

Wild Birds as *Trypanosoma cruzi* reservoirs in Pampa biome, Rio Grande do Sul-Brazil: occurrence and molecular detection

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Abstract

Trypanosoma cruzi, the etiological agent of Chagas disease, is a zoonotic protozoan primarily associated with mammalian hosts. Birds have historically been considered refractory to *T. cruzi* infection due to innate resistance mediated by the complement system. However, recent evidence suggests an expanded host range. This study aimed to investigate the occurrence of *T. cruzi* in wild birds from the Pampa biome in southern Brazil using molecular methods. A total of 31 wild birds, comprising roadkill or animals received by wildlife rehabilitation centers in Pelotas and surrounding areas, Rio Grande do Sul, were sampled. Tissues including heart, liver, intestine, spleen, lungs, and brain were analyzed via Polymerase Chain Reaction targeting the parasite's kinetoplast DNA (kDNA). *Trypanosoma cruzi* was detected in 12 out of 31 individuals, representing an occurrence rate of 38.7% across diverse avian orders such as Gruiformes, Strigiformes, Anseriformes, and Passeriformes. The highest infection frequencies were observed in the heart, liver, and intestine, indicating a tissue tropism consistent with the disease's pathophysiology in mammals. These findings challenge the paradigm of avian resistance, demonstrating that wild birds can harbor *T. cruzi* in various organs. The detection of the parasite in multiple bird species highlights their potential role as novel reservoirs in the sylvatic and periurban transmission cycles. From a One Health perspective, this active circulation of *T. cruzi* in southern Brazilian avian populations underscores the impact of anthropogenic changes and the need for inclusive wildlife epidemiological surveillance.

1 INTRODUCTION

Brazil is known by its biologic richness, with different biomes and several differences in the environmental characteristics, but mainly by the large diversity of wild species that are present in the country. On a continental scenario, Brazil is one of the three nations in the Americas that have the greatest number of bird species. However, the country is in first place in the rank of threatened species in the world and has one of the highest rates of illegal bird commerce, fact that combined with different anthropic actions, as logging and illegal hunt, contribute to the transmission of pathogens, making these animals possible reservoirs of etiologic agents, as *Trypanosoma* sp. (Echenique et al. 2020).

Trypanosoma cruzi is one of the best-known species in the *Trypanosoma* genus. Belonging to the family Trypanosomatidae, it is a protozoan that causes Chagas disease, or American Trypanosomiasis, a zoonotic affection of great importance in the continent, especially in Brazil. Wild animals, especially marsupials, can be described as possible reservoirs of the parasite, acting in the agent's life cycle and transmission (Zetun et al. 2023).

Birds have been described as refractory to *T. cruzi*, especially poultry, due to a natural resistance to the parasite, characterized by the complement system action (Roque and Jansen 2014). Martínez-Hernández et al. (2022) brought a characterization of the parasite in wild species by molecular diagnosis, indicating the possibility of occurrence of the agent in wild birds. In addition, there are few

reports about the *T. cruzi* infection in birds, showing that the issue has huge importance in an One Health view.

Thus, this research aims to assess the occurrence of *Trypanosoma cruzi* in wild birds from the Pampa biome in southern Brazil by molecular assays.

2 MATERIALS AND METHODS

2.1 Samples collection and storage

The collected carcasses were taken into the Instituto de Patologia Veterinária at the Federal University of Pelotas (UFPEL) for necropsy to tissues and blood collection. A total of thirty-one wild birds from roadkill on highways or received by Núcleo de Reabilitação da Fauna Silvestre (NURFS) were analyzed from Pelotas and surrounding areas, Rio Grande do Sul, Brazil, from February 2025 to February 2026 (Figure 1).

Fragments of brain, intestine, heart, liver, ventricle, kidneys, lungs, striated skeletal muscle and spleen were collected. The samples were placed in 2 mL Eppendorf tubes for DNA extraction, using Quick-Zol™ reagent (Ludwig Biotecnologia Ltda., Alvorada, RS, Brazil) according to the manufacturer's instructions, and in 15 ml Falcon tubes with 10% glycerin for preservation of the left tissues, while total blood were stored in 0.4 ml EDTA tubes, and preserved in -80 °C or -20 °C freezers until processing at the Laboratório de Biologia Molecular Veterinário (LaBMol-Vet) from UFPEL. This study was conducted under authorization No. 0991790320260410 issued by the Brazilian Biodiversity Information and Authorization System (SisBio).

2.2 Molecular analysis (PCR)

For molecular detection, the Polymerase Chain Reaction (PCR) mixture contained 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 µM of primers forward and reverse (diluted at 10 pmol), 1.25 U Taq DNA polymerase, and 1 µL of template DNA at 100 ng (total volume of 25 µL per aliquote). A positive control available at LaBMol-Vet and negative control (nuclease-free water) were used per PCR reaction. The primers used in this research targeted the kinetoplast DNA (kDNA) of *T. cruzi*, which were 121-F (5' - AAA TAA TGT ACG GKG GAG ATG CAT GA - 3') and 122-R (5' - GGT TCG ATT GGG GTT GGT GTA ATA TA - 3') (Schijman et al. 2011). The cycling conditions followed: initial denaturation at 94 °C for two minutes, 35 cycles of denaturation at the same temperature for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 40 seconds, and final extension at 72 °C for seven minutes. After PCR, electrophoresis on a 1.5% agarose gel with ethidium bromide for specific bands visualization.

3 RESULTS

Among the 31 collected and necropsied animals, 12 were positive for *Trypanosoma cruzi*, corresponding to 38.7% of total individuals (Table 1). Each bird and samples collected with the results of PCR are

described in Table 2.

Table 1 - Distribution of bird species according to taxonomic order, number of animals examined, number of positive birds, and positivity rate.

HOST SPECIES	ORDER	TOTAL ANIMALS COLLECTED	TOTAL POSITIVE ANIMALS	POSITIVE RATE
<i>Aramides saracura</i>	Gruiformes	2	2	100%
<i>Bubo virginianus</i>	Strigiformes	1	1	100%
<i>Buteogallus meridionalis</i>	Accipitriformes	1	0	0%
<i>Caracara plancus</i>	Falconiformes	2	1	50%
<i>Chauna torquata</i>	Anseriformes	1	1	100%
<i>Coccyzus melacoryphus</i>	Cuculiformes	1	1	100%
<i>Colaptes melanochloros</i>	Piciformes	1	0	0%
<i>Columba livia</i>	Columbiformes	2	1	50%
<i>Falco peregrinus</i>	Falconiformes	1	1	100%
<i>Falco sparverius</i>	Falconiformes	1	0	0%
<i>Furnarius rufus</i>	Passeriformes	1	0	0%
<i>Molothrus bonariensis</i>	Passeriformes	1	0	0%
<i>Myiopsitta monachus</i>	Psittaciformes	6	1	16,6%
<i>Penelope obscura</i>	Galliformes	1	0	0%
<i>Pitangus sulphuratus</i>	Passeriformes	4	3	75%
<i>Progne tapera</i>	Passeriformes	1	0	0%
<i>Turdus rufiventris</i>	Passeriformes	1	0	0%
<i>Vanellus chilensis</i>	Charadriiformes	1	0	0%
<i>Zenaida auriculata</i>	Columbiformes	2	0	0%

Table 2 - Distribution of PCR-positive tissue samples according to species, animals' origin, and tissue type.

HOST SPECIES	GEOGRAPHIC ORIGIN	TISSUES SAMPLED	PCR-POSITIVE TISSUES
<i>Aramides saracura</i> 1	31°47'49.6"S 52°29'22.0"W	Heart, Ventricle, Lungs, Kidneys, Liver, Intestine, Brain	Liver, Intestine
<i>Aramides saracura</i> 2	31°46'36.6"S 52°24'57.0"W	Spleen, Heart, Ventricle, Lungs, Kidneys, Liver, Intestine, Brain, Blood	Spleen, Heart, Liver
<i>Bubo virginianus</i>	30°41'33.4"S 51°23'50.1"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	Spleen, Intestine
<i>Buteogallus meridionalis</i>	32°33'38.6"S 53°22'42.5"W	Heart, Skeletal Muscle, Lungs, Kidneys, Liver, Brain	-
<i>Caracara Plancus</i> 1	31°57'09.4"S 52°46'00.2"W	Heart, Ventricle, Skeletal Muscle, Lungs, Liver	Heart, Skeletal Muscle
<i>Caracara plancus</i> 2	31°46'39.6"S 52°20'32.2"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Chauna torquata</i>	31°48'26.413"S 52°19'16.55" W	Spleen, Heart, Ventricle, Lungs, Kidneys, Liver, Intestine, Brain	Spleen, Heart, Liver
<i>Coccyzus melacoryphus</i>	31°44'23.1"S 52°42'32.4"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain, Egg	Heart, Kidneys, Intestine
<i>Colaptes melanochloros</i>	31°47'14.8"S 52°24'41.9"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain, Blood	-
<i>Columba livia</i> 1	30°51'05.8"S 51°48'45.7"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	Heart, Skeletal Muscle, Kidneys, Liver, Intestine
<i>Columba livia</i> 2	31°48'03.1"S 52°24'31.2"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Falco peregrinus</i>	32°38'50.4"S 52°35'10.5"W	Heart, Ventricle, Lungs, Intestine, Brain	Lungs
<i>Falco sparverius</i>	31°46'16.5"S 52°20'27.9"W	Heart, Ventricle, Skeletal Muscle, Liver, Intestine	-
<i>Furnarius rufus</i>	31°46'42.3"S 52°24'56.1"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-

<i>Molothrus bonariensis</i>	31°45'20.2"S 52°15'01.8"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Myiopsitta monachus</i> 1	32°10'52.0"S 52°09'38.1"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Myiopsitta monachus</i> 2	32°10'11.9"S 52°11'07.0"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Myiopsitta monachus</i> 3	31°45'19.9"S 52°19'12.0"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Myiopsitta monachus</i> 4	32°05'02.4"S 52°06'20.5"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain, Testicle	-
<i>Myiopsitta monachus</i> 5	32°06'12.1"S 52°11'01.5"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain, Blood	-
<i>Myiopsitta monachus</i> 6	31°46'45.8"S 52°18'55.9"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	Liver, Intestine
<i>Penelope obscura</i>	31°43'42.8"S 52°36'25.3"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain, Blood	-
<i>Pitangus sulphuratus</i> 1	31°46'34.8"S 52°24'57.1"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	Spleen, Heart, Lungs, Kidneys, Liver, Intestine, Brain
<i>Pitangus sulphuratus</i> 2	31°45'45.0"S 52°24'16.7"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Brain	Heart, Ventricle, Skeletal Muscle, Brain
<i>Pitangus sulphuratus</i> 3	31°45'10.6"S 52°27'32.3"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Pitangus sulphuratus</i> 4	31°46'40.0"S 52°21'01.7"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	Heart, Skeletal Muscle, Brain
<i>Progne tapera</i>	32°10'08.4"S 52°11'11.6"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain, Testicle	-
<i>Turdus rufiventris</i>	31°19'23.8"S 54°06'40.5"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-

<i>Vanellus chilensis</i>	31°19'25.7"S 54°06'32.8"W	Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Zenaida auriculata</i> 1	31°45'35.7"S 52°15'46.6"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-
<i>Zenaida auriculata</i> 2	31°45'27.5"S 52°21'03.2"W	Spleen, Heart, Ventricle, Skeletal Muscle, Lungs, Kidneys, Liver, Intestine, Brain	-

Note: The collection of tissues varied due to tissue’s size or infeasibility at the time of necropsy by cause of death or advanced autolysis.

The highest frequencies of PCR-positive samples were observed heart (8/30; 26.6%), liver (6/29; 20.6%) and intestine (6/28; 21.4%), while spleen (4/19; 21.05%) and skeletal muscle (4/27; 14.8%) also had considerable results in the analyzed birds. Furthermore, kidneys (3/28; 10.7%) affected three different individuals from three different orders, while infected brains (3/28; 10.7%) were observed in three different animals of the same species. Lungs (2/30; 6.66%) and ventricle (1/30; 3.33%) were the least reported in this research, observing lung infection in two different individuals from different orders and ventricle infection in one *Pitangus sulphuratus* only.

PCR products from wild bird samples yielded the expected amplicon of approximately 330 bp, consistent with *Trypanosoma cruzi* amplification. In addition to PCR products, a commercial 100bp DNA Ladder (K9-100L, KASVI, Brazil) was used for assistance of band size comparison. A pattern in the posterior electrophoresis was demonstrated between 300 and 400 bp, according to base methodology (Supplementary Figures 1, 2 and 3).

4 DISCUSSION

It is believed that the *T. cruzi* has a sylvatic origin, which ancestors in past centuries came from bats and through them, spread to a considerable amount of wild species (Sampaio et al. 2025). The agent is consistently reported as mammals’ parasite, which can cause cardiomyopathies, myocarditis, heart failure, lethargy, anorexia, congestion, among others clinical signs in varied tissues (Sánchez Pérez et al 2024). Our study indicates that the host-range scenario can be expanded, providing indication that wild birds in Southern Brazil are possible hosts to *T. cruzi*.

The *T. cruzi* infection in wild birds is new to the scientific community. The only research that brings the parasite detection in a wild bird species is from Martínez-Hernández et al. (2022), who identified *T. cruzi* infection in a *Tyto furcata*. On the other hand, Campanha (2023) identified *Trypanosoma Polygranularis* and *Trypanosoma everetti* from samples of wild birds by molecular analysis. Furthermore, *Trypanosoma avium*, *Trypanosoma culicavium* and *Trypanosoma thomasbancrofti* are described as species that can be

identified in wild birds (Šlapeta et al. 2016; Galen et al. 2020), indicating that the *Trypanosoma* sp. is actively correlated to birds.

In a poultry view, Teixeira et al. (2023) identified that chicks from intentionally infected eggs by *T. cruzi* developed cardiomyopathys similar to chagas disease, but had no active parasitemia. In addition, Roellig et al. (2013) identified chickens' blood from triatomines' digestive system, indicating that these insects can feed from birds, however, no wild bird was used in the research. Furthermore, Teixeira et al. (2006) integrated a *T. cruzi*'s kDNA sequence into a chicken genome, allowing mutations that influenced the host's tissues, affections like the Chagas disease.

Wild birds are described as sentinels for diseases from other spectrums, such as the West Nile virus, assisting with the transmission and contributing to a bigger diversity of the etiologic agent (Mikhaleva et al. 2026). Our findings suggest a more active avian role in the *T. cruzi* infection than previously, supported by the prevalence of the parasite detection in wild birds in Pampa Biome, Rio Grande do Sul, Brazil (38.7% of analyzed animals) (Table 2). The percentage of positive animals aligns with reported results of infected wild birds in other regions with different biomes or divergent parasites, supporting the concept that these individuals can be associated to an enzootic role in the environment, even though most studies quote the refractory of *T. cruzi* in birds (Teixeira et al. 2006; Echenique et al. 2020).

The most frequently PCR-positive samples in our study were heart, liver and intestine, results that can be observed in reported studies with *T. cruzi* in wild animals species, which demonstrates a tissue tropism to cardiac and digestive organs and the capability to cause the cardiovascular and gastrointestinal systems affections in parasitemia (Busselman and Hamer 2022). Aligned with the findings of multiple organs infection, it is possible to reiterate the use of cardiac-related tissues as targets for molecular diagnosis in wildlife animals, in addition to the parasite's capacity of migration between varied systems in different stages of the disease, as reported in previous studies with sylvatic individuals (Rodriguez-Castellanos et al. 2025).

The occurrence of *T. cruzi* in the tested samples contrasts the need to amplify the host spectra, allowing new surveillance activities and the wild birds' input in the possible hosts class. In addition, it was possible to identify several species and different orders of infected animals, demonstrating an interspecific range of infection among wild birds (Table 1). This variation of infected species balance with previous reports of *Trypanosoma* sp. in birds, which occur based on contact with vectors, environment, feeding habits, and social behavior, projecting standards in the susceptibility of avian diseases (Santana et al. 2023).

Due to scarce literature about the avian *T. cruzi* infection, it is still not possible to affirm the pathogenicity of the parasite in these animals. However, Tarello (2005) reported in his study with *T. avium* in wild birds that the infected individuals presented anemia, anorexia, lethargy, dyspnea, weakness, incapacity of high flies and death. Despite the present research not analyzing natural behaviors of sampled animals, some clinical signs could be considered in *T. cruzi* infection in birds because of similarity to Chagas disease, such as weakness, lethargy and anemia, for example (Durães-Oliveira et al. 2024)

The occurrence of *T. cruzi* in various avian tissues characterizes the possibility of infection over certain conditions and the parasite's persistence postmortem long enough for its detection, since it is known that birds have an ordinary resistance to the agent, due to its intrinsic complement system's action. The use of molecular tools for diagnosis is valuable due to its high specificity and sensibility, which combined with the context of avian resistance allows the identification of new patterns of reservoirs and the surveillance of wildlife cycles (Echenique et al. 2020).

In the One Health context, anthropogenic factors proximate possible parasite hosts, such as habitat fragmentation, agricultural expansion and deforestation, reinforcing the maintenance of the parasite's sylvatic cycle and the persistence of *T. cruzi* in periurban areas of Brazilian southernmost (Rodriguez-Morales et al. 2025). Furthermore, the hunting of wild birds for human consumption also contributes to the transmission of diseases. Despite not being commonly reported and an environmental crime, it is a practice still considered as an infection form (Souza et al. 2023).

These findings indicate an emerging view of parasite hosting and new ecologic patterns with the maintenance of transmission by wild birds, with the parasite's widespread circulation in periurban and wild environments of southernmost regions of Brazil. The use of wildlife carcasses supports the idea of new sentinels and stimulates its research to surveillance of neotropical diseases, providing valuable epidemiologic information about the studied population and wild environment. The different tissue and species distribution demonstrated in the present study encourage further investigation for new zoonotic and ecological patterns in avian *T. cruzi* infection, influencing in the One Health context and conservation.

5 CONCLUSION

Our findings are characterized by the first report of *T. cruzi* infection in most of PCR-positive species described, and the second pointing to the parasite detection in wild birds, revealing an occurrence of 38.7% among these individuals. These findings expand current knowledge of *Trypanosoma cruzi* tissue tropism and potential sites of parasite persistence in wild birds, revealing an emerging ecological pattern in which diverse avian hosts may contribute to the maintenance of parasite transmission in periurban and wild environments of southern Brazil. This evidence highlights the importance of wild birds in the eco-epidemiology of Chagas disease and reinforces the need for further investigations within a One Health perspective.

Declarations

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Authors contribution

Gustavo C. F. Klann: data collection, data analysis, and interpretation, study design, study execution, preparation of the manuscript. William C. F. Zorrer, Bibiana D. de Freitas, Estefani Rinaldi: data collection, data analysis, and interpretation. Nilseia Feijó da Silva, Raqueli Teresinha Franca: data collection. Pedro M. M. de Albuquerque, Rodrigo C. Cunha, Nathieli B. Bottari: preparation of the manuscript.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Declaration of IA Statement

The authors used AI-assisted technologies (OpenAI DeepSeek) for initial literature search during the preparation of this manuscript. Following the use of these tools, the authors reviewed, modified, and validated all generated content and take full responsibility for the accuracy and integrity of the work.

Animal Ethics Declaration

Not applicable.

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Figures

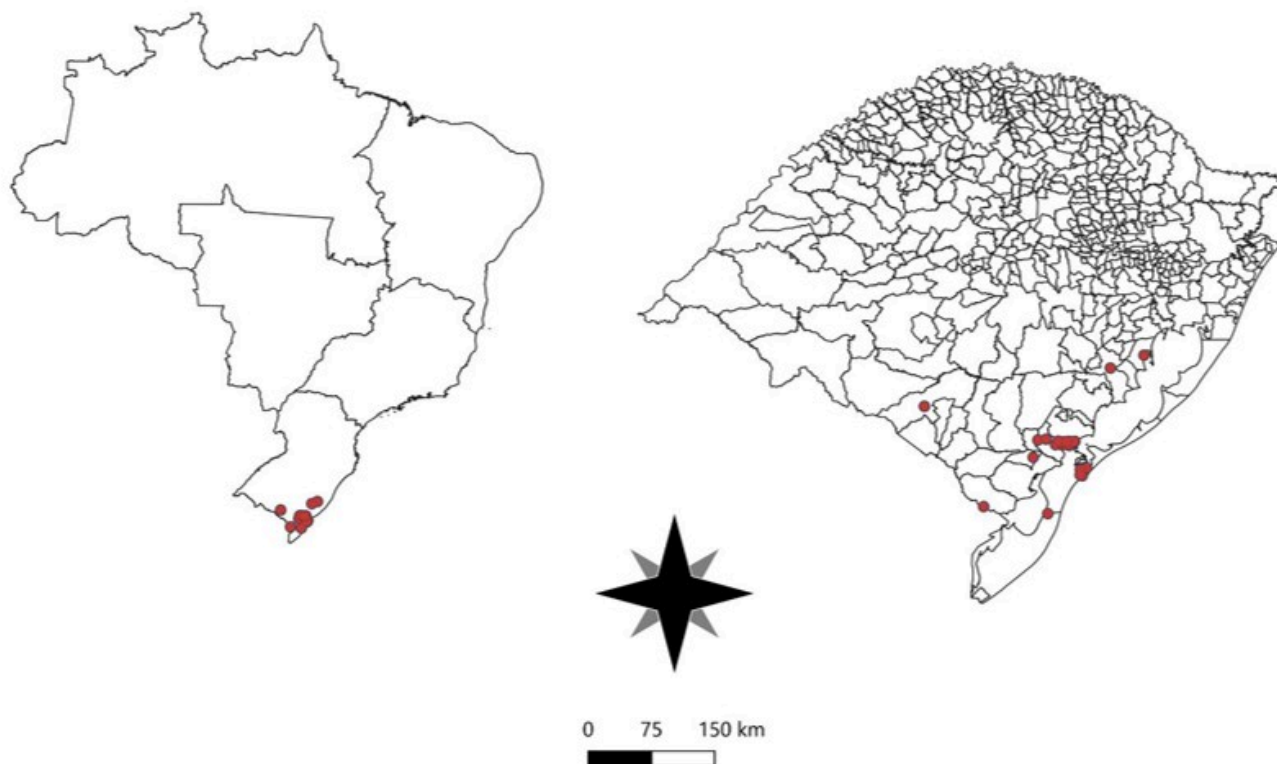


Figure 1

Wild birds' geographic origin in Rio Grande do Sul State, southern Brazil.

Note: Red dot indicates a wild bird collected for the research.

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