 2013), being the difference in FN due to a small-sized chromosome pair. The phylogenetic analysis showed *Galictis cuja* with high haplotypic diversity and a low nucleotidic diversity, confirming that this population expanded after a period of small population size. The network did not show population structure, suggesting connectivity between populations as previously showed for this species.

ACKNOWLEDGMENT

We thank the *Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres* field team for supporting fieldwork, to Vânia de Araujo Soares for collecting roadkill specimen from Distrito Federal, to Concer for support to *Caminhos da Fauna* Project, to Rayque Lanes for helping with maps. Work supported by grants to CR Bonvicino (CNPq 304498/2014-9, and FAPERJ E26/201.200/2017, in *Instituto Oswaldo Cruz*). We are grateful to Riccardo Castiglia and one unknown reviewer for their valuable suggestions and comments in a previous version of this manuscript.

REFERENCES

ANDRADE, A. F. B. & C. R. BONVICINO, 2003. A new karyologic variant of *Oecomys* (Rodentia: Sigmodontinae) and its phylogenetic relationship based on molecular data. **Genome** 46(2): 195-203. DOI: https://doi.org/10.1139/g02-123

BANDELT, H. J., P. FORSTER & A. RÖHL, 1999. Median-joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution** 16(1): 37-48. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a026036

BARBOSA, L. T., 2013. **Contribuições citogenéticas nas espécies *Galictis cuja* (Carnivora, Mustelidae) e *Scapteromys* (Rodentia, Cricetidae)**. Master Dissertation – Universidade Federal do Paraná, Curitiba.

BARROS, D. M., M. L. LORINI & V. G. PERSSON, 1990. Dioctophymosis in the Little Grison (*Galictis cuja*). **Journal of Wildlife Diseases** 26(4): 538-539. DOI: https://doi.org/10.7589/0090-3558-26.4.538

BORNHOLDT, R., K. HELGEN, K.-P. KOEPFLI, L. OLIVEIRA, M. LUCHERINI & E. EIZIRIK, 2013. Taxonomic revision of the genus *Galictis* (Carnivora: Mustelidae): species delimitation, morphological diagnosis, and refined mapping of geographical distribution. **Zoological Journal of the Linnean Society** 167(3): 449-472. DOI: https://doi.org/10.1111/j.1096-3642.2012.00859.x

BUENO, C., C. O. M. SOUSA & S. R. FREITAS, 2015. Habitat or matrix: which is more relevant to predict roadkill of vertebrates? **Brazilian Journal of Biology** 75(4), suppl. 1: S228-S238. DOI: http://dx.doi.org/10.1590/1519-6984.12614

CASELLA, J., N. C. CÁCERES, C. SANTOS GOULART, A. C. PARANHOS FILHO & P. N. S. CONCEIÇÃO, 2006. Uso de sensoriamento remoto e análise espacial na interpretação de atropelamentos de fauna entre Campo Grande e Aquidauana, MS. **Simpósio de Geotecnologias no Pantanal** 1: 321-326.

CASSENS, I., S. VICARIO, V. G. WADDELL, H. BALCHOWSKY, D. VAN BELLE, W. DING, C. FAN, R. S. MOHAN, P. C. SIMÕES-LOPES, R. BASTIDA, A. MEYER, M. J. STANHOPE & MILINKOVITCH, 2000. Independent adaptation to riverine habitats allowed survival of ancient lineages. **Proceedings of the National Academy of Sciences** 97(21): 11343-11347. DOI: https://doi.org/10.1073/pnas.97.21.11343

CHEREM, J. J., K. M. AMMERS, I. R. GHIZONI-JR. & A. MARTINS, 2007. Mamíferos de médio e grande porte atropelados em rodovias do Estado de Santa Catarina, sul do Brasil. **Revista Biotemas** 20(3): 81-96.

COELHO, I. P., A. KINDEL & A. V. P. COELHO, 2008. Roadkills of vertebrate species on two highways through the Atlantic Forest Biosphere Reserve, southern Brazil. **European Journal of Wildlife Research** 54: 689-699. DOI: https://doi.org/10.1007/s10344-008-0197-4

CORRÊA, P., C. BUENO, F. M. VIEIRA & L. C. MUNIZ-PEREIRA, 2016. Helminth parasites of *Galictis cuja* (Carnivora, Mustelidae), from localities in the Atlantic Forest of Brazil. **Brazilian Journal of Parasitology** 25(4): 407-413. DOI: https://doi.org/10.1590/s1984-29612016077

EIZIRIK, E., W. J. MURPHY, K.-P. KOEPFLI, W. E. JOHNSON, J. W. DRAGOO, R. WAYNE & S. J. O'BRIEN, 2010. Pattern and timing of diversification of the mammalian order Carnivora inferred from multiple nuclear gene sequences. **Molecular Phylogenetics and Evolution** 56(1): 49-63. DOI: https://doi.org/10.1016/j.ympev.2010.01.033

FERRIOLLI, F. & M. P. BARRETTO, 1969. Disease reservoirs and wild vectors of Trypanosoma cruzi. Natural infection of the ferret *Galictis cuja furax* (Thomas, 1907) caused by *T. cruzi*. **Revista do Instituto de Medicina Tropical de São Paulo** 11: 264-273.

FLUXUS, [s. d.]. Available at: https://www.fluxus-engineering.com/about.htm. Accessed on: December 2019.

FLYNN, J. J. & M. A. NEDBAL, 1998. Phylogeny of the Carnivora (Mammalia): congruence vs incompatibility among multiple data sets. **Molecular Phylogenetics and Evolution** 9(3): 414-426. DOI: http://dx.doi.org/10.1006/mpev.1998.0504

FLYNN, J. J., J. A. FINARELLI, S. ZEHR, J. HSU & M. A. NEDBAL, 2005. Molecular phylogeny of the Carnivora (Mammalia): assessing the impact of increased sampling on resolving enigmatic relationships. **Systematics Biology** 54(2): 317-337. DOI: http://dx.doi.org/10.1080/10635150590923326

FRANCO-DE-SÁ, J. F. O., F. C. W. ROSAS & E. FELDBERG, 2007. Cytogenetic study of the giant otter *Pteronura brasiliensis* Zimmermann 1780 (Carnivora, Mustelidae, Lutrinae). **Genetics and Molecular Biology** 30(4): 1093-1096. DOI: http://dx.doi.org/10.1590/S1415-47572007000600011

FREITAS, T. R. O., M. S. MATTEVI & L. F. B. OLIVEIRA, 1982. Karyotype characterization of lontra (*Lutra longicaudis longicaudis*, Mustelidae, Carnivora). **Mammalian Chromosomes Newsletter** 23(3): 91-96.

KOEPFLI, K. P. & R. K. WAYNE, 2003. Type I STS markers are more informative than cytochrome b in phylogenetic reconstruction of the Mustelidae (Mammalia: Carnivora). **Systematic Biology** 52(2003): 571-593. DOI: http://dx.doi.org/10.1080/10635150390235368

KOEPFLI, K. P., M. E. GOMPPER, E. EIZIRIK, C. C. HO, L. LINDEN, J. E. MALDONADO & R. K. WAYNE, 2007. Phylogeny of the Procyonidae (Mammalia: Carnivora): molecules, morphology and the great American interchange. **Molecular Phylogenetics and Evolution** 43(3): 1076-1095. DOI: http://dx.doi.org/10.1016/j.ympev.2006.10.003

KOEPFLI, K.-P., A. A. DEERE, G. J. SLATER, C. BEGG, K. BEGG, L. GRASSMAN, M. LUCHERINI, G. VERON & R. K. WAYNE, 2008. Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. **BMC Biology** 6: 10. DOI: https://doi.org/10.1186/1741-7007-6-10

KUROSE, N., R. MASUDA, T. AOR & S. WATANABE, 2000. Karyological differentiation between two closely related mustelids, the Japanese weasel *Mustela itatsi* and the Siberian weasel *Mustela siberica*. **Caryologia** 53(3-4): 269-275. DOI: https://doi.org/10.1080/00087114.2000.10589205

LEDJE, C. & U. ARNASON, 1996. Phylogenetic analyses of complete cytochrome *b* genes of the order Carnivora with particular emphasis on the Caniformia. **Journal of Molecular Evolution** 42: 135-144. DOI: https://doi.org/10.1007/BF02198839

LEVAN, A., K. FREDGA & A. A. SANDBERG, 1964. Nomenclature for centromeric position on chromosomes. **Hereditas** 52(2): 201-220. DOI: https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

MEGID, J., C. R. TEIXEIRA, A. CORTEZ, M. B. HEINEMANN, J. M. A. P. ANTUNES, F. FORNAZARI, F. B. RASSY & L. J. RICHTZENHAIN, 2013. Canine distemper virus infection in a lesser grison (*Galictis cuja*): first report and virus phylogeny. **Pesquisa Veterinária Brasileira** 33(2): 247-250. Available at: http://hdl.handle.net/11449/13376. Accessed on: December 2019.

MELO, L. A., 2008. **Detecção de *Leishmania* sp. em pequenos mamíferos silvestres e sinantrópicos no município de Belo Horizonte, MG**. Master Dissertation – Fundação Oswaldo Cruz, Belo Horizonte.

PESENTI, T. C., C. S. MASCARENHAS, C. KRÜGER, A. L. SINKOC, A. P. N. ALBANO, M. A. A. COIMBRA & G. MÜLLER, 2012. *Dioctophyma renale* (Goeze, 1782) collet-meygret, 1802 (Dioctophymatidae) in *Galictis cuja* (Molina, 1782) (Mustelidae) in Rio Grande Sul, Brazil. **Neotropical Helminthology** 6(2): 301-305.

RODRIGUES, S., L. AVILLA & S. A. K. AZEVEDO, 2015. Diversity and paleoenviromental significance of Brazilian fossil *Galictis* (Carnivora: Mustelidae). **Historical Biology, an International Journal of Paleobiology** 28(7): 907-912. DOI: https://doi.org/10.1080/08912963.2015.1055559

ROSA, A. O. & J. MAUHS, 2004. Atropelamento de animais silvestres na rodovia RS-040. **Caderno de Pesquisa, Série Biologia** 16(1): 35-42.

SAMBROOK, J., E. F. FRITSCH & T. MANIATIS, 1989. **Molecular cloning**: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SATO, J. J., M. WOLSAN, F. J. PREVOSTI, G. D'ELÍA, C. BEGG, K. BEGG, T. HOSODA, K. L. CAMPBELL & H. SUZUKI, 2012. Evolutionary and biogeographic history of weasel-like carnivorans (Musteloidea). **Molecular Phylogenetics and Evolution** 63(3): 745-757. DOI: https://doi.org/10.1016/j.ympev.2012.02.025

SOUSA, M. A. N. & P. C. MIRANDA, 2010. Mamíferos terrestres encontrados atropelados na rodovia BR-230/pb entre Campina Grande e João Pessoa. **BIOFAR - Revista de Biologia e Farmácia** 4: 72-82.

SU, L.-N., X.-C. LI, H.-Z. MENG, X.-Y. GAO, H. YIN & K. LI, 2015. Population genetic structure and historical demography of the ground beetle *Chlaenius costiger* in the Tsinling-Dabashan Mountains of central China. **Genetics and Molecular Research** 14(2): 3579-3589. DOI: https://doi.org/10.4238/2015.April.17.7

TAMURA, K., G. STECHER, D. PETERSON, A. FILIPSKI & S. KUMAR, 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. **Molecular Biology and Evolution** 30(12): 2725-2729. DOI: https://doi.org/10.1093/molbev/mst197

TORRES-CASTRO, M. A., R. A. MEDINA-PINTO, H. R. NOH-PECH, F. I. PUERTO & R. I. RODRÍGUEZ-VIVAS, 2019. Molecular identification of *Toxoplasma gondii* in roadkill wild animals in Yucatan, Mexico. **Veterinaria Mexico OA** 6(1). DOI: https://doi.org/10.22201/fmvz.24486760e.2019.1.511

TREMORI, T. M., 2018. **Identificação criminal de espécies da fauna silvestre por DNA mitocondrial**. Doctoral Dissertation – Universidade Estadual Paulista, Botucatu.

VIEIRA, E. M., 1996. Highway mortality of mammals in central Brazil. **Ciência e Cultura** 48(4): 270-272.

VIEIRA, F., L. C. MUNIZ-PEREIRA, S. SOUZA LIMA, A. H. A. MORAES NETO, P. R. GONCALVES & J. L. LUQUE, 2012. *Crenosoma brasiliense* sp. n. (Nematoda: Metastrongyloidea) parasitic in lesser grison, *Galictis cuja* (Molina, 1782) (Carnivora, Mustelidae) from Brazil, with a key to species of *Crenosoma* Molin, 1861. **Folia Parasitologica** 59(3): 187-194. DOI: https://doi.org/10.14411/fp.2012.026

VIEIRA, F. M., L. C. MUNIZ-PEREIRA, S. SOUZA-LIMA, B. M. ROCHA & J. L. LUQUE, 2017. Parasitic nematodes of three species of wild carnivore mammals from Atlantic forest in the state of Minas Gerais, Brazil. **Revista Mexicana de Biodiversidad** 88(4): 801-806. DOI: https://doi.org/10.1016/j.rmb.2017.10.033

WOLSAN, M. & J. J. SATO, 2010. Effects of data incompleteness on the relative performance of parsimony and Bayesian approaches in a supermatrix phylogenetic reconstruction of Mustelidae and Procyonidae (Carnivora). **Cladistics** 26(2): 168-194. DOI: https://doi.org/10.1111/j.1096-0031.2009.00281.x

ZABOTT, M. V., S. B. PINTO, A. M. VIOTT, R. A. TOSTES, L. H. F. B. BITTENCOURT, A. L. KONELL & L. GRUCHOUSKEI, 2012. Ocorrência de *Dioctophyma renale* em *Galictis cuja*. **Pesquisa Veterinária Brasileira** 32(8): 786-788. DOI: https://doi.org/10.1590/S0100-736X2012000800018

Appendix 1. List of samples of *Galictis cuja*. *Voucher* numbers are between parentheses. Numbers in square brackets refers to localities in Figure 1. Asterisk (*) = specimens investigated for parasites in previous publication (Corrêa *et al*., 2016), ([P]) = specimens used in molecular analysis.

ARGENTINA

No locality (EF987754, no *voucher*); **Chubut**, **[3]** Peninsula Valdez -24.5000, -64.0000 (AB564025, *voucher* MC795).

BRAZIL

Distrito Federal: [2] Brasília, -15.7833, -47.9167 (CRB3283[P]).

Minas Gerais state: Juiz de Fora, **[4]** BR-040 km 775, -21.6608, -43.4342 (MN79510), **[5]** BR-040 km 779-783, -21.6917, -43.4483 (MN79153, CB1013, MN83597[P]), **[6]** BR-040 km 789, -21.7697, -43.4372 (MN79358*[,P]), **[7]** BR-040 km 796-797, -21.8111, -43.3906 (CB1012[P]), **[8]** BR-040 km 798, -21.8172, -43.3842 (MN79471*[,P]), Matias Barbosa, -21.8658, -43.3669 (MN79445*[,P]), **[9]** BR-040 km 796 (MN79363[P]), BR-040 km 805 (CB991[P]), km 807 (MN79162*); Simão Pereira, **[10]** BR-040 km 816 -21.9239, -43.3194 (MN79404*[,P]), **[11]** BR-040 km 823, -21.9747, -43.2853 (MN79407*[,P]), **[12]** BR-040 km 825, -21.9908, -43.2847 (CB1089[P]), **[13]** BR-040 km 827, -22.0022, -43.2767 (MN83564*[,P], MN79190*).

Rio de Janeiro state: Areal, **[20]** BR-040 km 42 (MN79314*); Cachoeiras de Macacu, **[26]** RJ-122 km 25, -22.4667, -42.6500 **[26]** km 25 (CB1321); Comendador Levy Gasparian, -22.0097, -43.2642 **[14]** BR-040 km 1-4 (MN79213, CB1237), **[15]** BR-040 km 10 (MN79154); Duque de Caxias, **[31]** BR-040 km 97, -22.5833, -43.2606 (MN79452), **[32]** BR-040 km 100-102, -22.5925, -43.2800 (MN79326*, MN69904), **[33]** BR-040 km 103, -22.6175, -43.2864 (MN79379*), **[34]** BR-040 km 106, -22.6442, -43.2836 (MN79421*); Guapimirim, -22.5298, -42.9788, **[29]** RJ-122 km 10-17 (CB1320, CB1310); Petrópolis, **[21]** BR-040 km 53, -22.3428, -43.1328 (CB1185[P], MN79216*), **[22]** BR-040 km 54, -22.3772, -43.1303 (MN79261*), [**24**] BR-040 km 67, -22.4361, -43.1747 (MN79308), **[25]** BR-040 km 69, -22.4428, -43.1914 (MN79443*), **[27]** BR-040 km 77, -22.4931, -43.2231 (MN79334), **[28]** km 78, -22.4997, -43.2292 (MN83573), **[30]** BR-040 km 71, -22.5631, -43.2314 (MN79501*[,P], MN83244, MN79550[P]); Sumidouro, **[19]** -22.0504, -42.6842 (MN74461[P]), Bairro da Volta (LBCE7780[P]), Piedade (LBCE6587[P]), Soledade (LBCE6619[P]), Vale do Encanto (LBCE5276[P], LBCE6437[P]), Vale do Pamparrão (LBCE7241[P]); Teresópolis, **[23]** -22.4241, -42.9680 (LBCE7949[P], LBCE7956[P], LBCE7963[P], LBCE7969[P]); Três Rios, **[16]** BR-040 km 11, -22.0469, -43.2006 (MN79247*[,P]), **[17]** BR-040 km 14, -22.0681, -43.1942 (MN79294*[,P]), **[18]** BR-040 km 15, -22.0744, -43.1892 (MN79436, CB1294).

Tocantins state: **[1]** Aurora do Tocantins, -12.7121, -46.4072 (fossil specimen, Rodrigues *et al*., 2015).