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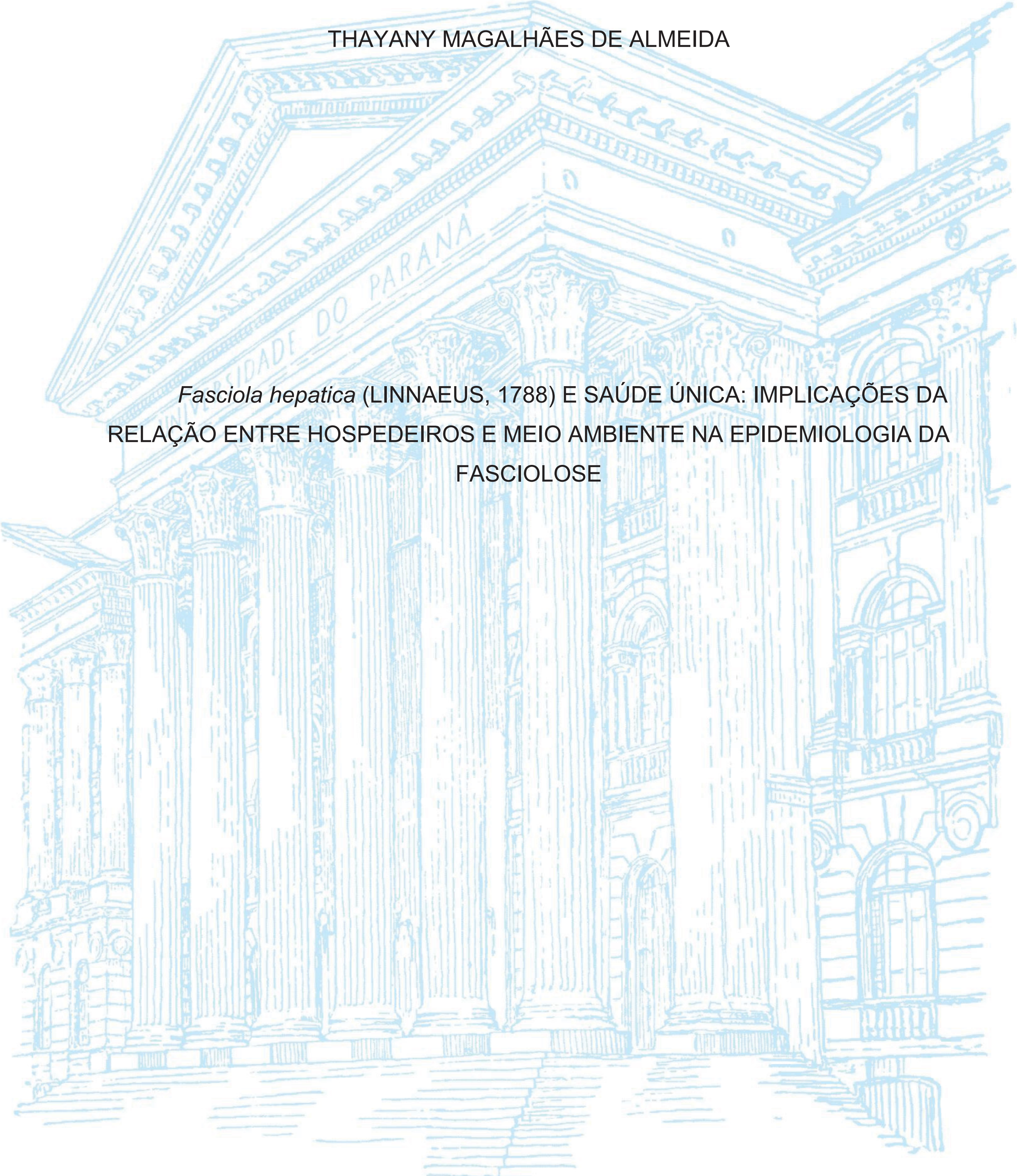
THAYANY MAGALHÃES DE ALMEIDA



Fasciola hepatica (LINNAEUS, 1788) E SAÚDE ÚNICA: IMPLICAÇÕES DA RELAÇÃO ENTRE HOSPEDEIROS E MEIO AMBIENTE NA EPIDEMIOLOGIA DA FASCIOLOSE

CURITIBA
2025

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Tese apresentada ao Programa de Pós-Graduação em Microbiologia, Patologia e Parasitologia, Setor de Ciências Biológicas da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Microbiologia, Parasitologia e Patologia.

Orientador: Prof. Dr. Marcelo Beltrão Molento

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação MICROBIOLOGIA, PARASITOLOGIA E PATOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **THAYANY MAGALHÃES DE ALMEIDA**, intitulada: ***Fasciola hepatica*** **(LINNAEUS, 1788) E SAÚDE ÚNICA: IMPLICAÇÕES DA RELAÇÃO ENTRE HOSPEDEIROS E MEIO AMBIENTE NA EPIDEMIOLOGIA DA FASCIOLOSE**, sob orientação do Prof. Dr. MARCELO BELTRÃO MOLENTO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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“Tudo está ligado... o que acontece com a Terra, acontece com os filhos da Terra”. (Chefe Seattle — tradução livre)

RESUMO

Fasciola hepatica, um trematódeo de importância médica e veterinária, é o agente causador da fasciolose e representa uma preocupação no contexto da Saúde Única. No Brasil, a infecção em humanos é frequentemente subdiagnosticada e subnotificada, enquanto, em ruminantes — especialmente bovinos e ovinos — apresenta alta endemicidade, com destaque para as regiões Sudeste e Sul. Considerando a expressiva produção pecuária nacional, este estudo teve como objetivo mapear a distribuição atual da fasciolose e identificar fatores associados à sua transmissão, com ênfase nas mudanças no uso da terra e na ocorrência e distribuição dos hospedeiros intermediários na América do Sul. Foram analisados dados de uso e cobertura do solo (MAPBIOMAS) e registros de inspeção de *F. hepatica* em fígados bovinos oriundos de abatedouros sob fiscalização do SIF/MAPA entre 2002 e 2020. Informações sobre a ocorrência de *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis* e *Galba truncatula* foram obtidas por meio de revisão bibliográfica e da Coleção Malacológica do Instituto Oswaldo Cruz (IOC). A modelagem de nicho ecológico, baseada em algoritmos de aprendizado de máquina, foi utilizada para estimar áreas de potencial distribuição dessas espécies. Mais de dois milhões de fígados bovinos apresentaram infecção por *F. hepatica*. Na região Norte, observou-se um aumento expressivo da área de pastagens ($p = 0,000001$), acompanhado por redução proporcional da cobertura florestal. As regiões Centro-Oeste e Norte concentraram o maior número de bovinos abatidos (> 290 milhões), sendo que o número de municípios com registros de fígados infectados passou de 194, em 2002, para 747, em 2020. Esses resultados sugerem que a expansão da fasciolose pode estar relacionada às alterações no uso da terra e ao intenso transporte de gado contaminado. Na modelagem, *P. columella* e *G. cubensis* apresentaram ampla adequabilidade ambiental, principalmente influenciada pela precipitação, enquanto *G. schirazensis* e *G. truncatula*, mais sensíveis à temperatura, mostraram distribuição restrita a áreas de maior altitude. Observou-se sobreposição de nicho entre *P. columella* e *G. cubensis* (0,46), e entre *G. schirazensis* e *G. truncatula* (0,56), com áreas ambientalmente favoráveis identificadas na Argentina, Peru, Bolívia e Brasil. No território brasileiro, *P. columella* apresentou alta adequabilidade nos biomas Cerrado e Amazônia, regiões impactadas pelo desmatamento e pela conversão de áreas nativas em pastagens. *Galba cubensis* também demonstrou grande potencial como hospedeiro intermediário no país, com ampla distribuição prevista. Diante desse cenário, torna-se evidente a expansão de *F. hepatica* por meio de seus hospedeiros intermediários e definitivos, ressaltando a necessidade de revisão dos mecanismos de fiscalização sanitária relacionados ao trânsito de bovinos. O controle da movimentação de gado pode subsidiar estratégias para restringir a ocupação desordenada de novas áreas. Ademais, medidas urgentes devem ser adotadas para conter a transmissão da fasciolose em regiões com populações vulneráveis e hospedeiros silvestres, sobretudo considerando a crescente produção bovina em áreas ilegais, como terras indígenas e unidades de conservação, o que eleva os riscos sanitários e ecológicos e favorece a introdução de novos patógenos em ambientes de elevada biodiversidade.

Palavras-chave: Fasciolose, Desmatamento, Produção pecuária, Lymnaeidae, Modelagem de nicho ecológico.

ABSTRACT

Fasciola hepatica, a trematode of medical and veterinary importance, is the causative agent of fascioliasis and represents a concern within the One Health framework. In Brazil, human infection is often underdiagnosed and underreported, while in ruminants—especially cattle and sheep—it shows high endemicity, particularly in the Southeast and South regions. Considering the country's extensive livestock production, this study aimed to map the current distribution of fascioliasis and identify factors associated with its transmission, with emphasis on land-use changes and the occurrence and distribution of intermediate hosts in South America. Land-use and land-cover data (MAPBIOMAS) and records of *F. hepatica* infection in cattle livers from slaughterhouses under SIF/MAPA inspection between 2002 and 2020 were analyzed. Occurrence records of *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula* were obtained through a literature review and from the Mollusk Collection of the Oswaldo Cruz Institute (IOC). Ecological niche modeling, using machine learning algorithms, was employed to estimate the potential distribution areas of these species. Over two million cattle livers were found to be infected with *F. hepatica*. In the Northern region, pasture areas increased significantly ($p = 0.000001$), accompanied by a proportional reduction in forest cover. The Central-West and Northern regions recorded the highest number of slaughtered cattle (> 290 million), with infected livers reported in 194 municipalities in 2002 and 747 in 2020. These findings suggest that the expansion of fascioliasis may be related to land-use changes and the intense movement of infected livestock. In the models, *P. columella* and *G. cubensis* showed broad environmental suitability, mainly influenced by precipitation, whereas *G. schirazensis* and *G. truncatula*, more sensitive to temperature, exhibited a more restricted distribution in higher altitude areas. Niche overlap was observed between *P. columella* and *G. cubensis* (0.46), and between *G. schirazensis* and *G. truncatula* (0.56), with environmentally suitable areas identified in Argentina, Peru, Bolivia, and Brazil. In Brazil, *P. columella* showed high suitability in the Cerrado and Amazon biomes, which are heavily affected by deforestation and pasture expansion. *Galba cubensis* also demonstrated strong potential as an intermediate host, with wide predicted distribution across the country. Given this scenario, the expansion of *F. hepatica* through its intermediate and definitive hosts becomes evident, highlighting the need to revise sanitary control mechanisms related to cattle transport. Monitoring livestock movement may support strategies to limit the unregulated occupation of new areas. Furthermore, urgent control measures must be implemented to prevent fascioliasis transmission in regions with vulnerable populations and wildlife hosts, especially in the context of increasing cattle production in illegal areas, such as Indigenous lands and conservation units, which raises both sanitary and ecological risks and favors the introduction of new pathogens in highly biodiverse environments.

Keywords: Fasciolosis, Forest deforestation, Cattle production, Lymnaeidae Ecological niche modeling.

Sumário

1. INTRODUCTION

Fasciola hepatica is a parasitic flatworm of the class Trematoda, belonging to the family Fasciolidae, which also includes two other recognized species: *Fasciola gigantica* and *Fasciola nyanzae* (Bargues et al., 2022). The latter was more recently described from infections in hippopotamus livers in Africa; however, to date, there is no evidence of its epidemiological relevance for human infections (Bargues et al., 2022). In contrast, *F. hepatica* and *F. gigantica* are etiological agents of medical and veterinary importance, with a recognized impact on both human health and animal production (Mas-Coma et al., 2009; Bargues et al., 2022).

The life cycle of *Fasciola* species involves freshwater gastropods from the family Lymnaeidae as intermediate hosts (Alda et al., 2021). Infection of snails occurs through the penetration of miracidia, larval forms released into the environment following egg development and hatching. Inside these hosts, the parasite undergoes asexual multiplication, giving rise to cercariae that are released into aquatic environments and encyst as metacercariae. This resistant form enables the parasite to survive under adverse environmental conditions until ingestion by a vertebrate host through contaminated water or food (Mas-Coma et al., 2009; Alba et al., 2020).

The dynamics of the life cycle are strongly influenced by environmental factors, including both landscape and climatic conditions (Alba et al., 2020). Pasture areas, temperature, and precipitation, for instance, have direct effects on the biology and ecology of snail hosts, as well as on the free-living stages of the parasite (Bennema et al., 2017; Galavani et al., 2024).

Ingestion of metacercariae can lead to the development of fasciolosis, whose clinical severity depends on multiple factors, such as the infective dose (mild or massive infection), the strain involved, and the host's immunological and nutritional status (Mas-Coma et al., 2009; Alba et al., 2020). It is a zoonosis with wide geographic distribution, with reports on all continents except Antarctica (Bargues et al., 2022). This broad distribution is strongly associated with the presence of vector snails and the parasite-host interaction. Species of the genus *Galba*, for example, are highly susceptible to infection by *F. hepatica*, which accounts for the highest global burden of infections, with records from the Americas, Europe, Africa, Asia, and Oceania (Vazquez et al., 2018; Bargues et al., 2022). In contrast, *F. gigantica* is restricted to Africa and Asia and is more commonly associated with *Radix* snails, whose distribution is also limited to those continents (Vazquez et al., 2018; Bargues et al., 2022).

In addition to the diversity of invertebrate hosts, several vertebrate species may serve as definitive hosts, including humans and wild animals (Mas-Coma et al., 2009; Alba et al., 2020). However, infections in domestic animals such as cattle, sheep, goats, and water buffalo are more frequently observed and studied. This is due to the economic impact caused by losses in meat and milk production, reinforcing the veterinary importance of the disease (Molento et al., 2020).

Human infections, in turn, remain largely underdiagnosed and underreported (Sabourin et al., 2018; Alba et al., 2020). Exceptions are observed in hyperendemic areas, such as the Bolivian Altiplano, where mass treatment campaigns are occasionally conducted to reduce disease prevalence and impact (Bargues et al., 2021). In Brazil, on the other hand, knowledge about human infections by *F. hepatica* is still limited and generally inferred from infection data in animals (Alba et al., 2020). Animals slaughtered in federally inspected slaughterhouses (SIF/MAPA) undergo liver evaluation, where the presence of the parasite or compatible lesions, such as bile duct fibrosis, may be recorded (MAPA, 2024).

Currently, the reemergence of fasciolosis has been observed in various regions of the world, driven by multiple factors including biological, environmental, social, scientific, and political aspects (Alba et al., 2020). In this context, recognizing fasciolosis as a One Health issue is fundamental. The disease should not be addressed solely in terms of livestock production losses but must also consider animal welfare and impacts on human health. Additionally, the expanding occurrence of *F. hepatica* has been associated with the growth of livestock production and deforestation, especially in environmentally sensitive areas (McMannus et al., 2016; Alba et al., 2020).

Considering this scenario, characterized by increasing deforestation and livestock expansion in already fragile biomes such as the Amazon, Cerrado, and Pantanal (McMannus et al., 2016; Alba et al., 2020), along with the intensification of cattle production over the years (MAPA, 2024; MapBiomas), the central objective of this thesis was to investigate the distribution of *Fasciola hepatica* in Brazil, in a context of intense land-use change, and to address fasciolosis as a One Health issue. Moreover, it aimed to identify areas potentially susceptible to human transmission and occurrence through the analysis of cattle infection and ecological niche modeling for four epidemiologically relevant vector snail species in South America. The snail species whose ecological niches were modeled in this work were *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula*.

2. CHAPTER 1 – GEOGRAPHIC EXPANSION OF *Fasciola hepatica* (LINNAEUS, 1758) DUE TO CHANGES IN LAND USE AND COVER IN BRAZIL

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ABSTRACT

Fasciolosis is caused by parasites of the genus *Fasciola,* affecting animals and humans worldwide. In South America, the disease is a result of infection with *Fasciola hepatica,* and although animal infections are more frequently reported, the full extent of the impact on human health due to underdiagnosis remains uncertain. This study analyzed changes in land use and the distribution of *F. hepatica* in bovine livers in Brazil over 18 years. Data on land use and land cover were collected from the Mapbiomas Project. Data on 414,481,963 slaughtered cattle and condemned livers due to *F. hepatica* infection were obtained from 4,433 municipalities. Joinpoint analysis was used to study the time series, and the Susceptible-Infected-Recovered (SIR) model was utilized to explore the behavior of *F. hepatica* infection. In the North, pasture areas significantly increased (P = 0.000001), while forested areas decreased (P = 0.000001). The midwestern and northern regions concentrated the highest number (> 290 million) of cattle slaughtered in Brazil. More than 2 million bovine livers were infected by *F. hepatica*. The infected cattle originated from 194 municipalities in 2002, increasing to 747 in 2020. We consider that the changes in land use and intense cattle transportation may have caused the expansion of *F. hepatica*. The SIR model analyzed the spread of the disease looking at all six biomes: Caatinga, Amazon Forest, Cerrado, Pantanal, Atlantic Forest, and Pampa. Moreover, this infection not only threatens the health of animals but is also a major concern to biodiversity and vulnerable human communities in South America. Emblematic biomes such as the Amazon basin already face challenges with logging, desertification, and loss of biodiversity. Therefore, strategies for mitigating infection should include controlling illegal pasture areas, establishing health inspections of animal transport, quarantine of newly arrived animals, and livestock zoning, as well as clear One Health policies.

Keywords: Deforestation; Zoonosis; Epidemiology; Fasciolosis; Cattle

2.1 INTRODUCTION

Fasciola hepatica is responsible for causing food-borne trematode infection worldwide. The infection affects domestic and wild animals and humans. In addition to *F. hepatica*, two other species are known, *Fasciola gigantica* and *Fasciola nyanzae*

(Alba et al., 2020; Bargues et al., 2022), but only *F. hepatica* and *F. gigantica* have been associated with infections in humans (Mas-Coma et al., 2009; Sabourin et al., 2018; Lalor et al., 2021). The parasite affects the liver and uses freshwater mollusks of the Lymnaeidae family (*Radix* in Africa and Asia and *Galba/Fossaria* in Europe, Asia, Africa, the Americas, and Oceania) as intermediate hosts (IH) (Bargues et al., 2022). The main species of mollusk known in the transmission cycle of *F. hepatica* in Brazil is *Pseudosuccinea columella* (Say, 1817). This IH is distributed in the southern and southeastern regions of Brazil, with few occurrences in other regions (Medeiros et al., 2014).

Infection by *Fasciola* spp. can cause chronic lesions in the biliary tract, depending on the number of ingested eggs and the host's immune and nutritional status (Mas-Coma et al., 2009). However, clinical signs are common in other diseases, making diagnosis challenging, especially in humans. Apathy, abdominal pain, anemia, and severe weight loss can be observed (Bloemhoff et al., 2015; Lalor et al., 2021). The diversity of clinical signs and different onset times can interfere with the estimation of disease morbidity and mortality (Alba et al., 2020). An estimated 180 million people are at risk of infection, with a prevalence of 30 to 70 million cases of *Fasciola* spp. infection worldwide (Sabourin et al., 2018). Animal infection is highly prevalent in over 600 million cattle (Toet et al., 2014). The infection leads to global losses of US $3.2 billion per year due to reduced weight gain and milk production (Alba et al., 2020). Annual losses in Brazil are estimated at US$ 210 million (Molento et al., 2020).

Considered a Neglected Tropical Disease (NTD) (Rojas et al., 2010; Mas-Coma et al., 2019; Alba et al., 2020), fasciolosis has ceased to be a secondary concern and has become a parasitic disease of notable importance, gaining prominence on the World Health Organization's agenda as a priority disease to be eradicated or controlled (WHO, 2013). However, controlling the disease is extremely challenging due to its complex epidemiology, which can be influenced by environmental factors (climate and land use), social factors (low-income countries), and biological factors (ecological invasion by species such as cattle) (Mas-Coma et al., 2019; Alba et al., 2020).

Changes in land use practices exert an important influence on the distribution and prevalence of the disease by favoring increased opportunities for interaction between the parasite and its hosts. Large areas of pastureland designated for livestock farming may include bodies of water, large-scale drainage systems, ditches, and irrigation systems, often used to meet the demands of beef production (Robinson et al., 2011). This creates favorable environments for the development of the intermediate host (IH) close to the definitive host (DH), resulting in an increased probability of infection when the parasite is present (Alba et al., 2020).

In Brazil, land use and land cover are heavily influenced by livestock production, resulting in the creation of extensive pasture areas, primarily driven by the movement of cattle from the southern and southeastern regions to the northern part of the country. McMannus et al. (2016) reported an increase of up to 40% in the cattle population in the north, a movement led by humans. Deforestation for the establishment of pasture and agricultural production areas has been a consequence of this expansion, encroaching upon fragile biomes (Skidmore et al., 2021). Another consequence, besides deforestation, is the sanitary risks associated with this movement of cattle without authorization, inspection at interstate borders, and the lack of rigorous veterinary sanitary protocols. Control measures contribute to increasing animal safety and preventing the spread of diseases (e.g., tuberculosis and foot-and-mouth disease). Unfortunately, little to no inspection of neglected diseases is being carried out in Brazil or other regions. In this context, infected animals can be moved to areas with no disease records (Machado et al., 2019; Dadar et al., 2022). In Australia, the state of Western Australia is a region that is free from *F. hepatica* infection, maintained through compulsory fasciolicide treatment of all animals entering the state (Palmer et al., 2014).

The movement of cattle without inspection to new pasture areas produces, in addition to impacts on environmental and animal health, impacts on human health. Human fasciolosis is generally associated with areas of animal infection (Mas-Coma et al., 1999; Esteban et al., 2002; Ashrafi et al., 2015). The occurrence of *F. hepatica* in humans in the Brazilian Amazon (Maciel et al., 2018) indicates the active spread of the parasite to northern Brazil. The exploitation of land in the southern Amazon region, including protected areas and indigenous lands, for cattle production (Garcia et al., 2017) can result in underdiagnosis due to limited medical access and scarce laboratory facilities in remote areas (M.B. Molento, personal communication), hiding the risk of spreading the disease (Ekwem et al., 2021). The objective of this study was to examine the impacts of modification in land use and cover practices over 18 years and their implications for the temporal changes in the distribution of *F. hepatica* in Brazil.

2.2 MATERIAL AND METHODS

2.2.1 Study area

Brazil occupies more than 851 million hectares (ha) divided into five geopolitical regions (Fig. 1A). The country has six inland biomes: the Amazon Forest, Cerrado, Caatinga, Pampa, Pantanal, and Atlantic Forest (Fig. 1B) (IBGE - https://www.ibge.gov.br/geociencias/informacoes-ambientais/vegetacao/15842-biomas.html).

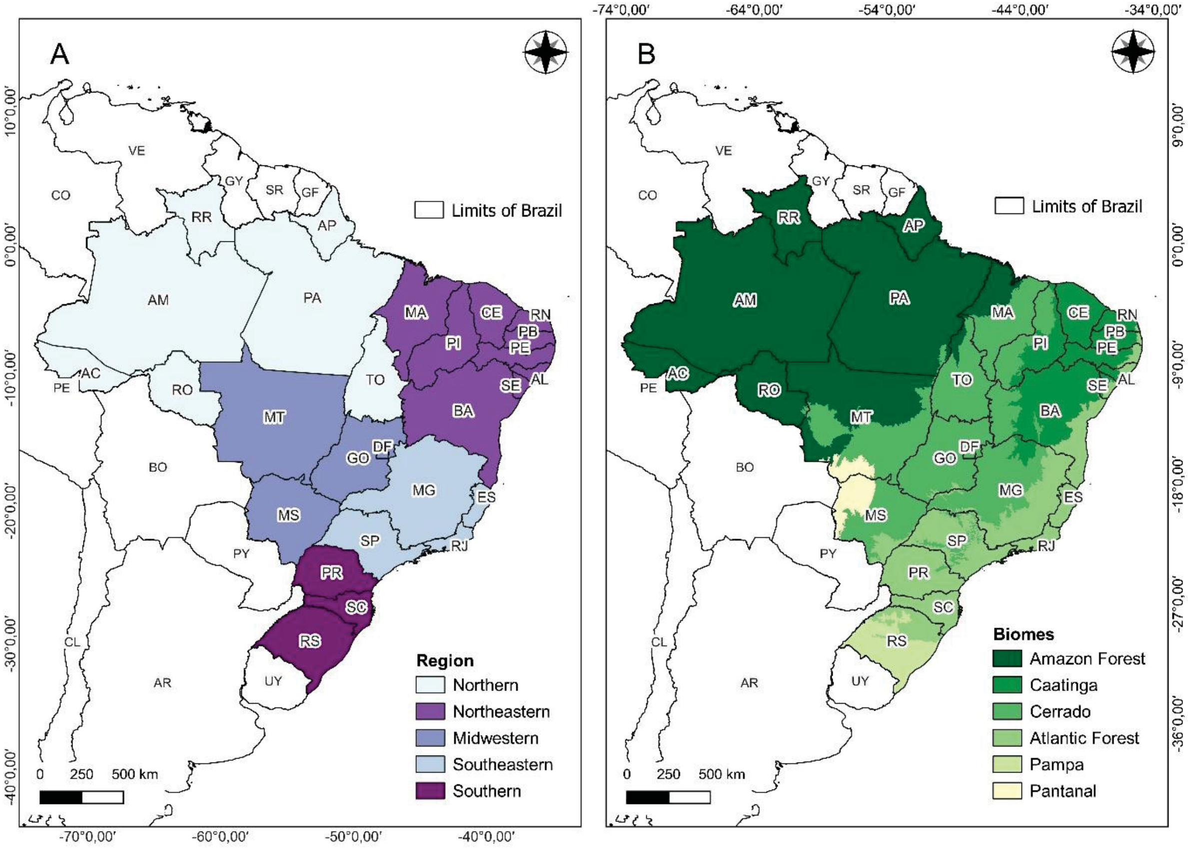

Figure 1. Locations of geographic regions (A) and biomes (B) in Brazil. AC, Acre; AL, Alagoas; AP, Amapá; AM, Amazonas; BA, Bahia; CE, Ceará; DF, Distrito Federal; ES, Espírito Santo; GO, Goiás; MA, Maranhão; MT, Mato Grosso; MS, Mato Grosso do Sul; MG, Minas Gerais; PA, Pará; PB, Paraíba; PR, Paraná; PE, Pernambuco; PI, Piauí; RJ, Rio de Janeiro; RN, Rio Grande do Norte; RS, Rio Grande do Sul; RO, Rondônia; RR, Roraima; SC, Santa Catarina; SP, São Paulo; SE, Sergipe; TO, Tocantins.

2.2.2 Land use and coverage data

The land use and land cover data were obtained from 2002 to 2020 from Collection 7.1 (2022) of the MapBiomas Project, Brazil (https://plataforma.brasil.mapbiomas.org/), with a spatial resolution of 30 m. The download of the images and the area statistics (in ha) for the selected geometries and periods were obtained through Toolkits, prepared in the Google Earth Engine (GEE) (https://earthengine.google.com/) as indicated by the platform itself. Landscape statistics were obtained for forested areas, pastures for livestock activities, and crop areas in the five geographical regions.

2.2.3 Data on slaughtered and infected cattle

Data on the number of slaughtered cattle and bovine livers infected by *F. hepatica* were obtained from publicly available data from the Federal Meat Inspection Service of the Ministry of Agriculture, Livestock, and Supply (SIF/Mapa - https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-animal/sif), Brazil. Data collection from 2002 to 2020, with 2002 set as the lower limit due to the unavailability of information for earlier years, and 2020 set as the upper limit because information was only available before that date at the time the data were collected. Bovine livers are inspected according to the guidelines that standardize the techniques, facilities, and equipment necessary for the evaluation of meat quality (Mapa, 2017 - https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-animal/empresario/arquivos/copy2_of_TOMOdebovinosemPDF.pdf,). This inspection includes visual evaluation and palpation of the livers and, when the presence of the parasite is identified, the organ is condemned and discarded.

The potential absence of information on a specific municipality does not indicate the absence of *F. hepatica* (infected bovines) but rather the lack of available data provided by SIF/Mapa. Information about meat inspection was used to correlate municipal geocodes, names of municipalities, state, number of cattle slaughtered in each municipality, and number of bovine livers infected with *F. hepatica*. The following equation was used to estimate the annual prevalence (%) of infected cattle from the total number of slaughtered cattle by municipality (Cauaneque et al., 2022):

Prevalence = absolute number of infected cattle / absolute number of slaughtered cattle x 100

2.2.4 Susceptible-Infected-Recovered (SIR) epidemiological model

The Susceptible-Infected-Recovered (SIR) model was used to demonstrate how biomes could influence the level of infectivity of *F. hepatica* (Table 1). Four conditions were simulated, considering the type of vegetation, climate, and livestock practices associated with land use in each biome (Souza Jr. et al., 2020). The first condition (A) represents the Caatinga biome. Condition B represents the Amazon, condition C brings together the Cerrado and Pantanal biomes, and condition D represents the Atlantic Forest and Pampas. The grouping of biomes in the same condition considered their environmental characteristics and their proximity in geopolitical divisions.

In addition to defining the conditions to be simulated, some model parameters were established, such as the infection rate, recovery rate, and the number of initially infected animals. Differences in the prevalence of condemned bovine livers identified in this study and reports from Bennema et al. (2014) in different regions served as the basis for determining the parameter values. Other parameters of the SIR model were incorporated. The interaction level of 0.001 was defined for all four conditions (fixed values). The infection level ranged from 0.15 (low) to 0.35 (high), and the recovery level varied from 0.15 (high) to 0.02 (low). Initial assumptions considered time to be equal to zero plus one (t = 0 + 1) with 1000 animals. The following equation was used for the SIR model:

$$\text{SIR} = \frac{\Delta S}{\Delta t} = -aSI + uI$$

where S is the number of susceptible individuals, a is a constant for the level of disease contact (dispersal/host-parasite interaction), I is the number of infected individuals, t is time in days, the minus sign assumes that the number of healthy individuals will decrease, u is a parameter representing the rate of loss of infection in infected animals and is proportional to the inverse of the life expectancy of the parasite, and uI represents the increase in susceptible individuals caused by the loss of parasites due to death by treatment in infected animals. We assumed that recovered cattle could become reinfected; however, the recovery rate would depend on the simulated condition.

Table 1. Description of characteristics of land use and cover and climatic zones of six biomes in Brazil used as representative environmental conditions in the construction of four Susceptible-Infected-Recovered (SIR) epidemiological models for *Fasciola hepatica* in cattle.

Model	Biome	Land cover	Land use	Climate	Infection rate	Recovery rate	Number of cattle with initial infections
A	Caatinga	Woody and deciduous forests Almost 50% of the original has been converted	Livestock, crop, non-timber forestry and urbanization	(B)^a, Bs.	0.15	0.15	25
B	Amazon	Dense upland forests, seasonal forests, igapó forests, flooded fields, floodplains and savannas Almost 20% of the original has been converted	Livestock, crop, logging and non-timber forestry	(A)^a, Af, Am and Aw.	0.25	0.10	50
C	Cerrado	Mosaic between extensive formations of savannas and forest fields Almost 50% of the original has been converted	Livestock, crop and logging for charcoal production	(A)^a, Af, Am, Aw, As, (C) Cf and Cw.	0.30	0.07	100
	Pantanal	Savanna, grassland and wetland	Livestock and crop	(A)^a, Af, Am and Aw.			
D	Atlantic Forest	Isolated forest fragments, remnants of secondary growth, surrounded by areas of agricultural cultivation, pastures, forest plantations, urban areas and infrastructure	Livestock, crop, urbanization and forest plantation	(A), Af, Am, Aw, As, (C)^a, Cf and Cw.	0.35	0.02	150
	Pampa	Natural fields with scattered bushes, trees and rocky outcrops	Livestock (native pasture), crop, forestry and urbanization	(C)^a, Cf.			

[a] The largest territorial extension in the climatic zone.

(A), tropical zone; Af, without dry season; Am, monsoon; Aw, with dry winter; As, with dry summer; (B), dry zone; Bs, semi-arid zone; (C), humid subtropical zone; Cf, oceanic climate, without dry season; Cw, with dry winter.

2.2.5 Analysis of georeferenced data

Data from the Mapbiomas project and SIF were georeferenced using the Sirgas 2000 Coordinate System as a reference and open-source QGIS v. 3.28 (Common Gateway Interface, http://qgis.osgeo.org). Thematic maps were subsequently generated to represent the information during the study period. These maps were developed using the continuous cartographic base of municipalities in Brazil at a scale of 1:250,000.

The Jenks classification, also known as "natural breaks" or "break optimization", was used to generate the classes of slaughtered animals represented in the legends of the figures. The Jenks classification is a statistical method used to separate data into classes or groups, minimizing variation within each class and maximizing variation between classes (Chen et al., 2013). The Jenks classification was not applied to label or separate groups based on the number of infected cattle as some municipalities had a value of 0. We, therefore, defined class intervals to cover a minimum of one case (1-10) to many cases (>500).

2.2.6 Statistical analysis of time series

Spreadsheets containing data on land use and coverage, *F. hepatica* prevalence, and the number of municipalities with records of infected livers were loaded into the Joinpoint Regression Program v. 5.0.2 (https://surveillance.cancer.gov/joinpoint/) for modeling and trend analysis. Trends were analyzed, considering the prevalence of livers infected by *F. hepatica* within the number of slaughtered cattle, the percentage of positive municipalities in the number of municipalities with slaughtered cattle, and the ratio of pasture and forest areas to the total area.

The annual percentage change (APC) and the average annual percentage change (AAPC) were calculated for each epidemic indicator (Clegg et al., 2009) using the equation:

$$APC / AAPC = (e^{\beta} - 1) \times 100$$

where β is the regression coefficient, and e is the error. A t-test was used to identify significant APCs. No Joinpoint was indicated if APC = AAPC, which could suggest a monotonic increasing or decreasing trend in the data. $P < 0.05$ was considered statistically significant.

2.2.7 Data accessibility

The raw data obtained in this study has been made available in Excel spreadsheet format and published in the Mendeley Data repository (https://data.mendeley.com/preview/ngkk8dkz9t?a=07a3330f-598d-46ae-9ba9-9358a98f9d3c). Access to the data can be obtained through the DOI: 10.17632/ngkk8dkz9t.2.

2.3 RESULTS

2.3.1 Changes in land use and cover

In the time series analysis, different trends were identified in the sizes of classified pasture areas (Table 2) intended for livestock farms and forestry areas (Supplementary Table S1) for each geographic region. Trends are reported for the entire period examined (2002 - 2020), together with distinct trends observed over narrower time frames, as identified through Joinpoint analysis.

Table 2. Variation in the size of areas in Brazil classified as pasture between 2002 and 2020.

Region	Period	APC	CI (95%)	Test statistic (*t*)	*P*	Trend
South	2002-2004	-2.45[a]	-3.86 to -1.01	-3.918	0.004429	Decreasing
	2004-2009	-4.75[a]	-5.23 to -4.27	-22.311	0.000000	Decreasing
	2009-2015	-0.25	-0.63 to 0.12	-1.524	0.165832	Stable
	2015-2020	-1.55[a]	-1.93 to -1.64	-9.189	0.000016	Decreasing
	2002-2020	-2.12[a]	-2.34 to -1.89	-18.203	0.000001	Decreasing
Southeast	2002-2006	-1.49[a]	-1.74 to -1.25	-14.0868	0.000001	Decreasing
	2006-2012	-2.05[a]	-2.23 to -1.86	-25.292	0.000000	Decreasing
	2012-2015	-1.19[a]	-2.07 to -0.29	-3.069	0.015354	Decreasing
	2015-2020	-1.65[a]	-1.86 to -1.44	-18.214	0.000000	Decreasing
	2002-2020	-1.67[a]	-1.82 to -1.52	-21.473	0.000001	Decreasing
Midwest	2002-2006	0.77[a]	0.37 to 1.18	4.484	0.002043	Increasing
	2006-2011	-0.36	-0.75 to 0.03	-2.125	0.066298	Stable
	2011-2014	-1.61[a]	-2.86 to -0.34	-2.930	0.018978	Decreasing
	2014-2020	-0.77	-0.99 to -0.55	-8.006	0.000043	Decreasing
	2002-2020	-0.45[a]	-0.68 to -0.23	-3.97	0.000070	Decreasing
North	2002-2005	5.19[a]	4.39 to 5.99	15.353	0.000000	Increasing
	2005-2008	2.58[a]	1.15 to 4.04	4.178	0.003087	Increasing
	2008-2013	0.47[a]	0.03 to 0.90	2.499	0.036956	Increasing
	2013-2020	1.06[a]	0.88 to 1.24	13.694	0.000001	Increasing
	2002-2020	1.82[a]	1.56 to 2.08	13.883	0.000001	Increasing
Northeast	2002-2020	0.5569	-0.83 to 1.96	0.842	0.410996	Stable

[a] significant APC value; APC, annual percent change; CI, confidence interval.

For the southern region of the country, the results indicated a significant decrease (P = 0.000001) in areas classified as pasture throughout the period (2002-2020) (Table 2). Regarding forested areas, different trends over the 18 years were identified (Supplementary Table S1). Between 2002 and 2009, a significant increase (P = 0.000002) in forested areas occurred, followed by a stable period (2009 – 2014), and a significant reduction (P = 0.007585) between 2017 and 2020.

In the southeastern region, there was a significant reduction in pasture areas (P = 0.000001) between 2002 and 2020. Forested areas in this region remained stable from 2002 to 2014 but exhibited a significant increase (P = 0.000009) between 2014 and 2020 (Supplementary Table S1). In the midwestern region, both pasture areas (P = 0.000070) and forested areas (P < 0.000001) showed a significant decrease during the analyzed period (2002 - 2020), with a significant upward trend (P = 0.002043) in pasture areas between 2002 and 2006.

The areas classified as pasture for livestock activity and forested areas in the northern region exhibited different trends. In this region, pasture areas significantly increased (P = 0.000001), while forest areas decreased (P = 0.000001). This region had a pasture area of 28,441,858 ha in 2002, which had increased to 39,512,011 ha by 2020 (data not shown). Furthermore, in the time series analysis, pasture areas in the northeastern region showed stability (P = 0.410996), but there was a significant decrease in forested areas (P = 0.000001). The gradual decrease in pasture areas in several regions was due to the rapid transition from livestock production to crop production (Fig. 2).

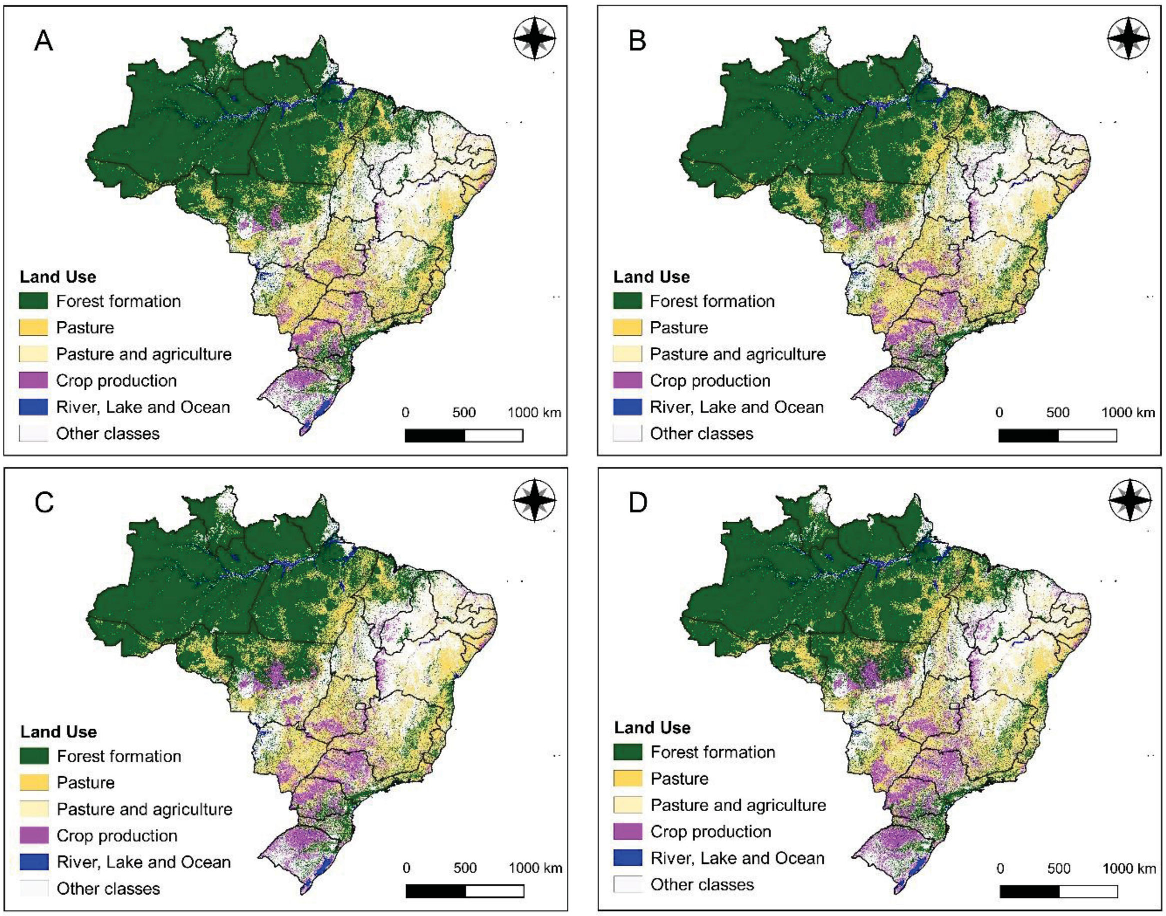

Figure 2. Spatial distribution of land use and cover (forest formation, pastures, and crop production) in Brazil during 2002 (A), 2003-2008 (B), 2009-2014 (C), and 2015-2020 (D).

2.3.2 Changes in the geographic distribution of cattle slaughter

The information regarding the number of cattle slaughtered in registered federal slaughterhouses was available for 4,433 out of the 5,570 (80%) Brazilian municipalities. The analysis of SIF/Mapa data revealed that 414,481,963 cattle were slaughtered between 2002 and 2020, with 26,105,237 animals slaughtered in 2013, the year with the highest number of slaughters. In 2002, the animals originated from 1,922 municipalities, however, as of 2003, more than 3,000 municipalities were registered as places of origin for these animals (Supplementary Table S2).

Considering the geographical regions of the country, the midwestern region had the highest number of registered bovine slaughters (200,645,331) (Table 3; Fig. 3). This number increased substantially over the period in the region to 2,070,633 slaughtered animals in 2002, and more than 10 million animals in 2020.

The northern region ranks second in bovine slaughters, with a total of 89,334,992 registered between 2002 and 2020. At the beginning of this period, 738,244 bovines were recorded in 2002, increasing to 2,114,457 the following year, reaching the peak in 2018 with 6,076,815 bovines slaughtered (Table 3). Furthermore, a substantial increase in the number of municipalities with recorded slaughters is observed in the region (Fig. 3A-D). The southeastern, southern, and northeastern regions, respectively, occupy the remaining positions in relation to the number of bovine slaughters.

Table 3. Annual description of the total number of slaughtered cattle in Brazil infected with *Fasciola hepatica* and the number of municipalities with records of infected livers by geographic region between 2002 and 2020.

Year	Slaughtered cattle					Infected livers (Positive municipalities)				
	North	Northeast	Midwest	South	Southeast	North	Northeast	Midwest	South	Southeast
2002	738,244	392,274	2,070,633	630,491	204,827	0	3 (1)	0 (0)	19,378 (193)	0 (0)
2003	2,114,457	660,504	8,601,985	1,679,891	1,441,770	0	0	3 (2)	74,274 (313)	49 (27)
2004	3,302,728	707,153	10,693,526	2,350,022	3,424,632	1 (1)	0	19 (15)	131,623 (380)	124 (53)
2005	3,844,581	754,587	11,786,504	2,578,539	3,629,518	0	0	44 (19)	152,979 (401)	92 (31)
2006	4,895,116	879,567	12,529,826	2,669,281	4,105,619	0	0	76 (10)	166,318 (377)	111 (27)
2007	5,472,425	989,450	12,046,197	1,906,842	4,731,753	0	0	199 (10)	86,025 (343)	376 (38)
2008	4,579,023	933,642	10,385,385	1,788,573	4,643,700	0	0	31 (18)	71,179 (349)	246 (46)
2009	4,635,620	776,741	10,414,755	1,713,852	4,066,287	0	0	125 (28)	71,379 (316)	147 (24)
2010	4,737,328	818,940	10,155,487	2,137,559	4,100,247	0	0	23 (9)	86,461 (339)	362 (30)
2011	4,883,477	797,348	10,596,072	1,882,406	3,657,268	0	0	178 (15)	102,331 (334)	150 (31)
2012	5,024,742	801,114	11,978,614	1,822,692	3,900,984	0	0	27 (14)	98,142 (308)	307 (30)
2013	5,590,453	913,672	13,034,671	1,947,537	4,618,904	3 (2)	0	43 (19)	89,492 (350)	260 (23)
2014	5,432,011	1,057,328	12,111,855	2,061,286	4,990,924	0	0	103 (39)	100,680 (387)	451 (34)
2015	5,393,583	966,890	10,418,172	1,783,732	4,381,991	0	0	88 (37)	85,670 (369)	577 (33)
2016	5,724,635	875,291	10,085,198	1,617,840	3,903,413	2 (1)	0	62 (28)	109,058 (357)	199 (10)
2017	5,765,916	766,275	10,643,722	1,730,833	4,268,676	2 (1)	0	37 (22)	139,676 (414)	149 (23)
2018	6,076,815	782,926	11,261,226	1,964,060	4,507,670	4 (2)	0	58 (27)	224,191 (505)	619 (77)
2019	5,859,310	727,316	11,459,708	1,754,699	4,737,334	3 (2)	4 (2)	59 (10)	182,367 (580)	1,585 (86)
2020	5,264,528	549,204	10,371,795	1,708,236	4,307,530	1 (1)	0	87 (22)	41,207 (483)	6,048 (241)
Total	89,334,992	15,150,222	200,645,331	35,728,371	73,623,047	16 (6)	7 (3)	1,262 (174)	2,032,430 (909)	11,852 (367)

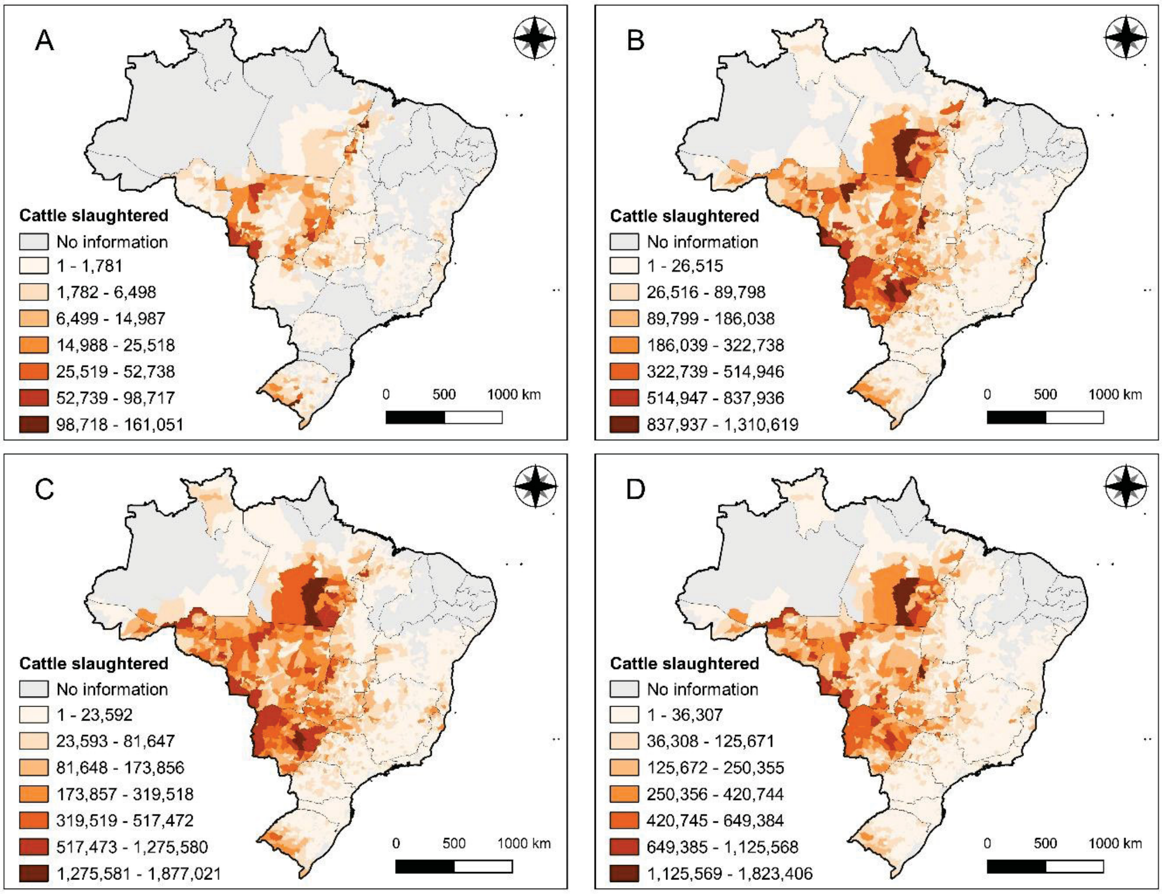

Figure 3. Spatiotemporal distribution of records of cattle slaughtered in Brazil between 2002 and 2020 by municipality. (A) 2002, (B) 2003-2008, (C) 2009-2014, and (D) 2015-2020.

2.3.3 Prevalence and changes in the distribution of *F. hepatica* in bovine livers

From 2002 to 2020, more than 2 million bovine livers were infected by *F. hepatica*. In 2002, the lowest number of infected livers was recorded (193,381; 0.48%), while in 2018, the highest number was observed (224,872; 0.91%). In terms of the prevalence of infected livers relative to the number of slaughters, 2020 saw the lowest prevalence in 18 years (Supplementary Table S2). Temporal analysis revealed that the prevalence of bovine livers infected by *F. hepatica* remained stable over the 18 years from 2002 to 2020. However, there was a significant decreasing trend (P = 0.019456) from 2002 to 2015, which then increased again in 2018 (Supplementary Table S3).

The southern region of the country stood out for the high number of livers infected by *F. hepatica*, totaling 2,032,430 cases. In 2018, the region recorded a total of 224,191 infected livers, but a decrease was observed in 2020 when this number decreased to 41,207 cases. In the southeastern region, a total of 11,582 infected livers were recorded. In 2020, there was a substantial increase in the number of bovine livers infected by *F. hepatica* in the region compared with previous years; 6,048 infected bovine livers were recorded (Table 3). In Supplementary Table S4, it is possible to consult in detail the prevalence in the states of the southern region and São Paulo (southeastern region). In the northern region of the country, bovine livers infected by *F. hepatica* were sporadic, with the first record in this time series in 2004 and then in 2013 (three cases). From 2016 to 2020, the region recorded cases of infected cattle every year, at the end of the period totaling 16 bovine livers infected by *F. hepatica* registered in SIF/Mapa. The midwestern region presented a total of 1,262 records of infected bovine livers. In the northeast, there were only seven cases (Table 3).

The temporal analysis identified an increase in the percentage of municipalities of origin of bovines with livers infected by *F. hepatica*. Temporal variations were observed in the period from 2002 to 2020 (Supplementary Table S3). A significant trend (P = 0.000203) of an increase in the percentage of these municipalities was observed between 2016 and 2020 (95% confidence interval of 9.99 - 22.36) (Supplementary Table S3; Fig. 4).

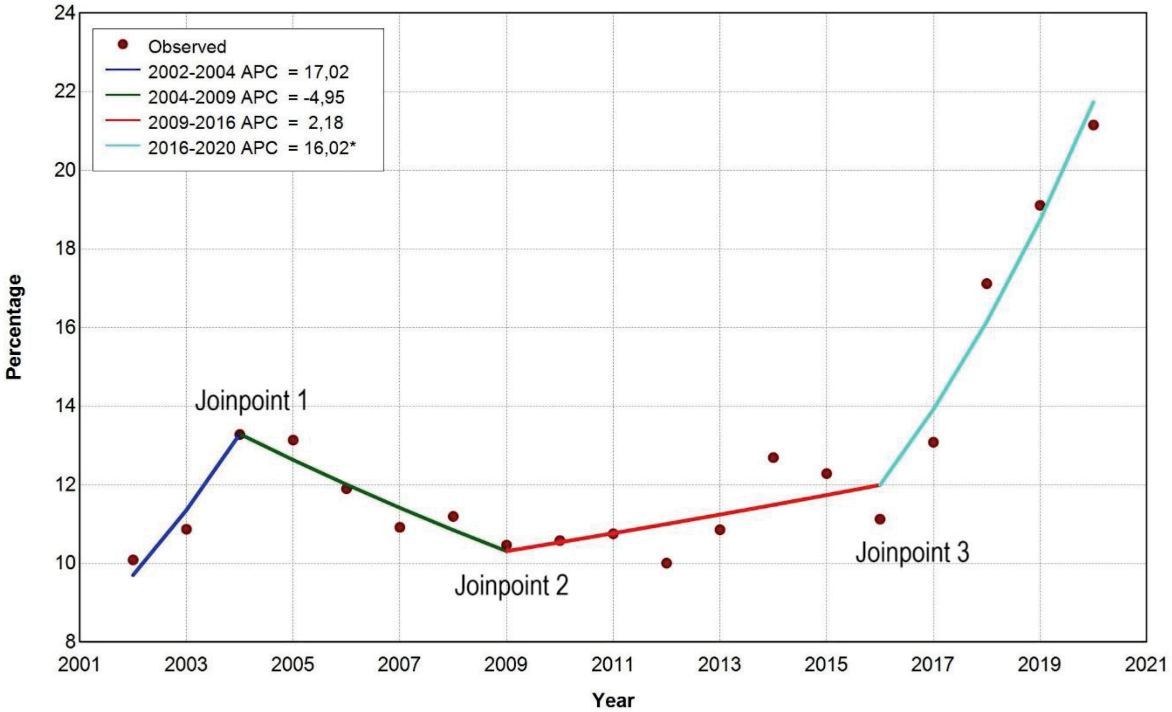

Figure 4. Percentage of municipalities in Brazil with records of *Fasciola hepatica* in bovine livers in relation to the total number of municipalities, with information on cattle slaughtered between 2002 and 2020.
* significant APC value.

The infected cattle originated from 194 municipalities in 2002 (10.09%; 194/1,922) (Fig. 4A), mainly in the southern region (state of Rio Grande do Sul), except for one municipality in the northeastern region (state of Paraíba) (Table 3; Fig. 5). In the year 2019, infected cattle were recorded in 680 municipalities (19.11%; 680/ 3,558), distributed across all regions. By 2020, the number of municipalities with infected cattle increased to 747 (21.16%; 747/3,531) (Figs. 4, 5). In total, *F. hepatica* was found in bovine livers from 1,459 municipalities throughout the analyzed period, spanning 15 states and all geographic regions.

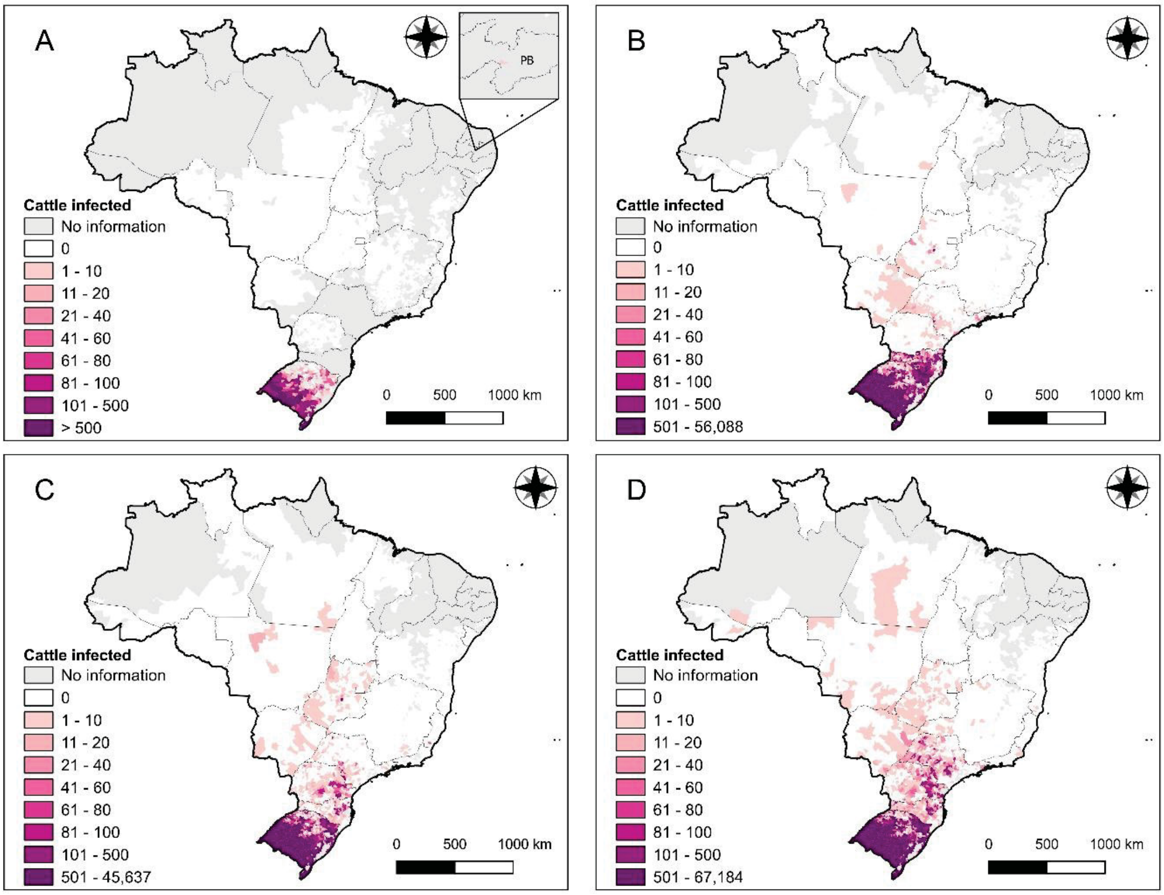

Figure 5. Spatiotemporal distribution of records of bovine livers infected with *Fasciola hepatica* in Brazil between 2002 and 2020 by municipality. (A) 2002; the municipality of Livramento, in Paraíba (PB), where a case of bovine with a liver infected by *Fasciola hepatica* was registered (2002), is enlarged in the top right corner. (B) 2003-2008, (C) 2009-2014 and (D) 2015-2020.

Records of *F. hepatica* in bovine livers expanded to the southern, southeastern, midwestern, and northern regions of the country (Fig. 5A - D). In 2003, infected cattle originated from 27 municipalities in the southeastern region. By 2020, this number had increased to 241 municipalities. In the midwestern region, only two municipalities were the source of infected cattle at the beginning of the period, and by 2020, this number had risen to 22 municipalities. Over 18 years, cattle infected by *F. hepatica* came from 147 municipalities in this region. According to SIF/MAPA data, the presence of *F. hepatica* in bovine livers in the northern region was first reported in 2004, in a single municipality in Pará. In 2013, another case of the disease was recorded in the same state. Starting from 2016, municipalities in Pará began to report cases of infected bovine livers. Furthermore, cases were identified in other municipalities in the northern region, one in Amazonas in 2018 and another in Acre in 2019. In total, the northern region had 16 cases of infected cattle in six municipalities.

In the northeastern region, in 2019, four cases of infected cattle were reported in Bahia, in addition to three cases recorded in Paraíba in 2002.

2.3.4 Modeling *F. hepatica* infection in cattle across Brazilian biomes

The SIR model determined scenarios of infection and recovery from *F. hepatica* in the six biomes, identifying the variations in infection rates and distinct patterns of disease expansion. Infection was low in the semi-arid climatic zones, as in the case of the Caatinga biome (Fig. 6A), with a limited spread of fasciolosis, however, it was better able to spread/adapt in tropical areas with hot and humid summers, as observed in the Amazon Forest (Fig. 6B) and to the Cerrado and Pantanal (Fig. 6C), indicated by higher infection rates. The model also demonstrated that disease prevalence was considerably higher, with low recovery in the oceanic and more temperate Atlantic Forest and Pampa biomes (Fig. 6D). We attributed this trend to the large presence of infected cattle and IH. These data provided valid insights into the dynamics of the disease under different ecological conditions, but the range of biological possibilities along the edges of these areas is very broad due to the transient meteorological conditions.

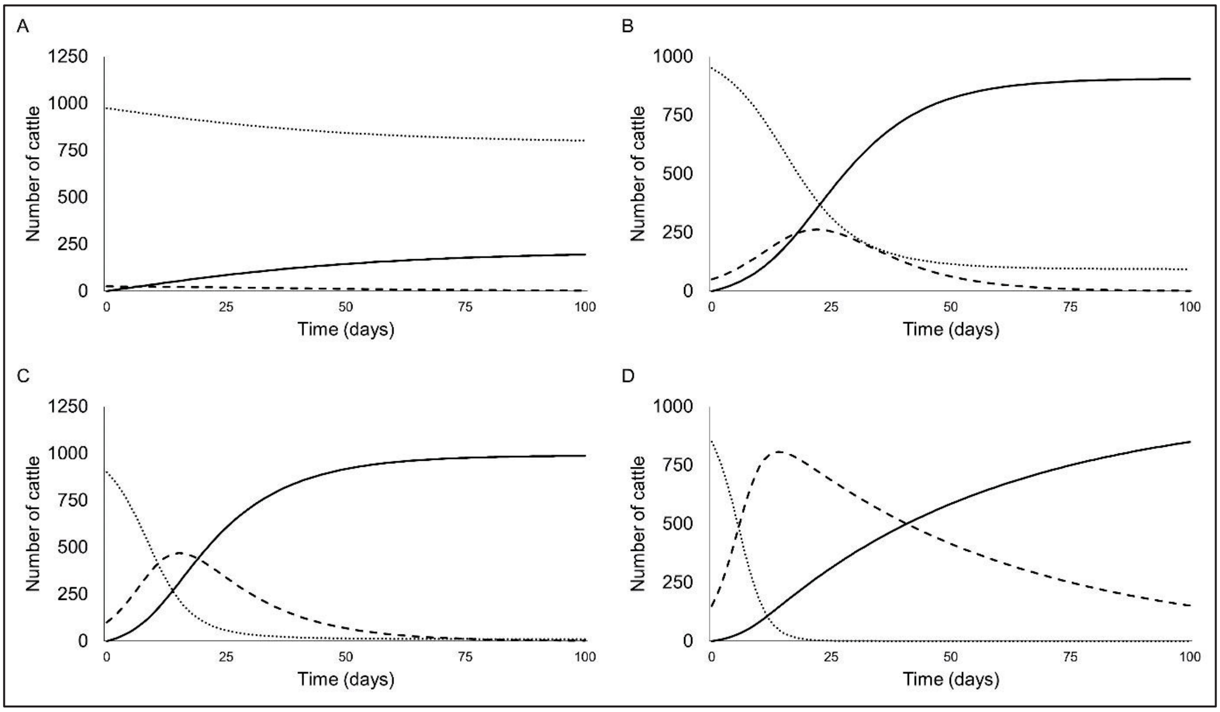

Figure 6. Representation of the Susceptible-Infected-Recovered (SIR) epidemiological model of bovine fasciolosis in biomes Caatinga (A), Amazon Forest (B), Cerrado and Pantanal (C), and Atlantic Forest and Pampa (D) in Brazil. The lines represent susceptible (solid gray), recovered (solid black), and infected (dashed black) animals.

2.4 DISCUSSION

This is the first known study that analyzes an 18-year historical series of land use and land cover changes, as well as changes in the distribution of *F. hepatica* in bovine livers from federal slaughterhouses in Brazil. Our results indicated that pasture areas and forest formations exhibited different trends across geographical regions. In the northern region of the country, there was an increase in pasture areas between 2002 and 2020, together with a reduction in areas classified as forest during the same period. In the midwestern region of the country, there was an increase in pasture areas for livestock activities between 2002 and 2006, but these areas decreased in the following years as many of them were converted into cultivable areas (i.e., soybean and corn).

Our results indicated an increase in the number of cattle slaughtered in the northern and midwestern regions. These findings indicate the vast introduction, dispersal, and displacement of beef cattle. França et al. (2021) demonstrated strong positive and significant associations between Brazilian livestock production and deforestation. The authors also comment that the majority of recent pasture expansion in Brazil has replaced Amazonian forests. This anthropogenic alteration of natural habitats can greatly affect the transmission of *F. hepatica*, as it creates favorable ecosystems, increasing the contact between mollusks and DH (Robinson et al., 2011; Grabner et al., 2014; Vázquez-Perera et al., 2015; Alba et al., 2020). Landscape changes due to livestock production have major impacts on ecosystems, especially on grasslands that contain bodies of water, drainage channels, ditches, and large-scale river systems (Alba et al., 2020).

Between 2002 and 2020, more than 2 million bovine livers (out of more than 400 million inspected) were infected with *F. hepatica*. The number of affected cattle was lowest in 2002 and highest in 2018. The southern and southeastern regions had a high prevalence of cattle infected by *F. hepatica*. The temperate climate of these regions and the characteristics of low altitude and high humidity play important roles in the occurrence of *F. hepatica* (Dutra et al., 2010). Paraense (1982) reported that *P. columella* was mainly distributed in the southern region. Medeiros et al. (2014) also observed that *P. columella* was widely distributed in the southern and southeastern regions, reporting only a few records in the states in the northern (Amazon Forest), midwestern (Pantanal/swamp areas), and northeastern (Cerrado/Savanna) regions/biomes.

The low numbers of cases of *F. hepatica* in bovine livers in the northeastern, northern, and midwestern regions can be attributed to the high temperatures frequently recorded in these areas. Although lymnaeids have a wide geographical distribution (Vázquez-Perera et al., 2018), local climatic factors influence their distribution (Medeiros et al., 2014; Alba et al., 2020). Furthermore, in stressful situations, such as trematode infections, the thermal threshold of mollusks is lower, reducing the populations of IH (Diaz-Morales et al., 2022). However, it is relevant to consider the presence of *P. columella* in Paraíba (northeastern region) (Medeiros et al., 2014) and in the municipalities of Manaus and Benjamin Constant, in the state of Amazonas, located in the northern region of the country (Paraense, 1983). Documented records of natural infection of *P. columella* by *F. hepatica* have emerged in Canutama, in the state of Amazonas (Maciel et al., 2018). We should also consider that other mollusks such as *Galba viatrix*, *Galba truncatula*, and *Galba cubensis* are suitable hosts for *F. hepatica* (Sanabria et al., 2012; Vázquez-Perera et al., 2015). All these mollusk species have been documented in Brazil, with a greater distribution in the southern and southeastern regions of the country (Medeiros et al., 2014).

Our results also indicated an increase in the number of municipalities of origin of cattle with livers infected by *F. hepatica*. In 2002, cattle infected with *F. hepatica* originated from 194 municipalities, mainly from the southern region, increasing to 747 municipalities in 2020. The parasite was present in the livers of cattle from 909 municipalities in the South, 367 in the Southeast, 174 in the Midwest, six in the North, and three in the Northeast. In this context, infection with *F. hepatica* may become a future concern in the northeastern, midwestern, and northern regions as, despite the low prevalence of livers infected by *F. hepatica*, municipalities in these regions report cases of infection. Land use and land cover have been influenced by the extensive movement of cattle from the southern and southeastern (endemic regions) to the midwestern and northern regions of Brazil (non-endemic regions) (McMannus et al., 2016). The expansion of the parasite's distribution areas involves a complex set of ecological interactions and adaptations, even for exotic organisms in new areas (Alba et al., 2020).

The increased prevalence and/or distribution of *F. hepatica* in cattle livers is of great concern for both animal and human health (Kelley et al., 2020). The following factors influence the epidemiology of the disease: (i) biological - the parasite's ability to survive, adapt and spread to different hosts (Spratt and Presidente, 1981; Rondelaud et al., 2001; Mas-Coma et al., 2009; Alba et al., 2020); (ii) environmental - degradation (pasture/livestock) and land changes (Robinson et al., 2011; Alba et al., 2016; Mehmood et al., 2017); and (iii) social - eating habits, culture and human migration (Macpherson et al., 2005; Mas-Coma et al., 2009, Alba et al., 2020). Its (re)emergence, however, is associated with multiple integrated and concomitant factors that affect disease transmission and expansion (Alba et al., 2020).

An important weakness identified by our study was the limitation of state border inspections in preventing the spread of the disease. A potential problem from the transport of animals is the spread of viruses, bacteria, or parasites from endemic areas to regions with little or no occurrence of these pathogens, including important anthropozoonotic parasites (Fèvre et al., 2006; Motta et al., 2017). Ekwem et al. (2021) reported a similar risk when transporting dairy cattle in eastern Africa. The association between the transport of cattle positive for *F. hepatica* and the geographic spread of fasciolosis in Brazil is based on important epidemiological elements. In general, Fasciola is a generalist parasite, so their survival would be compromised if their host tolerance were specific, as assumed by the Stockholm Paradigm (Brooks et al., 2022).

In Australia, the state of Western Australia is free from *F. hepatica* as farmers have a strict procedure required for the entry and movement of cattle from other states. Ranchers must adhere to importation conditions, including liver fluke testing at a Department of Primary Industries and Regional Development (DPIRD, Australia) - approved laboratory (Palmer et al., 2014; DPIRD, 2022 - https://www.agric.wa.gov.au/livestock-biosecurity/liver-fluke-testing-and-laboratory-requirements-western-australia). Another example is the spread of 'frontier malaria' to municipalities where the expansion of the agricultural frontier occurs, due to a combination of biological, environmental, and socioeconomic factors conducive to transmission (Souza et al., 2019).

A fundamental element in the structure of the livestock industry is the transport of animals between different areas, aiming to offer adequate grazing conditions and transport to slaughterhouses (Schirdewahn et al., 2020). This practice requires essential biosecurity approaches to mitigate the risks associated with cattle production and movement (Schirdewahn et al., 2020; Ekwem et al., 2021). The DPRID in Australia is dedicated to preventing the introduction of pests and diseases into the states and territories of the country. The application of quarantine measures for animals is considered one of the crucial biosecurity requirements in the context of the import and export of animals in this region. In the Brazilian context, there is a lack of effective inspection and control actions in the transport of cattle in relation to infection by *F. hepatica*.

The expansion of livestock into new areas, including illegal livestock (Skidmore et al., 2021), has important effects in addition to the direct consequences associated with the infection, such as high morbidity and mortality leading to large economic losses. The environmental impacts of the introduction of *F. hepatica* in fragile ecosystems such as the Amazon Forest, Pantanal, and Cerrado have not been fully understood. We need also to stress that the spread of pathogens to new areas is often associated with a major loss of biodiversity (Cardinale et al., 2012; Alba et al., 2020). The SIR model was useful for defining the impact of fasciolosis in different biomes and may be used to help design control programs after a regular diagnostic protocol is implemented. In situations where the infection rate is high, and treatment is inadequate, untreated animals shed eggs, contaminating the pastures. These eggs can remain viable, allowing the infection of other animals after the egg hatches in the environment, and the generation of infective metacercariae released by IH, amplifying the occurrence of the infection (Alba et al., 2020). Dutra et al. (2010), Aleixo et al. (2015), and Silva et al. (2020) determined different infection levels (low, medium, and high) of *F. hepatica* in different biomes and seasons, requiring monitoring throughout the year. Silva et al. (2016) reported that rainfall was strongly correlated with a higher occurrence of fasciolosis in southern Brazil and that La Niña weather patterns affected the positivity index of bovine fasciolosis by changes in air temperature and rainfall patterns.

Based on the risk factors that were observed from the present data, that influence the epidemiology of fasciolosis, we included a list of assumptions that could have contributed to the expansion of *F. hepatica* in the different biomes. (i) The lack of parasite control before the transport of cattle (Palmer et al, 2014); (ii) increased resistance to triclabendazole (Coyne et al., 2020); (iii) the wide distribution of Lymnaeidae mollusk species (Vázquez-Perera et al., 2018); (iv) the scarcity of studies on the morphological identification and the medical importance of mollusks, making it difficult to identify critical areas (Medeiros et al., 2014); (v) the presence of other susceptible hosts including humans, horses, capybaras, etc.; (vi) the high adaptability of the parasite to new environments and IHs (Alba et al., 2020); (vii) the abundance of water bodies that are accessible to animals (MapBiomas, 2022); (viii) the inadequate inspection for animal diseases at state borders (Tildesley et al., 2019, Ibeji et al., 2022); (ix) the non-reported cases of fasciolosis (Nguyen et al., 2011; Alba et al., 2020); and (x) the significant change in land use and cover in recent years.

In conclusion, the data from this work demonstrate that fasciolosis must be treated not only as a livestock health problem but from a One Health perspective. Data on land use and cover demonstrated a reduction in forest areas and an increase in pasture areas in regions where livestock have been recently introduced (midwestern and northern regions of Brazil). Moreover, we have associated changes in the landscape with the geographic distribution of *F. hepatica* during the last two decades, based on reports of *F. hepatica* in bovine livers (SIF/Mapa). This approach also highlighted the importance of state-level livestock inspections to control the spread of this parasite in diverse biomes and fragile ecological niches. Accurate diagnosis of the disease also plays a crucial role in identifying priorities for interventions to control *F. hepatica* in animals and in countless human communities around the world.

2.5 REFERENCES

Alba, A., Vázquez, A.A., Sánchez, J., Fraga, J., Martínez, E., Hernández, H., Marcet, R., Figueredo, M., Sarracent, J., 2016. Assessment of the FasciMol-ELISA in the detection of the trematode *Fasciola hepatica* in field-collected *Galba cubensis*: a novel tool for the malacological survey of fasciolosis transmission. Parasit. Vectors. 9(22). DOI: 10.1186/s13071-016-1303-1.

Alba, A., Vazquez, A.A., Hurtrez-Boussès, S., 2020. Towards the comprehension of fasciolosis (re-) emergence: an integrative overview. Parasitology. 148, 385-407. DOI: 10.1017/S0031182020002255.

Aleixo, M.A., Freitas, D.F., Dutra, L.H., Malone, J., Martins, I.V.F., Molento, M.B., 2015. *Fasciola hepatica*: epidemiology, perspectives in the diagnostic and the use of geoprocessing systems for prevalence studies. Semin. Cienc. Agrar. 36, 1451-66. DOI: 10.5433/1679-0359.2015v36n3p1451.

Ashrafi, K., Valero, M.A., Peixoto, R.V., Artigas, P., Panova, M., Mas-Coma, S., 2015. Distribution of *Fasciola hepatica* and *F. gigantica* in the endemic area of Guilan, Iran: relationships between zonal overlap and phenotypic traits. Infect. Genet. Evol. 31, 95-109. DOI: 10.1016/j.meegid.2015.01.009.

Bargues, M.D., Halajian, A., Artigas, P., Luus-Powell, W.J., Valero, M.A., Mas-Coma, S., 2022. Paleobiogeographical origins of *Fasciola hepatica* and *F. gigantica* in light of new DNA sequence characteristics of *F. nyanzae* from hippopotamus. Front. Vet. Sci. 9:990872. DOI: 10.3389/fvets.2022.990872.

Bennema, S.C., Scholte, R.G.C., Molento, M.B., Medeiros, C., Carvalho, O.S., 2014. *Fasciola hepatica* in bovines in Brazil: data availability and spatial distribution. Rev. Inst. Med. Trop. São Paulo. 56, 35-41. DOI: 10.1590/S0036-46652014000100005.

Bloemhoff, Y., Forbes, A., Danaher, M., Good, B., Morgan, E., Mulcahy, G., Sekiya, M., Sayers, R., 2015. Determining the prevalence and seasonality of *Fasciola hepatica* in pasture-based dairy herds in Ireland using a bulk tank milk ELISA. Ir. Vet. J. 68. DOI: 10.1186/s13620-015-0042-5.

Brooks, D., Boeger, W., Hoberg, E., 2022. The Stockholm paradigm: lessons for the emerging infectious disease crisis. MANTER: J Parasit Biodivers. 22. DOI: 10.32873/unl.dc.manter22.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature. 486, 59-67. DOI: 10.1038/nature11148.

Cauaneque, A.R.F., Azevedo, D.L., Costa, E.F., Borba, M.R., Corbellini, L.G., 2022. Epidemiological analyses of cattle carcasses affected by cysticercosis and hydatidosis in the State of Rio Grande do Sul from 2014 to 2018. Pesqui. Vet. Bras. 42, e06805. DOI: 10.1590/1678-5150-PVB-6805.

Chen, J., Yang, S.T., Li, H.W., Zhang, B., Lv, J.R., 2013. Research on geographical environment unit division based on the method of natural breaks (Jenks). Int. Arch. Photogramm. Remote. Sens. Spat. Inf. Sci. XL-4, 47-50. DOI: 10.5194/isprsarchives-XL-4-W3-47-2013.

Clegg, L.X., Hankey, B.F., Tiwari, R., Feuer, E.J., Edwards, B.K., 2009. Estimating average annual percent change in trend analysis. Stat. Med. 28, 3670-3682. DOI: 10.1002/sim.3733.

Coyne, L.A., Bellet, C., Latham, S.M., Williams, D., 2020. Providing information about triclabendazole resistance status influences farmers to change liver fluke control practices. Vet. Rec. 187, 357-357. DOI: 10.1136/vr.105890.

Dadar, M., Omar, S.S., Shahali, Y., Fakhri, Y., Godfroid, J., Khaneghah, A.M. 2022. The prevalence of camel brucellosis and associated risk factors: a global meta-epidemiological study. Qual. Assur. Saf. Crops. Foods. 14, 55-93. DOI: 10.15586/qas.v14i3.1088.

Díaz-Morales, D.M., Bommarito, C., Vajedsamiei, J., Grabner, D.S., Rilov, G., Wahl, M., Sures, B., 2022. Heat sensitivity of the first host and cercariae may restrict parasite transmission in a warming sea. Sci. Rep. 12(1), 1174. DOI: 10.1038/s41598-022-05139-5.

Dutra, L.H., Molento, M.B., Naumann, C.R.C., Biondo, A.W., Fortes, F.S., Savio, D., Malone, J.B., 2010. Mapping risk of bovine fasciolosis in the south of Brazil using geographic information systems. Vet. Parasitol. 169, 76-81. DOI: 10.1016/j.vetpar.2009.

Ekwem, D., Morrison, T.A., Reeve, R., Enrigth, J., Buza, J., Shirima, G., Mwajombe, K., Lembo, T., Hopcraft, K.G.C., 2021. Livestock movement informs the risk of disease spread in traditional production systems in East Africa. Sci. Rep. 11(1), 16375. DOI: 10.1038/s41598-021-95706-z.

Esteban, J.G., Gonzalez, C., Bargues, M.D., Angles, R., Sanchez, C., Naquira, C., Mas-Coma, S., 2002. High fascioliasis infection in children linked to a man-made irrigation zone in Peru. Trop. Med. Int. Health. 7, 339-48. DOI: 10.1046/j.1365-3156.2002.00870.x.

Fèvre, E.M., Bronscoort, B.M.C., Hamilton, K.A., Cleveland, S., 2006. Animal movements and the spread of infectious diseases. Trends Microbiol. 14, 125-31. DOI: 10.1016/j.tim.2006.01.004.

Garcia, E., Ramos-Filho, F.S.V., Mallmann, G.M., Fonseca, F., 2017. Costs, benefits and challenges of sustainable livestock intensification in a major deforestation frontier in the Brazilian Amazon. Sustainability. 9, 158. DOI: 10.3390/su9010158.

Grabner, D.S., Mohamed, F.A.M.M., Nachev, M., Méabed, E.M.H., Sabry, A.H.A., Sures, B., 2014. Invasion biology meets parasitology: a case study of parasite spill-back with Egyptian *Fasciola gigantica* in the invasive snail *Pseudosuccinea columella*. PLoS One. 9(2), e88537. DOI: 10.1371/journal.pone.0088537.

Ibeji, J.U., Mwambi, H., Iddrisu, A.K., 2022. Bayesian spatio-temporal modelling and mapping of malaria and anemia among children between 0 and 59 months in Nigeria. Malar. J. 21(311). DOI: 10.1186/s12936-022-04319-y.

Kelley, J.M., Rathinasamy, V., Elliott, T.P., Rawlin, G., Beddoe, T., Stevenson, M.A., Spithill, T. W., 2020. Determination of the prevalence and intensity of *Fasciola hepatica* infection in dairy cattle from six irrigation regions of Victoria, South-eastern Australia, further identifying significant triclabendazole resistance on three properties. Vet. Parasitol. 277, 109019. DOI: 10.1016/j.vetpar.2019.109019.

Lalor, R., Cwiklinski, K., Calvani, N.E.D., Dorey, A., Hamon, S., Corrales, J.L., Dalton, J.P., DeMarco-Verissimo, C., 2021. Pathogenicity and virulence of the liver flukes *Fasciola hepatica* and *Fasciola gigantica* that cause the zoonosis Fasciolosis. Virulence. 12, 2839-2867. DOI: 10.1080/21505594.2021.1996520.

Machado, G., Vilalta, C., Recamonde-Mendoza, M., Corzo, C., Torremorell, M., Perez, A., Vander-Waal, K., 2019. Identifying outbreaks of Porcine Epidemic Diarrhea virus through animal movements and spatial neighborhoods. Sci. Rep. 9(1), 457. DOI: 10.1038/s41598-018-36934-8.

Maciel, M.G., Lima, W.S., Almeida, F.L.M., Coelho, L.I.A.R.C., Araújo, G.A.N., Lima, M.G., Maciel, L.H.G., Pereira, C.A.J., Maciel, T.C.S., Guerra, J.A.O., Santana, R.A.G., Guerra, M.G.V.B., 2018. Cross-sectional serological survey of human fascioliasis in Catunama municipality in Western Amazon, Brazil. J. Parasitol. Res. 8, 6823638. DOI: 10.1155/2018/6823638.

Macpherson, C.N., 2005. Human behavior and the epidemiology of parasitic zoonoses. Int. J. Parasitol. 35, 1319-1331. DOI: 10.1016/j.ijpara.2005.06.004.

Mas-Coma, S., Anglés, R., Esteban, J.G., Bargues, M.D., Buchon, P., Franken, M., Strauss, W., 1999. The Northern Bolivian Altiplano: a region highly endemic for human fascioliasis. Trop. Med. Int. Health. 4, 454-467. DOI: 10.1590/S1984-29612021014.

Mas-Coma, M.S., Valero, M.A., Bargues, M.D., 2009. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology, and control. Adv. Parasitol. 69, 41-146. DOI: 10.1016/S0065-308X(09)69002-3.

Mas-Coma, S., Valero, M.A., Bargues, M.D., 2019. Fascioliasis. Adv. Exp. Med. Biol. 1154, 71-103. DOI: 10.1007/978-3-030-18616-6_4.

McMannus, C., Barcellos, J.O.J., Formenton, B.K., Hermuche, P.M., Carvalho, O.A., Guimarães, R., Gianezini, M., Dias, E.A., Lampert, V.N., Zago, D., Neto, J.B., 2016. Dynamics of cattle production in Brazil. PLoS One. 11, e0147138. DOI: 10.1371/journal.pone.0147138.

Medeiros, C., Scholte, R.G.C., D'ávila, S., Caldeira, R.L., Carvalho, O.S., 2014. Spatial distribution of Lymnaeidae (Mollusca, basommatophora), intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematoda, digenea) in Brazil. Rev. Inst. Med. Trop. São Paulo. 56, 235-252. DOI: 10.1590/S0036-46652014000300010.

Mehmood, K., Zhang, H., Sabir, A.J., Abbas, R.Z., Ijaz, M., Durrani, A.Z., Saleem, M.H., Ur Rehman, M., Iqba, I.M.K, Wang, Y., Ahmad, H.I., Abbas, T., Hussain, R., Ghori, M.T., Ali, S., Khan, A.U., Li, J., 2017. A review on epidemiology, global prevalence and economic losses of fasciolosis in ruminants. Microb. Pathog. 109, 253-262. DOI: 10.1016/j.micpath.2017.06.006.

Molento, M.B., Dutra, L.H., Pritsch, I.C., Garbin, V.P., Pereira, A.M., Gavião, A., Gabriel, A.L., De Sousa, R.S., Viana, J.G.A., 2020. *Fasciola hepatica* infection in cattle and the use of simulation models for endemic areas. J. Helminthol. 94, e185. DOI: 10.1017/S0022149X2000067X.

Motta, P., Porphyre, T., Handel, I., Hamman, S.M., Ngwa, V.N., Tanya, V., Morgan, K., Christeley, R., Bronsvoort, B.M.C., 2017. Implications of the cattle trade network in Cameroon for regional disease prevention and control. Sci. Rep. 7, 1-13. DOI: 10.1038/srep43932.

Nguyen, T.G.T., Le, T.H., Dao, T.H.T., Tran, T.L.H., Praet, N., Speybroeck, N., Vercruysse, J., Dorny, P., 2011. Bovine fasciolosis in the human fasciolosis hyperendemic Binh Dinh province in Central Vietnam. Acta Trop. 117, 19-22. DOI: 10.1016/j.actatropica.2010.09.003.

Palmer, D.G., Lyon, J., Palmer, M.A., Forshaw, D., 2014. Evaluation of a copro-antigen Elisa to detect *Fasciola hepatica* infection in sheep, cattle and horses. Aust. Vet. J. 92, 357-361. DOI: 10.1111/avj.12224.

Paraense, W.L., 1982. *Lymnaea rupestris* sp. n. from Southern Brazil (Pulmonata: Lymnaeidae). Mem. Inst. Oswaldo Cruz. 77, 437-443. DOI: 10.1590/S0074-02761982000400011.

Paraense, W.L., 1983. *Lymnaea columella* in northern Brazil. Mem. Inst. Oswaldo Cruz. 78, 477-482. DOI: 10.1590/s0074-02761983000400011.

Rondelaud, D., Vignoles, P., Abrous, M., Dreyfuss, G., 2001. The definitive and intermediate hosts of *Fasciola hepatica* in the natural watercress beds in central France. Parasitol. Res. 87, 475-478. DOI: 10.1007/s004360100385.

Rojas, L., Vazquez, A., Domenech, I., Robertson, L.J., 2010. Fascioliasis: can Cuba conquer this emerging parasitosis? Trends Parasitol. 26, 26-34. DOI: 10.1016/j.pt.2009.10.005.

Robinson, T.P., Thornton P.K., Franceschini, G., Kruska, R.L., Chiozza, F., Notenbaert, A., Cecchi, G., Herrero, M., Epprecht, M., Fritz, S., You, L., Conchedda, G., See, L., 2011. Global livestock production systems. In: Food and Agriculture Organization of the United Nations (FAO) and International Livestock Research Institute (ILRI). ISBN 978-92-5-107033-8 .

Sabourin, E., Alda, P., Vázquez, A., Hurtrez-Boussès, S., Vittecoq, M., 2018. Impact of human activities on fasciolosis transmission. Trends Parasitol. 34, 891-903. DOI: 10.1016/j.pt.2018.08.004.

Sanabria, R., Mouzet, R., Courtioux, B., Vignoles, P., Rondelaud, D., Dreyfuss, G., Cabaret, J., Romero, J., 2012. Intermediate snail hosts of French *Fasciola hepatica*: *Lymnaea neotropica* and *Lymnaea viatrix* are better hosts than local

Galba truncatula. Parasitol. Res. 111, 2011-2016. DOI: 10.1007/s00436-012-3049-9.

Schirdewahn, F., Lentz, H.H.K., Colizza, V., Koher, A., Hovel, P., Vidondo, B., 2021. Early warning of infectious disease outbreaks on cattle-transport networks. PLoS One. 16(1), e0244999. DOI: 10.1371/journal.pone.0244999.

Silva, A.E.P., Freitas, C.C., Dutra, L.V., Molento, M.B., 2016. Assessing the risk of bovine fasciolosis using linear regression analysis for the state of Rio Grande do Sul, Brazil. Vet. Parasitol. 217, 7-13. DOI: 10.1016/j.vetpar.2015.12.021.

Silva, A.E.P., Freitas, C.C., Dutra, L.V., Molento, M.B., 2020. Correlation between climate data and land altitude for *Fasciola hepatica* infection in cattle in Santa Catarina, Brazil. Rev. Bras. Parasitol. Vet. 29(3), e008520. DOI: 10.1590/s1984-29612020065.

Skidmore, M.E., Moffette, F., Rausch, L., Christie, M., Munger, J., Gibbs, H.K., 2021. Cattle ranchers and deforestation in the Brazilian Amazon: production, location, and policies. Glob. Environ. Change. 68, 102280. DOI: 10.1016/j.gloenvcha.2021.102280.

Souza, P.F., Xavier, D.R., Mutis, M.C.S., Mota, M.C., Peiter, P.C., Matos, V.P., Magalhães, M.A.F.M., Barcellos, C., 2019. Spatial spread of malaria and economic frontier expansion in the Brazilian Amazon. PLoS One. 14, e0217616. DOI: 10.1371/journal.pone.0217615.

Souza-Jr, C.M., Shimbo, J.Z., Rosa, M.R., Parente, L.L., Alencar, A.A., Rudorff, B.F.T., Hasenack, H., Matsumoto, M., Ferreira, L.G., Souza-Filho, P.W.M., de Oliveira, S.W., Rocha, W.F., Fonseca, A.V., Marques, C.B., Diniz, C.G., Costa, D., Monteiro, D., Rosa, E.R., Vélez-Martin, E., Weber, E.J., Lenti, F.E.B., Paternost, F.F., Pareyn, F.G.C., Siqueira, J.V., Viera, J.L., Neto, L.C.F., Saraiva, M.M., Sales, M.H., Salgado, M.P.G., Vasconcelos, R., Galano, S., Mesquita, V.V., Azevedo, T., 2020. Reconstructing three decades of land use and land cover changes in Brazilian biomes with Landsat archive and earth engine. Remote Sens. 12, 2735. DOI: 10.3390/rs12172735.

Spratt, D.M., Presidente, P.J., 1981. Prevalence of *Fasciola hepatica* infection in native mammals in southeastern Australia. Aust. J. Exp. Biol. Med. Sci. 59, 713-721. DOI: 10.1038/icb.1981.62.

Tildesley, M.J., Brand, S., Pollock, E.B., Bradbury, N.V., Werkman, M., Keeling, M.J., 2019. The role of movement restrictions in limiting the economic impact of livestock infections. Nat. Sustain. 2, 834-840. DOI: 10.1038/s41893-019-0356-5.

Toet, H., Piedrafita, D.M., Spithill, T.W., 2014. Liver fluke vaccines in ruminants: strategies, progress and future opportunities. Int. J. Parasitol. 44, 915-927. DOI: 10.1016/j.ijpara.2014.07.011.

Vázquez-Perera, A.A., Sánchez, J., Alba, A., Pointier, J.P., Hurtrez-Boussès, S., 2015. Natural prevalence in Cuban populations of the lymnaeid snail *Galba cubensis* infected with the liver fluke *Fasciola hepatica*: small values do matter. Parasitol. Res. 114, 4205-4210. DOI: 10.1007/s00436-015-4653-2.

Vázquez-Perera, A.A., Alda, P., Lounnas, M., Sabourin, E., Alba, A., Pointier, J.P., Hurtrez-Bòusses, S., 2018. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: digenea): a worldwide review. CABI Reviews. 13, 1-15. DOI: 10.1079/PAVSNNR201813062.

World Health Organization. 2013. Sustaining the drive to overcome the global impact of neglected tropical diseases: second WHO report on neglected tropical diseases. WHO Technical Report Series. ISBN 978 92 4 156454 0.

2.6 SUPPLEMENTARY MATERIAL

Table S1. Variation in the size of areas in Brazil classified as forest between 2002 and 2020.

Region	Period	APC	CI (95%)	Test statistic (*t*)	*P*	Trend
South	2002-2009	0.26[a]	0.21 to 0.31	12.295	0.000002	Increasing
	2009-2014	0.09	-0.02 to 0.21	1.903	0.093521	Stable
	2014-2017	0.43[a]	0.06 to 0.79	2.721	0.026171	Increasing
	2017-2020	-0.27 [a]	-0.46 to -0.09	-3.543	0.007585	Decreasing
	2002-2020	0.15 [a]	0.08 to 0.22	4.582	0.000005	Increasing
Southeast	2002-2007	-0.05	-0.13 to 0.02	-1.715	0.124670	Stable
	2007-2010	0.15	-0.20 to 0.51	1.013	0.340715	Stable
	2010-2014	-0.01	-0.19 to 0.16	-0.193	0.851542	Stable
	2014-2020	0.25[a]	0.19 to 0.31	9.887	0.000009	Increasing
	2002-2020	0.09[a]	0.02 to 0.15	2.737	0.006201	Increasing
Midwest	2002-2005	-1.94[a]	-2.16 to -1.73	-20.007	0.000000	Decreasing
	2005-2018	-0.32[a]	-0.35 to -0.29	-26.541	0.000000	Decreasing
	2018-2020	0.42	-0.04 to 0.88	1.995	0.071367	Stable
	2002-2020	-0.51[a]	-0.57 to -0.45	-17.263	0.000001	Decreasing
North	2002-2006	-0.45[a]	-0.50 to -0.40	-19.889	0.000000	Decreasing
	2006-2009	-0.21[a]	-0.39 to -0.04	-2.855	0.021304	Decreasing
	2009-2012	-0.05	-0.22 to 0.12	-0.684	0.513303	Stable
	2012-2020	-0.20[a]	-0.22 to -0.18	-24.015	0.000000	Decreasing
	2002-2020	-0.23[a]	-0.27 to -0.20	-12.475	0.000001	Decreasing
Northeast	2002-2004	-0.74[a]	-0.99 to -0.50	-7.026	0.000001	Decreasing

[a] significant APC value

Supplementary Table S2. Annual distribution of total cattle slaughtered, livers infected with *Fasciola hepatica*, correspondence percentage, and the number of municipalities with registration of the parasite between 2002 and 2020 in Brazil.

Year	Slaughtered cattle	Infected livers	Prevalence (%)	Municipalities	Positive municipalities	Percentage (%)
2002	4,036,469	19,381	0.48	1,922	194	10.09
2003	14,498,607	74,326	0.51	3,145	342	10.87
2004	20,478,061	131,767	0.64	3,380	449	13.28
2005	22,593,729	153,115	0.68	3,432	451	13.14
2006	25,079,409	166,505	0.66	3,478	414	11.90
2007	25,146,667	86,600	0.34	3,581	391	10.92
2008	22,330,323	71,456	0.32	3,688	413	11.20
2009	21,607,255	71,651	0.33	3,515	368	10.47
2010	21,949,561	86,846	0.40	3,571	378	10.59
2011	21,816,571	102,659	0.47	3,532	380	10.76
2012	23,528,146	98,476	0.42	3,516	352	10.01
2013	26,105,237	89,798	0.34	3,628	394	10.86
2014	25,653,404	101,234	0.39	3,623	460	12.70
2015	22,944,368	86,335	0.38	3,572	439	12.29
2016	22,206,377	109,321	0.49	3,558	396	11.13
2017	23,175,422	139,864	0.60	3,515	460	13.09
2018	24,592,697	224,872	0.91	3,569	611	17.12
2019	24,538,367	184,018	0.75	3,558	680	19.11
2020	22,201,293	47,343	0.21	3,531	747	21.16
Total	414,481,963	2,045,567	---	---	---	---

Supplementary Table S3. Temporal changes in the prevalence of livers infected by *Fasciola hepatica* and the number of municipalities with records of infected livers of cattle between 2002 and 2020 in Brazil.

Variable	Period	APC	CI (95%)	t-test	*P* value	Trend
Infected livers	2002-2015	-4.80[a]	-8.51 to -0.95	-2.733	0.019456	Decreasing
	2015-2018	41.93	-24.60 to 167.67	1.218	0.248538	Stable
	2018-2020	-39.87	-73.20 to 34.93	-1.385	0.193456	Stable
	2002-2020	-3.3198	-17.76 to 9.65	-0.525	0.599296	Stable
Positive municipalities	2002-2004	17.02	-10.74 to 53.42	1.338	0.217645	Stable
	2004-2009	-4.94	-10.88 to 1.38	-1.814	0.107206	Stable
	2009-2016	2.18	-1.37 to 5.86	1.405	0.197549	Stable
	2016-2020	16.01[a]	9.99 to 22.36	6.426	0.000203	Increasing
	2002-2020	4.58[a]	1.13 to 22.36	2.617	0.008865	Increasing

[a] significant APC value

APC, annual percent change; CI, confidence interval

Supplementary Table S4. Description of the total number of slaughtered cattle, infected livers and the prevalence (%) of *Fasciola hepatica* from 2002 to 2020 in São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul, Brazil.

Year	São Paulo (southeastern region)			Paraná (southern region)			Santa Catarina (southern region)			Rio Grande do Sul (southern region)		
	Sla.	Inf.	Prev. (%)	Sla.	Inf.	Prev. (%)	Sla.	Inf.	Prev. (%)	Sla.	Inf.	Prev. (%)
2002	18	0	0.00	54,815	0	0.00	0	0	0.00	575,676	19,378	3.37
2003	797,321	49	0.01	951,118	5	0.00	78,469	1,120	1.43	650304	73,149	11.25
2004	2,040,505	103	0.01	1,269,460	13	0.00	120,992	4,605	3.81	959,570	127,005	13.24
2005	2,368,462	92	0.00	1,306,762	0	0.00	152,285	6,084	4.00	1,119,492	146,895	13.12
2006	2,373,344	111	0.00	1,323,568	0	0.00	141,008	6,489	4.60	1,204,705	159,829	13.27
2007	2,525,968	376	0.01	1,125,846	4	0.00	104,372	4,169	3.99	676,624	81,852	12.10
2008	2,197,256	246	0.01	983,359	25	0.00	99,860	2,966	2.97	705,354	68,188	9.67
2009	1,963,216	147	0.01	880,098	101	0.01	97,829	1,670	1.71	735,925	69,608	9.46
2010	2,029,169	310	0.02	1,075,415	246	0.02	94,193	145	0.15	967,951	86,070	8.89
2011	1,927,579	131	0.01	894,606	286	0.03	92,098	147	0.16	895,702	101,898	11.38
2012	1,971,768	292	0.01	1,013,196	447	0.04	87,662	45	0.05	721,834	97,650	13.53
2013	2,018,082	260	0.01	1,118,464	484	0.04	98,032	462	0.47	731,041	88,546	12.11
2014	2,108,358	450	0.02	1,210,494	410	0.03	104,513	1,228	1.17	746,279	99,042	13.27
2015	1,765,126	566	0.03	1,011,109	370	0.04	111,669	923	0.83	660,954	84,377	12.77
2016	1,785,640	199	0.01	806,241	427	0.05	107,481	1,112	1.03	704,118	107,519	15.27
2017	1,894,287	147	0.01	875,014	824	0.09	111,016	1,310	1.18	744,803	137,542	18.47
2018	2,069,292	619	0.03	956,231	1,608	0.17	114,367	959	0.84	893,462	221,624	24.81
2019	2,323,915	1,579	0.07	895,289	1,052	0.12	109,769	1,250	1.14	749,641	180,065	24.02
2020	2,140,702	6,033	0.28	849,146	808	0.10	122,540	2,200	1.80	736,550	38,199	5.19

	36,300,008	11,710		18,600,231	7,110		1,948,155	36,884		15,179,985	1,988,436	

Sla., slaughtered cattle; Inf., infected livers; Prev., prevalence.

3. CHAPTER 2 – DISTRIBUTION AND ENVIRONMENTAL CHARACTERISTICS OF LYMNAEIDAE (MOLLUSCA, BASOMMATOPHORA), INTERMEDIATE HOSTS OF *Fasciola hepatica*, IN SOUTH AMERICA

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ABSTRACT

Distribution of intermediate hosts directly influences the transmission of *Fasciola hepatica*, as the presence of these species in specific areas determines the receptiveness to transmission. This review compiled occurrence records of four epidemiologically relevant limneid species—*Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula*—to assess their geographical distribution and the associated environmental conditions. A search in six databases resulted in 15,233 studies, of which 103 were selected based on eligibility criteria. A total of 969 occurrence records were documented across 46 countries and three French administrative regions. *Pseudosuccinea columella* and *G. cubensis* showed widespread distribution in the Caribbean and the Americas, while *G. schirazensis* and *G. truncatula* were also recorded in Africa, Europe, and Asia. In South America, 493 non-duplicate records were analyzed, revealing distinct altitude and climate preferences among the species. *P. columella* and *G. cubensis* were predominantly found at altitudes of up to 2,000 m, while *G. schirazensis* and *G. truncatula* were more common at higher elevations, particularly in the Andean region. Kruskal-Wallis and Dunn tests confirmed significant differences in environmental variables between species, except for precipitation between *G. cubensis* and *G. truncatula* ($p = 0.172$) and altitude between *G. schirazensis* and *G. truncatula* ($p = 0.057$). The results highlight the ecological flexibility of these mollusks and their potential to sustain *F. hepatica* transmission in various environmental conditions. The data also highlights the difficulty in controlling fascioliasis based on this complex web of adaptative IH, posing a major a challenge to public health and livestock welfare.

Keywords: Lymnaeidae; Environment distribution; adaptability; disease risk; fasciolosis.

3.1 INTRODUCTION

Lymnaeidae snails (Gastropoda: Lymnaeidae) are freshwater mollusks characterized by dextrally coiled shells, widely distributed across tropical and temperate regions. They inhabit a variety of freshwater environments, including temporary and permanent bodies of water, typically shallow, clear, and with low water flow (Vázquez et al., 2018; Bargues et al., 2022). Their remarkable ecological plasticity allows them to occupy a variety of ecosystems, facilitating the spread of *Fasciola* spp., causing fasciolosis, a zoonosis of medical and veterinary relevance (Vázquez et al., 2018; Alda et al., 2021). However, not all species serve as intermediate hosts (IH) for the parasite, and among those that harbor some life stages of the parasites, there is considerable variation in their transmission potential (Soler et al., 2023).

Approximately 30 species of Lymnaeidae are estimated to serve as intermediate hosts for *Fasciola hepatica* and *Fasciola gigantica* (Alba et al., 2020). Among them, some genera stand out for their higher susceptibility to harboring these species of *Fasciola*. The genus *Galba*, for example, is predominantly associated with *F. hepatica*, while *Radix* is more frequently related to *F. gigantica* (Vázquez et al., 2018).

Species of *Galba* have high invasive potential and a great capacity for dispersal due to self-fertilization, allowing them to occur in various regions worldwide. This species is particularly present in extreme high-altitude conditions, as observed with *Galba truncatula* in the Bolivian Altiplano (Lounnas et al., 2017; Bargues et al., 2021). This characteristic, combined with biological factors related to the parasite, significantly contributes to the global burden of fasciolosis caused by *F. hepatica* (Alba et al., 2020). The distribution of *F. hepatica* is recorded on all continents except Antarctica, as well as various oceanic islands. In contrast, *F. gigantica* has a more restricted distribution, limited to tropical and subtropical regions of Asia and Africa, with a limited impact on global public and veterinary health (Bargues et al., 2022; Vázquez et al., 2018).

In South America, the human and animal transmission of *F. hepatica* primarily involves species from the *Galba/Fossaria* group, such as *G. truncatula, G. cubensis,* and *G. schirazensis*, which are widely distributed in endemic high-altitude areas in Peru, Colombia, and Ecuador (Alda et al., 2021; Bargues et al., 2021). On the other hand, in lowland areas, where fasciolosis is a veterinary problem and human infection occurs only in isolated cases, as in Brazil, the disease is frequently associated with the presence of Lymnaeidae outside the *Galba/Fossaria* group, such as *Pseudosuccinea columella* (Pritsch et al, 2018; Bargues et al., 2021).

The distribution of fasciolosis, closely linked to the presence of its hosts, is modulated by environmental factors that also influence the occurrence and abundance of Lymnaeidae, such as water velocity, soil type, precipitation, evapotranspiration, and temperature (Dutra et al., 2010; Alba et al., 2020). Understanding the geographic distribution and the factors shaping the presence of Lymnaeidae is essential for guiding fasciolosis control and prevention programs (Bargues et al., 2021). This is especially important when considering that the global distribution of *Galba* species is poorly known, due to the inaccuracy of records based on morphological data (Alda et al., 2021; Soler et al., 2023)

Considering the importance of environmental factors in the presence of intermediate hosts, the use of tools such as Geographic Information Systems (GIS) enables the integration of detailed environmental data collected from sampling sites to map distribution areas and expand the understanding of ecological determinants. The present study aimed to update and map the spatial distribution of intermediate host mollusks of *F. hepatica* in South America and discuss the environmental conditions associated with their presence.

3.2 MATERIAL AND METHODS

3.2.1 Overview

This study aimed to analyze the geographical distribution of four lymnaeid species of importance in the transmission of *F. hepatica*, namely *P. columella* and *G. cubensis*, considering *G. neotropica* as a synonymous species (Alda et al., 2021), *G. schirazensis* and *G. truncatula*. The documented occurrence records were investigated in three ways: 1) through the creation of a scoping review protocol in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P). This protocol was developed before the start of the research and was reviewed and approved by all team members; 2) by the occurrence data provided by the Malacological Collection of the Oswaldo Cruz Institute (IOC); and 3) occurrences documented in a comprehensive study on the distribution of Lymnaeidae mollusks in Brazil (Medeiros et al., 2014).

All references included in the scoping review and geographic coordinates were made available in the Mendeley Data repository, under DOI: 10.17632/r2cc38vz8k.5 (https://data.mendeley.com/datasets/r2cc38vz8k/1)

3.2.2 Eligibility criteria

The scoping review aimed to identify primary studies that reported collection sites for the four Lymnaeidae species. Eligible studies met the following criteria: (a) they provided specific geographic coordinates for the collection sites; (b) mollusk specimens were identified at the species level; (c) publications in any language were considered, although those not in English or Portuguese were included only if the file format allowed text selection for translation; and (d) the sources encompassed dissertations, theses, epidemiological bulletins, technical documents (grey literature), and scientific articles.

During the literature review, two categories of studies were identified as relevant: (1) studies that involved mollusk collection in the field, followed by identification using morphological and/or molecular methods, typical of classical field ecology approaches; and (2) studies that, although not focused on ecology, used mollusks as experimental models in laboratory settings, including molecular, physiological, and immunoassays. Although not aimed at fauna or ecology surveys, the latter provided useful information about the collection sites of Lymnaeidae used in the experiments.

Material exclusion was based on; a) studies that were not available in full; b) studies that could not be translated; c) studies that did not specify the study area or geographic coordinates; d) studies that did not include Lymnaeidae among the invertebrate fauna; e) studies with taxonomic identification only up to the family or genus level; and f) studies with species records from breeding/aquaculture.

3.2.3 Information sources

A search for studies meeting the eligibility criteria was conducted through a detailed search in journal repositories, such as Google Scholar, LILACS, PubMed, Science Direct, Scielo, and Scopus. All bibliographic sources were retrieved on August 12, 2023.

3.2.4 Search

Broad terms such as "Lymnaeidae" were used in the study search strategy. Alternative terms related to "occurrence," "ecology," and "fascioliasis" were included to increase the sensitivity of the search. No language or publication period restrictions were applied. This initial search strategy was developed collaboratively by all members of the review team.

Complete search syntax for the six databases was as follows: 1. (lymnaeidae OR lymnaeids) AND (occurrence OR distribution OR collected OR captured OR samples OR sampled OR sampling); 2. (lymnaeidae OR lymnaeids) AND (ecology OR biology); 3. (lymnaeidae OR lymnaeids) AND (fauna OR description OR described); 4. (lymnaeidae OR lymnaeids) AND ("intermediate host" OR vector OR snail); 5. (lymnaeidae OR lymnaeids) AND (Fasciola OR trematoda OR trematode OR helminth OR helminths); 6. (lymnaeidae OR lymnaeids) AND (infection OR "experimental infection" OR "natural infection" OR susceptibility OR sensibility); 7. (lymnaeidae OR lymnaeids) AND (fluke OR epidemiology OR diseases OR fascioliasis).

3.2.5 Selection of sources of evidence

Citations retrieved from the databases were imported into the Mendeley reference manager, where duplicate records were identified and removed. Remaining citations were then transferred to Rayyan, where a second check for duplicate studies was conducted, followed by an assessment of their eligibility. Initially, studies were selected based on titles and abstracts to identify those potentially relevant. Subsequently, they were independently reviewed in full to verify if they met the eligibility criteria.

Initial screening by title and abstract selected studies based on the following criteria: a) original studies; b) studies documenting the occurrence of mollusks/gastropods/macroinvertebrates or Lymnaeidae (more generic terms were considered when species were not listed in the abstract); c) studies indicating field collections or using the species as experimental models. Studies were excluded if they: a) lacked available abstracts; b) documented other mollusk groups, such as *Biomphalaria*; or c) were fossil records of Lymnaeidae.

3.2.6 Data charting process and synthesis of results

Initially, the raw occurrence points were filtered based on the species identification method. Since species of the *Galba* genus are considered cryptic (Alda et al., 2021), only points confirmed through molecular biology were included in the analysis. Subsequently, occurrences were filtered according to the area of interest, excluding those outside South America.

After the initial filtering, additional spatial filters were applied to minimize the influence of variables, such as biases related to sampling effort, which may impact both occurrence density and environmental characteristics of the sampled areas (Testai et al., 2023; Varela et al., 2014). The applied filters included: i) removal of duplicate coordinates; ii) exclusion of points located less than 1 km apart; and iii) removal of occurrences in pixels with no data.

Environmental layers of altitude, maximum and minimum temperature, and accumulated precipitation were extracted from the Google Earth Engine (GEE) platform (https://earthengine.google.com/), with median values calculated for each variable over the period from 1974 to 2019, corresponding to the collection period of the occurrence points. Temperature and precipitation data were obtained from the IDAHO_EPSCOR/TERRACLIMATE collection, with a resolution of 4638.3 m, while altitude data were extracted from the USGS/SRTMGL1_003 collection, with a 1 km resolution.

The occurrence points were georeferenced in the WGS-84 geocentric reference system (EPSG:4326). The extraction of environmental values from the layers in the pixels corresponding to the occurrence points was performed using the Point Sampling Tool (https://plugins.qgis.org/plugins/pointsamplingtool/) in QGIS.

Descriptive statistical analyses were performed using the R software. The Kruskal-Wallis test, suitable for non-parametric data, was applied to assess differences between species. When significant differences were identified, the Dunn test was conducted as a post-hoc analysis to determine which species pairs exhibited specific differences (Dunn, 1964).

3.3 RESULTS

3.3.1 Study eligibility

Bibliographic search conducted across six journal databases resulted in 15,233 studies (Fig. 1). After the duplicate check, 10,716 studies were excluded. Subsequent screening of titles and abstracts led to the exclusion of another 2,452 studies. Full texts of 1,694 studies were assessed for eligibility. Of these, 296 were excluded because they were not available in English or Portuguese (files that did not allow text copying for translation), 625 because taxonomic identification was limited to the family level, and 229 for addressing Lymnaeidae snail species not relevant to this study. In total, 103 studies were included in this systematic review

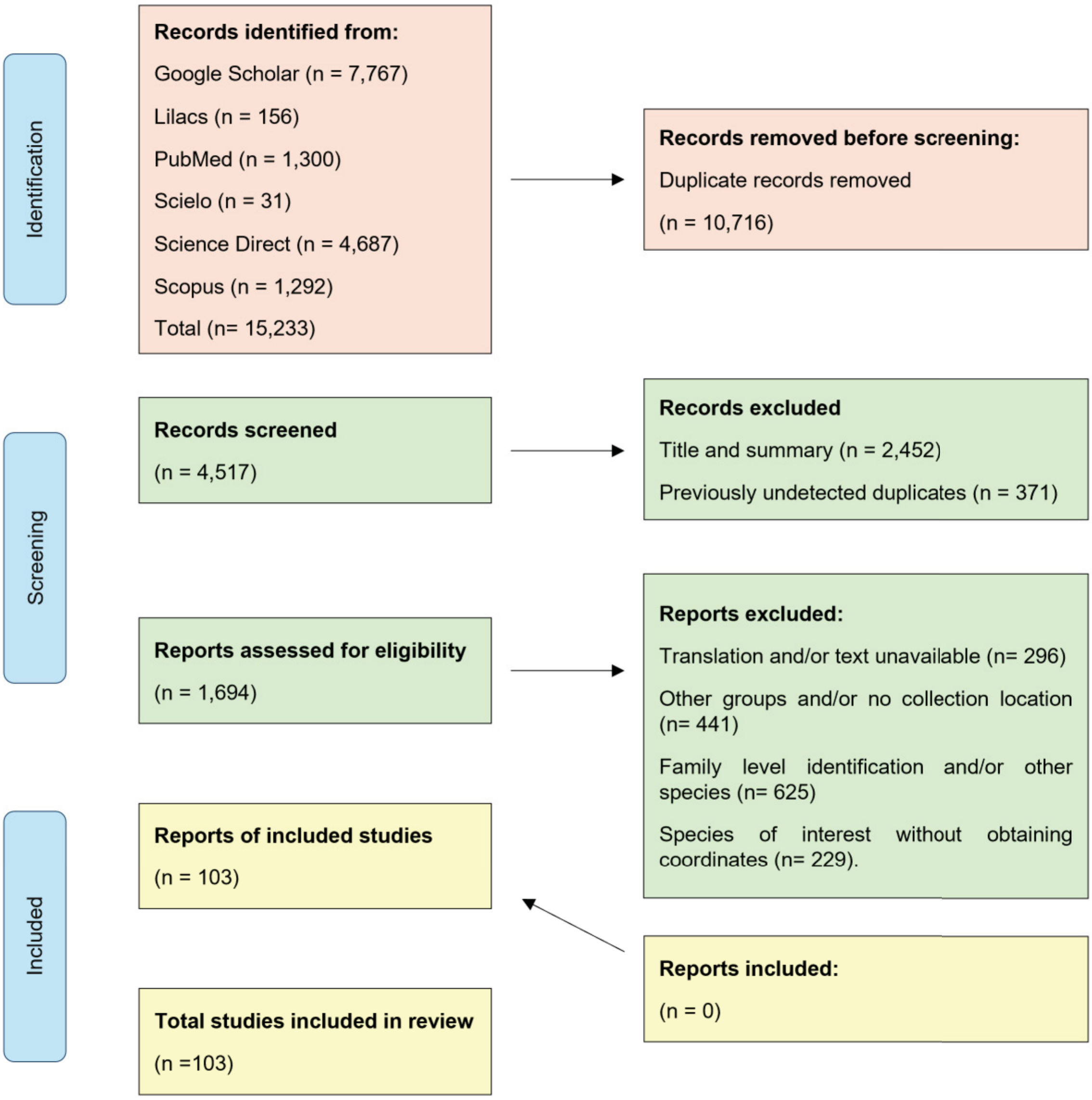

Figure 1. Research flowchart and bibliographic screening.

3.3.2 Worldwide geographic distribution

A total of 969 occurrence records for four limneid species were documented, spanning 46 countries and three French administrative regions (Guadeloupe, Réunion Island, and Martinique) (Fig. 2). Among these, *P. columella* accounted for 403 records, *G. cubensis* for 169, *G. schirazensis* for 130, and *G. truncatula* for 267. Following the exclusion of records lacking molecular confirmation, 120 records for *G. cubensis*, 130 for *G. schirazensis*, and 144 for *G. truncatula* were retained.

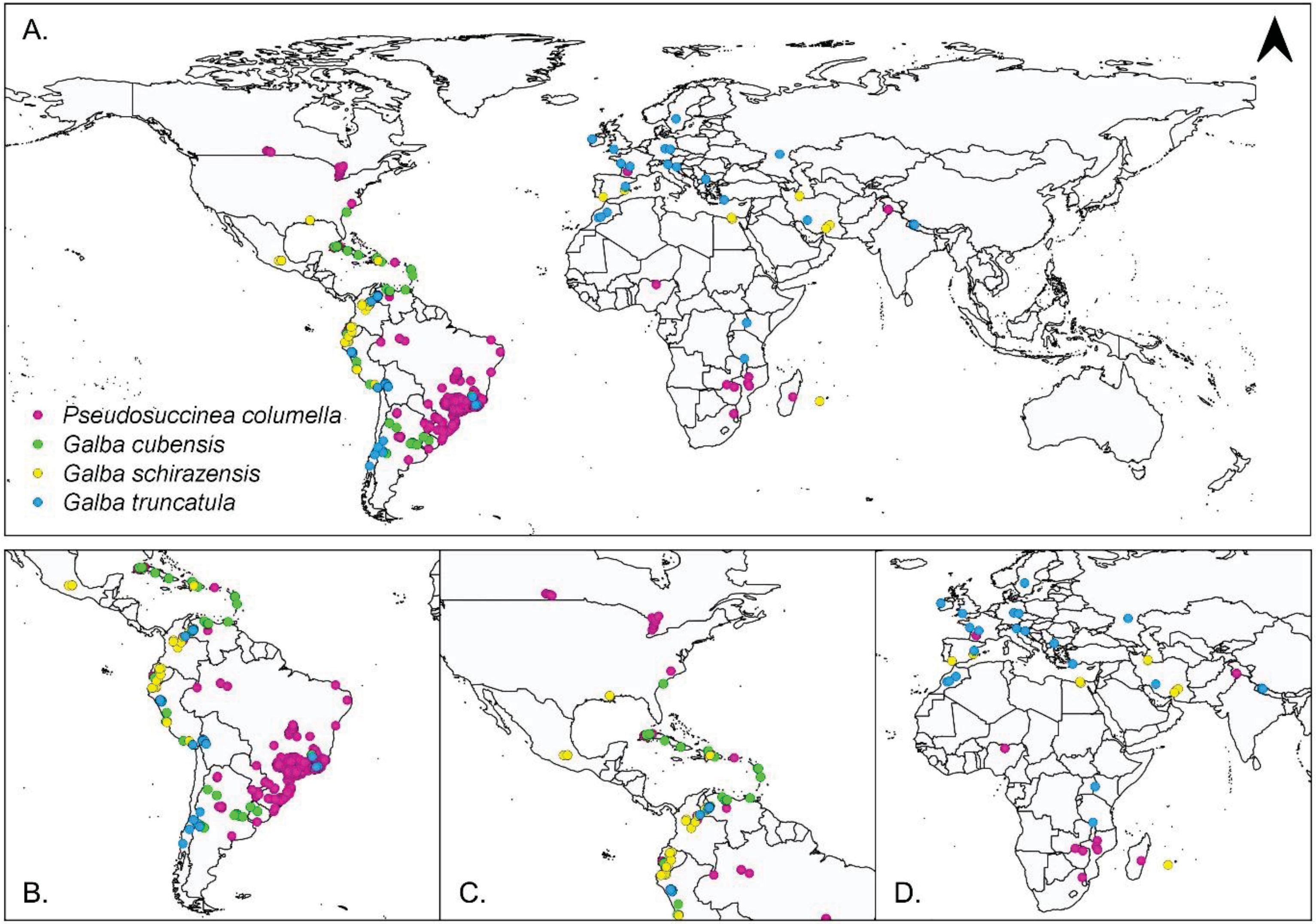

Figure 2. Occurrence points of *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula*, compiled from a literature review and the Malacological Collection of the Oswaldo Cruz Institute. (A) Global overview; and inserts of (B) South America; (C) Central and North America, and the Caribbean; (D) Africa, Europe and the Middle East.

Pseudosuccinea columella has been recorded in 19 countries, with a distribution ranging from Canada in North America to Argentina in South America. The species has also been reported in Africa (Malawi, Nigeria, South Africa, Zimbabwe), Europe (France), and Asia (India). As for the *Galba* species with molecular confirmation, the distributions have been observed: *G. cubensis* in 13 countries, with a distribution limited to the Americas, primarily concentrated in the Caribbean, Central America and Argentina; *G. schirazensis* in 12 countries; and *G. truncatula* in 23 countries. The latter two species have a broader geographic distribution, with notable presence in the Old World, compared to *P. columella* and *G. cubensis* (View Mendeley Data; DOI: 10.17632/r2cc38vz8k.5).

3.3.3 Geographic distribution in South America and environmental conditions

After applying spatial filters for South America, 493 non-duplicate occurrence points were retained, distributed as follows: 328 for *P. columella*, 43 for *G. cubensis*, 49 for *G. schirazensis*, and 73 for *G. truncatula*. Both *P. columella* and *G. cubensis* exhibit widespread distributions across South America, with records from seven countries: Ecuador, Argentina, Peru, Venezuela, Uruguay, Colombia, and Brazil. In contrast, *G. schirazensis* demonstrates a more restricted range, occurring in only four countries: Ecuador, Peru, Venezuela, and Colombia. *Galba truncatula*, identified in seven countries, is notable for its distribution in Andean regions, including Chile and Bolivia (Fig. 3).

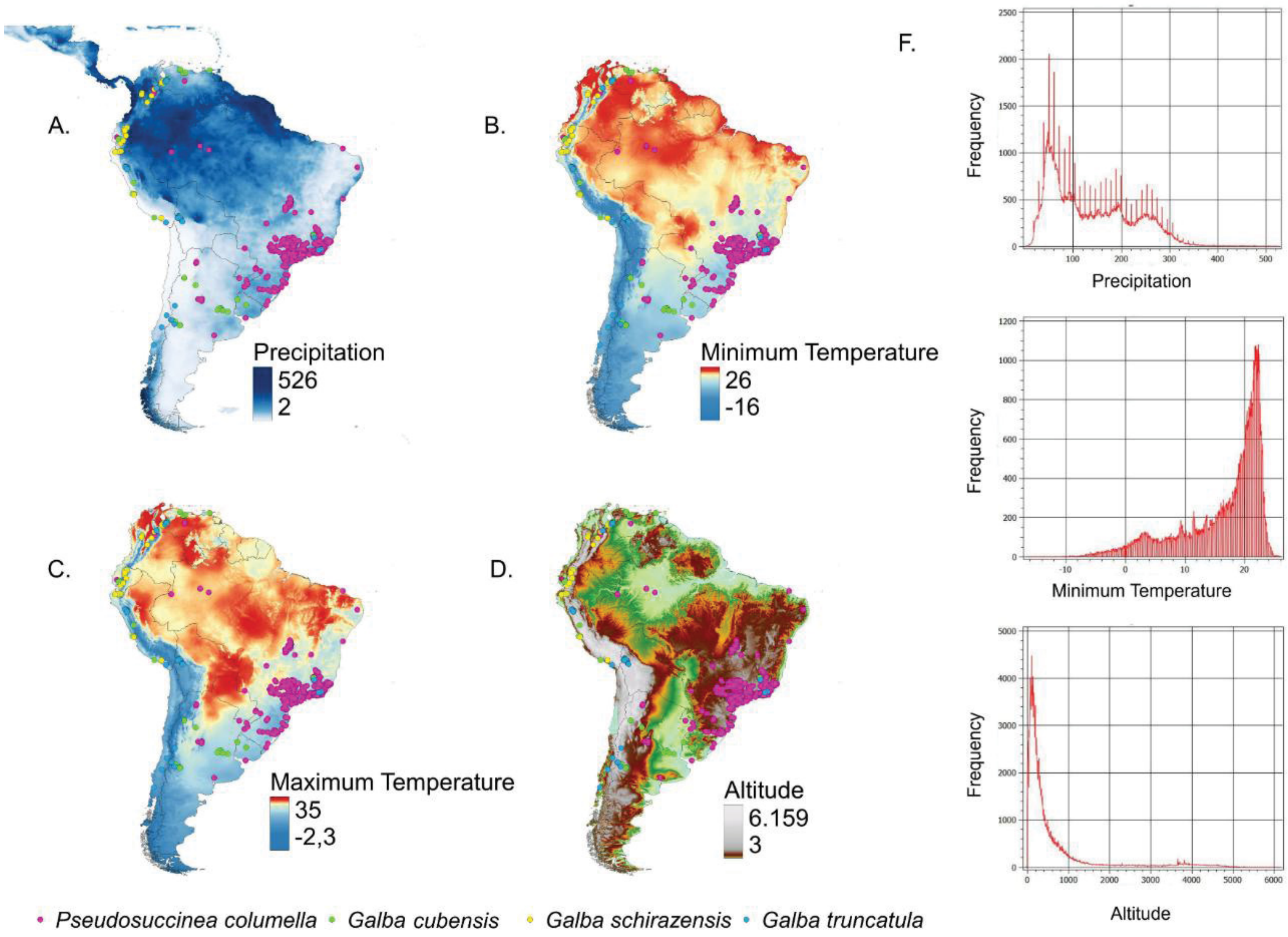

Figure 3. Occurrence of *Pseudosuccinea columella*, *Galba cubensis*, *Galba truncatula*, and *Galba schirazensis* based on precipitation (A), minimum temperature (B), maximum temperature (C), and altitude (D) in South America. (F) Frequency of pixels of environmental variables found across South America.

Pseudosuccinea columella (Fig. 4A) was recorded at altitudes up to 2,860 meters, with higher prevalence at altitudes up to 1,000 meters (Fig. 5A). The maximum temperature most frequently associated with the records was in the 27°C range. Regarding the minimum temperature, it ranged from 7 to 23°C, with the highest frequency of points distributed in areas with records between 15 and 20°C (Fig. 5B-C). Precipitation ranged from 7 to 247 mm (Fig. 5D).

Galba cubensis (Fig. 4B) was observed at altitudes above 4,300 meters, although the most frequent occurrence points were located at altitudes near 500 meters (Fig. 5A). Regarding the maximum temperature, records predominantly occurred in areas with temperatures around 25°C. For the minimum temperature, it ranged from -0.95°C (at a point in Peru) to 23°C, with most points falling within the 10 to 15°C range (Fig. 5B-C). Precipitation ranged from 0.5 to 212 mm (Fig. 5D).

Galba schirazensis (Fig. 4C), with a more restricted distribution, was found at altitudes ranging from 133 to 3,738 m above sea level (a.s.l.) (Fig. 5A). The maximum temperature most frequently observed was between 20 and 25°C, while the minimum temperature ranged from 2 to 19°C. Occurrence points were most frequently associated with areas where the minimum temperature was around 7°C (Fig. 5B-C). Precipitation ranged from 0 to 226 mm (Fig. 5D).

Galba truncatula (Fig. 4D) was recorded at altitudes ranging from 13 to 4,012 m with most occurrence points associated with altitudes between 3,500 and 4,000 m a.s.l. (Fig. 5A). The maximum temperatures for this species ranged from 11 to 25°C, while the minimum temperatures varied from 1 to 14°C, with the highest frequency of records in areas where the minimum temperature was 2°C (Fig. 5B-C). Precipitation ranged from 0 to 130 mm (Fig. 5D).

All values of the environmental variables associated with the occurrence points are presented in Table 1 of the supplementary material.

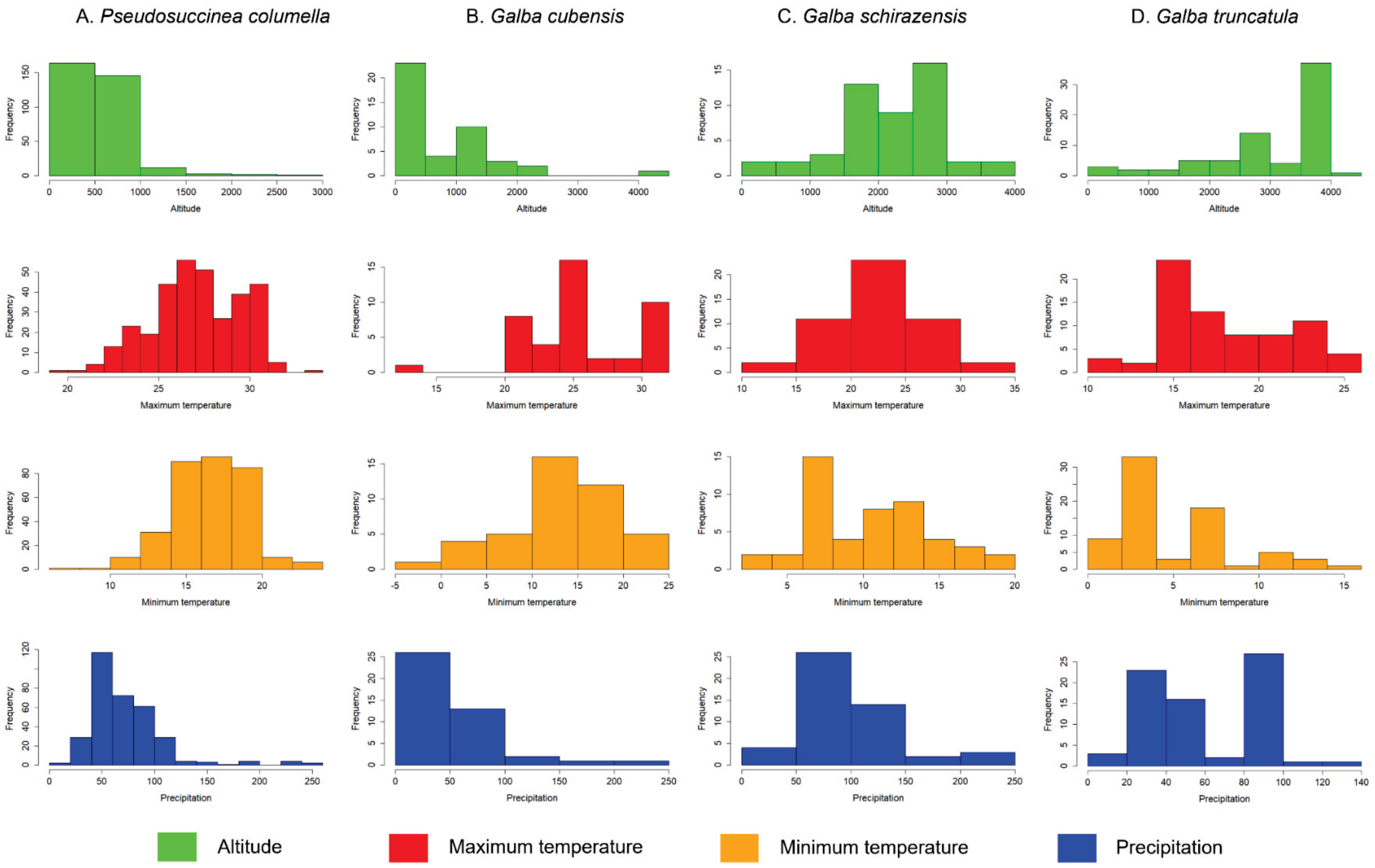

Figure 4. Distributions of altitude (green), maximum temperature (red), minimum temperature (orange), and precipitation (blue) extracted from the pixels corresponding to the occurrence points of *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula* in South America.

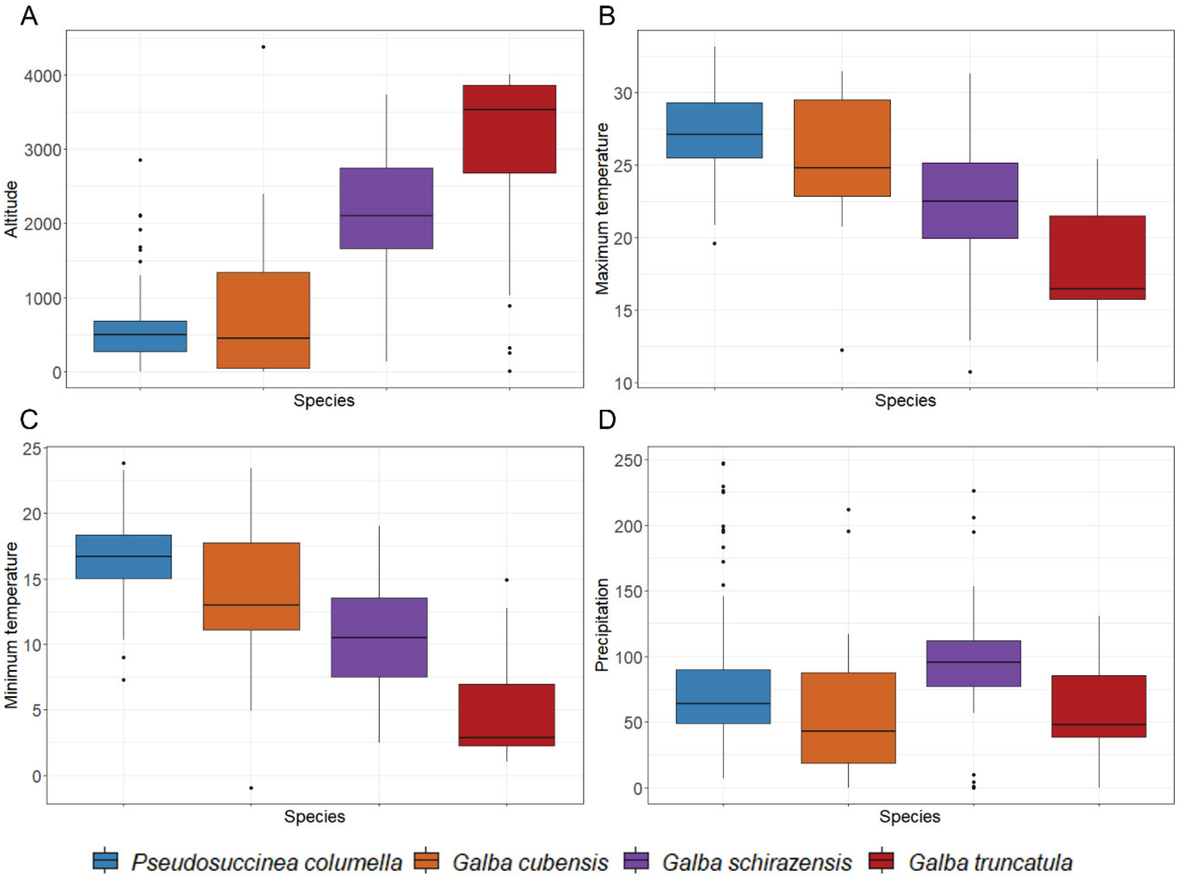

Figure 5. Boxplot analysis of environmental variables at the occurrence points of *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula*. (A) Altitude, (B) Maximum temperature, (C) Minimum temperature, and (D) Precipitation.

The Kruskal-Wallis tests revealed statistically significant differences among species for all variables analyzed ($p \leq 0.05$). These results suggest that snail species are significantly associated with variations in environmental factors such as altitude, temperature, and precipitation. Dunn test was performed to investigate which pairs of species showed significant differences (Table 1). Results demonstrated significant differences between all variables observed in the distribution when comparing *G. cubensis* and *G. schirazensis*. However, *G. cubensis* did not show a significant difference ($p = 0.172$) with *G. truncatula* regarding precipitation. *G. schirazensis* and *G. truncatula* also did not show a significant difference regarding altitude ($p = 0.057$), nor did *G. cubensis* and *P. columella* ($p = 0.289$). On the other hand, significant differences were found between *P. columella* and *G. schirazensis*, as well as between *P. columella* and *G. truncatula*, for all variables analyzed.

Table 1. Results of pairwise comparisons between species using the Dunn test for environmental variables.

Comparison between species	Altitude	Maximum temperature	Minimum temperature	Precipitation	Significant difference (all)
B - C	2.04e-10	6.90e-04	2.51e-03	1.03e-09	Yes
B - D	4.96e-17	1.99e-11	1.44e-10	1.72e-01	No*
C - D	5.79e-02	5.67e-04	3.51e-04	3.39e-09	No**
B - A	2.89e-01	1.53e-03	8.39e-05	1.92e-04	No**
C - A	3.83e-20	3.18e-06	2.80e-15	5.02e-06	Yes
D - A	3.85e-39	5.82e-42	2.44e-45	1.16e-03	Yes

Legend: (A) Pseudosuccinea *columella;* (B) *Galba cubensis;* (C) *Galba schirazensis;* (D) *Galba truncatula.*

* No significant difference was observed between the species for the precipitation variable (p = 0.172).

** No significant difference was observed between the species for the altitude variable (Comparison C--D, p = 0.057 and B-A, p = 0.289).

3.4 DISCUSSION

This study allowed the identification of the spatial distribution of four limneid species of epidemiological relevance in the transmission of *F. hepatica* in South America. The data also helped to characterize the environmental conditions associated with the occurrence sites of these mollusks. The distribution of these intermediate hosts may reflect the distribution of the disease, making these data essential for defining risk areas and focus point for health programs.

A total of 969 occurrences of the four species analyzed were recorded from different sources. *P. columella*, *G. schirazensis*, and *G. truncatula* exhibited broad distributions, being found in the Americas, Africa, Europe, and Asia. In contrast, *G. cubensis* was recorded exclusively in the Americas – with a wide presence in the Caribbean, with the exception of two specimens molecularly identified in Spain. Since the COI gene sequences of these specimens were identical to those of *G. cubensis* from Colombia, they were assigned to the same species (Schniebs et al., 2018).

Dunn analysis indicated that *G. schirazensis* and *G. truncatula* did not differ in terms of altitude (p = 0.057), nor did *G. cubensis* and *P. columella* (p = 0.289). However, differences were observed between *P. columella* and *G. schirazensis*, as well as between *P. columella* and *G. truncatula* for all variables analyzed. These results suggest that, although some species share similar environmental characteristics, *G. cubensis* is distinguished from other *Galba* species, except for precipitation, for which no significant difference was found compared to *G. truncatula* (p = 0.172). Additionally, *G. cubensis* has a wide altitudinal range, similar to that of *P. columella*.

Species identification within the genus *Galba* is challenging due to morphological similarities and high phenotypic plasticity, requiring molecular analyses for precise determination (Alda et al., 2021). The absence of altitudinal differences between *G. schirazensis* and *G. truncatula* further complicates the distinction between these species in the field, especially since both can coexist in the same region. This challenge is heightened by the fact that *G. truncatula* is widely recognized as a host for *F. hepatica* (Alda et al., 2021; Smith et al., 2024), while the role of *G. schirazensis* in transmission remains poorly established (Nasibi et al., 2021). In many cases, although the mollusks may harbor cercariae or be infected with *F. hepatica*, this does not guarantee that they are effectively intermediate hosts, meaning they are capable of supporting the full development of the parasite to the infective stage for vertebrate hosts (Caron et al., 2017; Alba et al., 2020).

The occurrence of these mollusks in dry climates and high altitudes, such as the Bolivian Altiplano, suggests a great ability to adapt to adverse environments (Smith et al., 2024). Furthermore, the ability of *G. schirazensis* to survive outside of water for extended periods reinforces its ecological flexibility (Lounnas et al., 2018). While *G. truncatula* can remain active at temperatures as low as 1.5°C, its activity and feeding significantly decrease below 5°C, reducing egg production. However, juveniles exposed to cold weather tend to grow faster and produce more eggs, suggesting that, in climates with harsh winters, reproduction in the spring may be sustained by these juveniles (Smith et al., 2024). In addition, the high rate of self-fertilization in the genus *Galba* promotes the rapid colonization of new habitats, enhancing and facilitating its dispersion (Schniebs et al., 2018).

The *Galba* group has been the subject of extensive taxonomic and biogeographic discussions. Issues related to its origin and synonymy remain debated (Schniebs et al., 2018; Alda et al., 2021). Some species of this group have epidemiological importance, such as *G. cubensis*, which acts as an intermediate host of *F. hepatica*, particularly in the Caribbean (Alba et al., 2015; Vázquez et al., 2015). This species is also considered a potential host for *Fascioloides magna* (Vignoles et al., 2014) and *Heterobilharzia americana*, a parasite affecting mammals, including horses (Corapi et al., 2015). In 2007, *G~~.~~ neotropica* was described by (Bargues et al., 2007) and later recorded in countries such as Peru, Argentina, Venezuela, and

Uruguay, being considered an intermediate host of *F. hepatica* (Schniebs et al., 2018; Alda et al., 2021). However, this species is now recognized as a synonym of *G. cubensis* (Alda et al., 2021) originally described in Cuba — Pfeiffer, 1839 —.

Galba truncatula — Müller, 1774 — is the primary host of fascioliasis in Europe, Asia, and Africa (Smith et al., 2024), and its origin remains a topic of debate. Although it was considered native to the Old World, molecular analyses suggest that the species may have originated in the Americas and later colonized Europe (Correa et al., 2010). Similarly, the origin of *G. schirazensis* — Küster, 1862 — remains uncertain. Bargues et al., (2011) propose that the species originated in the Middle East, initially expanding to Africa, Europe, and Asia, and later to the Americas, possibly associated with livestock transportation during the Spanish invasion colonization (Lounnas et al., 2018).

Pseudosuccinea columella — Say, 1817 — is native to North America (Correa et al., 2011) and, until the early 20th century, its distribution was limited to the eastern portion of the continent (Lounnas et al., 2017). In the last century, it was introduced to other continents, where it adapted to different environmental conditions, resulting in a broad distribution in Africa and South America, while its presence in Oceania remains restricted (Lounnas et al., 2017; Ngcamphalala et al., 2022). This species acts as an intermediate host for *F. hepatica* in several countries, such as Brazil (Medeiros et al., 2014), Cuba (Ngcamphalala et al., 2022), and Argentina (Martín et al., 2016), highlighting the need for continuous monitoring.

In addition to large-scale environmental conditions, regional climate dynamics influence mollusk abundance, with seasonal variations related to temperature, precipitation, and water availability (Vázquez et al., 2018; Alba et al., 2020). These variations affect parasitic transmission by altering the availability of intermediate hosts. However, in some regions, such as northern Corrientes province in Argentina, *P. columella* is more abundant in the winter, suggesting seasonal variations in its abundance depending on local environmental conditions (Ngcamphalala et al., 2022).

The collection of ecological data on these species from diverse regions and climates enables the assessment of variations in ecological preferences and the prediction of future climate change impacts (Alda et al., 2021; Smith et al., 2024) Distribution pattern analysis is crucial for identifying relevant species in different regions, especially considering the absence of distinct morphological traits to differentiate *Galba* species (Alda et al., 2020). In South America, the transmission of

F. hepatica involves several species from the *Galba* group. In endemic regions, different species of this group coexist, such as *G. schirazensis* and *G. truncatula* (Alda et al., 2020; Bargues et al., 2021). However, the composition of epidemiologically important species at high altitudes is distinct from lowlands, where fascioliasis is a veterinary concern associated with limneid species outside the *Galba* group, such as *P. columella* (Bargues et al., 2021)

Our results suggest that, in South America, *P. columella* and *G. cubensis* may be the main hosts of *F. hepatica*, with a potentially broader distribution than *G. schirazensis* and *G. truncatula*. This difference may be related to the ability of these species to occupy ecological niches at different altitudes, as they are more frequently recorded up to 2,000 meters. At higher altitudes, particularly in the Andean region, *G. schirazensis* and *G. truncatula* become more important, better adapting to the conditions of these areas.

3.5 REFERENCES

Alba A., Vázquez A. A., Hernández H., Sánchez J., Marcet R., Figueredo M., Sarracent J., Fraga, J. 2015. A multiplex PCR for the detection of *Fasciola hepatica* in the intermediate snail host *Galba cubensis*. Vet. Parasitol. 211: 195–200. https://doi.org/10.1016/j.vet-par.2015.05.012.

Alba, A., Vazquez, A.A., Hurtrez-Boussès, S. 2020. Towards the comprehension of fasciolosis (re-)emergence: An integrative overview. Parasitology. https://doi.org/10.1017/S0031182020002255.

Alda, P., Lounnas, M., Vázquez, A.A., Ayaqui, R., Calvopiña, M., Celi-Erazo, M., Dillon, R.T., González Ramírez, L.C., Loker, E.S., Muzzio-Aroca, J., Nárvaez, A.O., Noya, O., Pereira, A.E., Robles, L.M., Rodríguez-Hidalgo, R., Uribe, N., David, P., Jarne, P., Pointier, J.P., Hurtrez-Boussès, S. 2021. Systematics and geographical distribution of *Galba* species, a group of cryptic and worldwide freshwater snails. Mol Phylogenet Evol 157. https://doi.org/10.1016/j.ympev.2020.107035.

Bargues, M.D., Angles, R., Coello, J., Artigas, P., Funatsu, I.R., Cuervo, P.F., Buchon, P., Mas-Coma, S. 2021. One Health initiative in the Bolivian Altiplano human fascioliasis hyperendemic area: Lymnaeid biology, population dynamics, microecology and climatic factor influences. Revista Brasileira de Parasitologia Veterinaria 30. https://doi.org/10.1590/S1984-29612021014.

Bargues, M.D., Artigas, P., Khoubbane, M., Flores, R., Glöer, P., Rojas-García, R., Ashrafi, K., Falkner, G., Mas-Coma, S. 2011. *Lymnaea schirazensis*, an overlooked snail distorting fascioliasis data: Genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS One 6. https://doi.org/10.1371/journal.pone.0024567.

Bargues, M.D., Artigas, P., Mera Y Sierra, R.L., Pointier, J.P., Mas-Coma, S. 2007. Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and mitochondrial DNA. Ann Trop Med Parasitol 101, 621–641. https://doi.org/10.1179/136485907X229077.

Bargues, M.D., Halajian, A., Artigas, P., Luus-Powell, W.J., Valero, M.A., Mas-Coma, S. 2022. Paleobiogeographical origins of *Fasciola hepatica* and *F. gigantica* in light of new DNA sequence characteristics of *F. nyanzae* from hippopotamus.

Caron, Y., Celi-Erazo, M., Hurtrez-Boussès, S., Lounnas, M., Pointier, J.P., Saegerman, C., Losson, B., Benítez-Ortíz, W., 2017. Is Galba schirazensis (Mollusca, Gastropoda) an intermediate host of Fasciola hepatica (Trematoda, Digenea) in Ecuador? Parasite 24, 24.

Corapi, W. V., Eden, K.B., Edwards, J.F., Snowden, K.F. 2015. *Heterobilharzia americana* Infection and Congestive Heart Failure in a Llama (*Lama glama*). Vet Pathol 52, 562–565. https://doi.org/10.1177/0300985814540541.

Correa, A.C., Escobar, J.S., Durand, P., Renaud, F., David, P., Jarne, P., Pointier, J.P., Hurtrez-Boussès, S. 2010. Bridging gaps in the molecular phylogeny of the Lymnaeidae (Gastropoda: Pulmonata), vectors of Fascioliasis. BMC Evol Biol 10. https://doi.org/10.1186/1471-2148-10-381.

Dunn, O.J.,1964. Multiple Comparisons Using Rank Sums. Technometrics 6, 241–252. https://doi.org/10.1080/00401706.1964.10490181.

Lounnas, M., Correa, A.C., Vázquez, A.A., Dia, A., Escobar, J.S., Nicot, A., Arenas, J., Ayaqui, R., Dubois, M.P., Gimenez, T., Gutiérrez, A., González-Ramírez, C., Noya, O., Prepelitchi, L., Uribe, N., Wisnivesky-Colli, C., Yong, M., David, P., Loker, E.S., Jarne, P., Pointier, J.P., Hurtrez-Boussès, S. 2017. Self-fertilization, long-distance flash invasion and biogeography shape the population structure of *Pseudosuccinea columella* at the worldwide scale. Mol Ecol 26, 887–903. https://doi.org/10.1111/mec.13984.

Lounnas, M., Correa, A.C., Alda, P., David, P., Dubois, M.P., Calvopiña, M., Caron, Y., Celi-Erazo, M., Dung, B.T., Jarne, P., Loker, E.S., Noya, O., Rodríguez-Hidalgo, R., Toty, C., Uribe, N., Pointier, J.P., Hurtrez-Boussès, S. 2018. Population structure and genetic diversity in the invasive freshwater snail *Galba schirazensis* (Lymnaeidae). Can J Zool. 96, 425–435.

Martín, P.R., Ovando, X.M.C., Seuffert, M.E. 2016. First record of the freshwater snail *Pseudosuccinea columella* (Gastropoda: Lymnaeidae) in southern Pampas (Argentina) and assessment of future spread. Molluscan Res 36, 213–221. https://doi.org/10.1080/13235818.2015.1128602.

Medeiros, C., Scholte, R.G.C., D’Ávila, S., Caldeira, R.L., Carvalho, O. dos S. 2014. Spatial distribution of Lymnaeidae (Mollusca, Basommatophora), intermediate host of *Fasciola hepatica* linnaeus, 1758 (trematoda, digenea) in Brazil. Rev Inst Med Trop Sao Paulo 56, 235–252. https://doi.org/10.1590/S0036-46652014000300010.

Nasibi, S., Salahi Moghaddam, A., Ziaali, N., Akhlaghi, E., Mohammadi, M.A., Hanafi-Bojd, A.A., Fasihi Harandi, M. 2021. Molecular, Morphological, and Spatial Study of *Galba schirazensis* (Pulmonata, Lymnaeidae) from Southeastern Iran.

Ngcamphalala, P.I., Malatji, M.P., Mukaratirwa, S. 2022. Geography and ecology of invasive *Pseudosuccinea columella* (Gastropoda: Lymnaeidae) and implications in the transmission of *Fasciola* species (Digenea: Fasciolidae) - A review. J Helminthol. https://doi.org/10.1017/S0022149X21000717.

Pritsch, I., Beltrão Molento, M. 2018. Recount of reported cases of human fascioliasis in Brazil over the last 60 years. Revista de Patologia Tropical / Journal of Tropical Pathology 47, 75. https://doi.org/10.5216/rpt.v47i2.53636.

Schniebs, K., Glöer, P., Quiñonero-Salgado, S., Lopez-Soriano, J., Hundsdoerfer, A.K. 2018. The first record of *Galba cubensis* (L. Pfeiffer, 1839) (Mollusca: Gastropoda: Lymnaeidae) from open fields of Europe. Folia Malacol 26, 3–15. https://doi.org/10.12657/folmal.026.002.

Smith, C.D., Morgan, E.R., Jones, R.A. 2024. Environmental influences on the distribution and ecology of the fluke intermediate host *Galba truncatula*: A systematic review. Parasitology. https://doi.org/10.1017/S0031182024000957.

Soler, P., Abdala, A.M., Larroza, M. 2023. Genetic characterization and regional distribution of lymnaeid snails in northern Patagonia, Argentina. Vet Parasitol Reg Stud Reports 44. https://doi.org/10.1016/j.vprsr.2023.100919.

Vázquez A. A., Sánchez J., Alba A., Pointier J.-P., Hurtrez-Boussès, S. 2015. Natural prevalence in Cuban populations of the lymnaeid snail *Galba cubensis* infected with the liver fluke *Fasciola hepatica*: small values do matter. Parasitol. Res. 114: 4205–4210. https://doi.org/10.1007/s00436-015-4653-2.

Vázquez, A.A., Alda, P., Lounnas, M., Sabourin, E., Alba, A., Pointier, J.P., Hurtrez-Boussès, S. 2018. Lymnaeid snails hosts *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: Digenea): A worldwide review. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. https://doi.org/10.1079/PAVSNNR201813062.

Vignoles Ph., Novobilský A., Höglund J., Kašný M., Pancrác J., Dreyfuss G., Pointier J.-P., Rondelaud, D. 2014. *Lymnaea cubensis*, an experimental intermediate host for *Fascioloides magna*. Folia Parasitol. 61: 185–188. https://doi.org/10.14411/fp.2014.020.

3.6 SUPPLEMENTARY MATERIAL

Table S1. Environmental variable values extracted from the pixels corresponding to occurrence points of *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula* in South America.

Species	Altitude	Maximum temperature	Minimum temperature	Precipitation
Pseudosuccinea columella	445	30.35	18.8	87
Pseudosuccinea columella	1,920	22.4	11.25	107
Pseudosuccinea columella	2,107	21.6	13.2	195
Pseudosuccinea columella	2,860	22.5	7.3	83.5
Pseudosuccinea columella	73	33.15	23.3	91
Pseudosuccinea columella	703	27.55	16.65	42.5
Pseudosuccinea columella	74	27.15	16.4	59
Pseudosuccinea columella	2,110	22.3	13.7	199.5
Pseudosuccinea columella	1,214	25.7	11.65	21.5
Pseudosuccinea columella	341	21.85	9	56.5
Pseudosuccinea columella	495	24.75	11.5	44
Pseudosuccinea columella	1,489	24.7	10.5	20
Pseudosuccinea columella	1,025	23.45	10.4	35
Pseudosuccinea columella	242	28.75	15.3	56.5
Pseudosuccinea columella	86	26.75	15.6	74
Pseudosuccinea columella	9	22.75	12.85	65
Pseudosuccinea columella	11	30.55	20.55	7
Pseudosuccinea columella	7	29.95	21.55	22
Pseudosuccinea columella	72	27.1	16.5	59
Pseudosuccinea columella	183	29.15	15.3	58
Pseudosuccinea columella	53	26.25	15.45	86.5
Pseudosuccinea columella	52	26.3	15.4	86.5
Pseudosuccinea columella	507	24.65	11.4	39.5
Pseudosuccinea columella	742	23.65	10.35	44
Pseudosuccinea columella	54	29.1	22.35	154.5
Pseudosuccinea columella	53	31.55	22.9	247
Pseudosuccinea columella	1,685	25.85	15.3	226.5
Pseudosuccinea columella	1,641	25.85	15.3	226.5
Pseudosuccinea columella	1,131	25.5	17.15	129
Pseudosuccinea columella	977	27.9	19.05	116.5
Pseudosuccinea columella	1,100	26	18.85	120
Pseudosuccinea columella	1,297	26.4	15.5	172
Pseudosuccinea columella	665	27.1	15.6	59
Pseudosuccinea columella	709	27.05	15.55	61.5
Pseudosuccinea columella	806	27.5	16.85	51.5
Pseudosuccinea columella	230	25.15	15.2	75
Pseudosuccinea columella	831	20.85	12	103.5
Pseudosuccinea columella	649	21.75	12.85	96.5
Pseudosuccinea columella	98	23.7	14.75	82
Pseudosuccinea columella	50	23.7	15.9	86
Pseudosuccinea columella	82	26.75	15.3	91
Pseudosuccinea columella	9	25.05	16.05	84.5
Pseudosuccinea columella	454	23.75	13.25	97.5
Pseudosuccinea columella	11	23.75	15.05	86
Pseudosuccinea columella	69	24.1	15.7	87
Pseudosuccinea columella	12	25.4	16.2	77.5
Pseudosuccinea columella	65	24.85	15.75	90

Pseudosuccinea columella	25	24.45	16	97
Pseudosuccinea columella	6	24.5	15.95	110.5
Pseudosuccinea columella	561	23.8	13.05	95.5
Pseudosuccinea columella	250	23.95	14	98.5
Pseudosuccinea columella	9	23.35	16.3	95
Pseudosuccinea columella	562	24.55	12.9	88.5
Pseudosuccinea columella	7	24.3	16.6	101.5
Pseudosuccinea columella	937	19.6	10.55	114.5
Pseudosuccinea columella	1,001	22.15	12.4	94.5
Pseudosuccinea columella	896	22.65	13.2	102
Pseudosuccinea columella	713	25.2	11.95	69.5
Pseudosuccinea columella	97	25	16.8	119.5
Pseudosuccinea columella	926	22.8	12.5	86.5
Pseudosuccinea columella	859	22.9	12.95	83.5
Pseudosuccinea columella	615	27.95	15.2	41
Pseudosuccinea columella	726	27.5	16.75	47.5
Pseudosuccinea columella	446	30.8	19.1	41.5
Pseudosuccinea columella	646	26.35	14.75	33
Pseudosuccinea columella	469	27.9	16.4	52
Pseudosuccinea columella	585	28.85	18.05	36
Pseudosuccinea columella	652	27.25	15.05	56.5
Pseudosuccinea columella	413	30	17.6	45.5
Pseudosuccinea columella	542	27.7	15.7	33
Pseudosuccinea columella	500	28.15	16.1	57.5
Pseudosuccinea columella	883	26.6	16.7	49
Pseudosuccinea columella	561	29.8	18.1	50.5
Pseudosuccinea columella	568	27.15	15.35	39.5
Pseudosuccinea columella	597	25.8	14.45	38.5
Pseudosuccinea columella	728	24.85	14.35	54.5
Pseudosuccinea columella	546	25.45	15.65	68
Pseudosuccinea columella	340	30.55	19.25	43
Pseudosuccinea columella	696	27.2	15.9	59.5
Pseudosuccinea columella	597	29.05	17.35	51
Pseudosuccinea columella	698	24.4	13.85	50
Pseudosuccinea columella	602	26	15.25	40
Pseudosuccinea columella	884	26.25	15.7	54
Pseudosuccinea columella	502	30.05	18	56
Pseudosuccinea columella	718	27.2	14.35	32.5
Pseudosuccinea columella	552	27.15	16	42.5
Pseudosuccinea columella	613	26.55	15.3	38.5
Pseudosuccinea columella	571	29.65	18.2	46
Pseudosuccinea columella	462	30.8	18.9	36.5
Pseudosuccinea columella	518	27.7	16.3	41
Pseudosuccinea columella	624	27.25	15.25	37
Pseudosuccinea columella	799	27.9	16.55	48.5
Pseudosuccinea columella	907	25.45	16.95	50
Pseudosuccinea columella	663	27.65	16.7	58
Pseudosuccinea columella	692	26.85	15.8	52.5
Pseudosuccinea columella	30	27.15	17.75	93
Pseudosuccinea columella	370	30.65	18.75	36.5
Pseudosuccinea columella	563	29.3	17.5	51.5
Pseudosuccinea columella	530	30.25	18.45	51.5

Pseudosuccinea columella	733	23.5	13.2	52.5
Pseudosuccinea columella	584	29	18.5	42.5
Pseudosuccinea columella	448	30.9	19.2	46.5
Pseudosuccinea columella	641	25.95	14.55	36
Pseudosuccinea columella	6	25.35	18.85	196.5
Pseudosuccinea columella	810	26.25	15.7	48
Pseudosuccinea columella	440	30.25	18.2	54
Pseudosuccinea columella	899	22.65	12.55	56.5
Pseudosuccinea columella	466	30.15	18.05	36
Pseudosuccinea columella	421	30.7	18.95	35.5
Pseudosuccinea columella	532	26.35	15.4	40.5
Pseudosuccinea columella	421	29.9	17.8	49.5
Pseudosuccinea columella	611	26.6	14.9	43
Pseudosuccinea columella	615	27.55	15.15	31
Pseudosuccinea columella	531	29.2	17.3	53
Pseudosuccinea columella	725	26.15	14.5	47.5
Pseudosuccinea columella	102	25.3	17	183
Pseudosuccinea columella	847	26	13.7	40
Pseudosuccinea columella	581	26.4	15.65	54.5
Pseudosuccinea columella	607	28.85	17.25	49.5
Pseudosuccinea columella	684	25.65	14.85	66.5
Pseudosuccinea columella	818	23.4	13.2	53.5
Pseudosuccinea columella	405	30.4	18.35	40.5
Pseudosuccinea columella	452	30.4	18.6	36.5
Pseudosuccinea columella	593	28.25	16.85	50.5
Pseudosuccinea columella	517	29.85	18.75	31
Pseudosuccinea columella	44	26.2	17.35	145.5
Pseudosuccinea columella	614	28.5	17	49.5
Pseudosuccinea columella	450	30.55	18.35	49
Pseudosuccinea columella	560	29.55	18.35	47.5
Pseudosuccinea columella	428	30.7	18.75	36
Pseudosuccinea columella	528	28.65	17.05	52
Pseudosuccinea columella	490	30.55	18.85	45.5
Pseudosuccinea columella	528	30.6	18.85	43
Pseudosuccinea columella	490	28.9	16.5	28
Pseudosuccinea columella	545	29.9	18	52.5
Pseudosuccinea columella	618	27.1	15.05	32.5
Pseudosuccinea columella	449	29.9	17.6	43
Pseudosuccinea columella	925	25.85	13.9	43
Pseudosuccinea columella	774	26.7	17.25	49.5
Pseudosuccinea columella	1,006	25.65	16.6	51
Pseudosuccinea columella	820	23	12.9	55.5
Pseudosuccinea columella	679	23.7	13.3	51
Pseudosuccinea columella	423	30.65	19.25	44.5
Pseudosuccinea columella	665	26.35	14.9	100.5
Pseudosuccinea columella	591	28.55	17.4	59.5
Pseudosuccinea columella	523	29.85	18.15	47
Pseudosuccinea columella	495	29.9	18.2	48
Pseudosuccinea columella	544	29.05	17.35	49.5
Pseudosuccinea columella	423	30.7	19.1	43
Pseudosuccinea columella	520	29.95	17.75	40.5
Pseudosuccinea columella	934	22.75	12.95	97

Pseudosuccinea columella	15	26.75	17.65	98
Pseudosuccinea columella	881	23.65	13	52
Pseudosuccinea columella	590	28.4	16.9	48
Pseudosuccinea columella	410	29.9	17.7	44
Pseudosuccinea columella	606	27.3	15.45	42.5
Pseudosuccinea columella	373	30.4	18.2	41
Pseudosuccinea columella	434	29.8	17.65	53
Pseudosuccinea columella	885	22.1	12.75	100
Pseudosuccinea columella	548	26.9	16.1	56.5
Pseudosuccinea columella	603	24.7	14.3	45
Pseudosuccinea columella	432	30.35	18.55	39
Pseudosuccinea columella	575	29.3	17.55	52.5
Pseudosuccinea columella	349	30.7	19.15	41
Pseudosuccinea columella	775	27.5	16.45	63
Pseudosuccinea columella	698	28.3	16.85	58
Pseudosuccinea columella	402	30.55	19.2	44
Pseudosuccinea columella	379	30.7	19.15	43.5
Pseudosuccinea columella	569	25.15	15.4	66
Pseudosuccinea columella	674	23.75	13.35	46.5
Pseudosuccinea columella	675	28.2	16.7	54
Pseudosuccinea columella	611	25.2	14.5	40
Pseudosuccinea columella	556	29.6	17.9	49.5
Pseudosuccinea columella	551	28.65	17.05	52
Pseudosuccinea columella	546	29.25	17.6	49
Pseudosuccinea columella	605	29.05	17.35	48.5
Pseudosuccinea columella	615	27.5	15.35	33.5
Pseudosuccinea columella	512	29.9	18.4	48
Pseudosuccinea columella	497	27.4	16.25	44
Pseudosuccinea columella	414	30.2	18.1	53.5
Pseudosuccinea columella	580	29.3	17.6	50.5
Pseudosuccinea columella	624	25.4	15	51.5
Pseudosuccinea columella	30	26	18.7	75
Pseudosuccinea columella	554	24.75	14.7	123.5
Pseudosuccinea columella	682	24	14.1	101.5
Pseudosuccinea columella	124	27.75	17.2	68
Pseudosuccinea columella	38	26.55	17.7	92.5
Pseudosuccinea columella	283	28.2	17.55	72
Pseudosuccinea columella	490	25.7	15.25	90
Pseudosuccinea columella	284	26.95	15.7	81
Pseudosuccinea columella	198	25.65	17.2	87.5
Pseudosuccinea columella	41	27.15	18	74
Pseudosuccinea columella	514	25.3	15.05	94
Pseudosuccinea columella	754	24	13.85	93
Pseudosuccinea columella	470	25.2	15.1	99.5
Pseudosuccinea columella	581	23.5	14.15	120
Pseudosuccinea columella	26	26.75	18.6	90.5
Pseudosuccinea columella	55	28.65	18.4	72.5
Pseudosuccinea columella	150	28.2	17.6	70.5
Pseudosuccinea columella	366	27.05	17.35	104.5
Pseudosuccinea columella	205	28.1	16.95	77
Pseudosuccinea columella	29	26.65	18.15	113.5
Pseudosuccinea columella	17	26.7	17.75	117

Pseudosuccinea columella	16	26.25	18.5	73.5
Pseudosuccinea columella	461	24.9	14.8	94.5
Pseudosuccinea columella	673	23.5	13.95	98.5
Pseudosuccinea columella	201	27.45	16.1	76.5
Pseudosuccinea columella	277	27.8	16.65	85
Pseudosuccinea columella	50	26.65	18.9	89.5
Pseudosuccinea columella	886	22.25	12.65	99
Pseudosuccinea columella	34	27.35	18.3	109.5
Pseudosuccinea columella	575	24.35	14.35	94
Pseudosuccinea columella	389	26.2	15.55	109
Pseudosuccinea columella	261	26.15	16.7	132.5
Pseudosuccinea columella	622	23.6	14.05	104
Pseudosuccinea columella	392	25.8	15.35	92
Pseudosuccinea columella	221	27.8	16.5	85.5
Pseudosuccinea columella	428	26.65	15.6	86
Pseudosuccinea columella	774	25.2	16.8	90
Pseudosuccinea columella	165	28.05	17.9	74
Pseudosuccinea columella	757	24	14.1	119
Pseudosuccinea columella	7	27.3	18.55	108
Pseudosuccinea columella	40	26.3	18.3	91
Pseudosuccinea columella	886	21.75	12.65	130.5
Pseudosuccinea columella	746	23.1	13.7	98.5
Pseudosuccinea columella	393	26.5	15.8	111.5
Pseudosuccinea columella	878	27.15	15.35	63
Pseudosuccinea columella	1,142	23.9	13.8	70
Pseudosuccinea columella	617	25.4	14.7	98.5
Pseudosuccinea columella	920	25.85	14	78.5
Pseudosuccinea columella	893	26.35	14.65	73.5
Pseudosuccinea columella	812	27.2	15	73
Pseudosuccinea columella	1,302	23.65	14.1	80.5
Pseudosuccinea columella	718	26.95	15.8	42.5
Pseudosuccinea columella	1,002	25.3	14.85	59.5
Pseudosuccinea columella	846	26.3	14.2	80
Pseudosuccinea columella	723	26.85	16.35	46
Pseudosuccinea columella	485	30.55	19.15	21.5
Pseudosuccinea columella	953	26.6	14.85	75.5
Pseudosuccinea columella	866	25.2	15	69
Pseudosuccinea columella	904	26	14.75	64.5
Pseudosuccinea columella	714	27.5	16.45	48.5
Pseudosuccinea columella	816	27.3	16.25	41.5
Pseudosuccinea columella	814	26.95	15.35	51
Pseudosuccinea columella	879	26.45	14.25	79.5
Pseudosuccinea columella	909	26.65	16.2	53.5
Pseudosuccinea columella	1,066	25.4	14.9	59
Pseudosuccinea columella	377	27.35	16.85	61.5
Pseudosuccinea columella	766	26.2	14.9	53
Pseudosuccinea columella	735	27	16.45	44.5
Pseudosuccinea columella	864	26.75	14.9	67
Pseudosuccinea columella	380	28.7	18.15	62
Pseudosuccinea columella	936	27	14.85	56
Pseudosuccinea columella	741	27.55	16.7	43
Pseudosuccinea columella	688	25.1	14.75	73

Pseudosuccinea columella	176	27.6	16.7	83.5
Pseudosuccinea columella	181	27.4	17.25	70.5
Pseudosuccinea columella	120	28	18	72
Pseudosuccinea columella	127	27.75	17.3	74.5
Pseudosuccinea columella	618	25.8	14.4	85
Pseudosuccinea columella	134	28.3	17.7	77
Pseudosuccinea columella	163	27.65	17.5	70
Pseudosuccinea columella	629	25.5	14.25	81
Pseudosuccinea columella	326	26.9	16.45	75.5
Pseudosuccinea columella	35	27.1	18.95	69.5
Pseudosuccinea columella	61	27.75	18.95	51.5
Pseudosuccinea columella	636	24.95	14.75	85
Pseudosuccinea columella	585	28.4	19.25	72
Pseudosuccinea columella	668	28.6	18.6	71
Pseudosuccinea columella	616	28.8	19	59
Pseudosuccinea columella	807	27.25	16.9	64
Pseudosuccinea columella	870	28.15	17.35	96
Pseudosuccinea columella	902	27.45	17.95	77.5
Pseudosuccinea columella	608	28.35	19.15	72.5
Pseudosuccinea columella	495	29.45	19.9	74
Pseudosuccinea columella	211	31.15	19.85	45.5
Pseudosuccinea columella	24	30.65	23.85	50
Pseudosuccinea columella	566	26.95	18	47.5
Pseudosuccinea columella	65	31.55	21.85	229.5
Pseudosuccinea columella	20	31.25	22.55	225
Pseudosuccinea columella	36	31.55	22.85	247.5
Pseudosuccinea columella	3	25.05	15.8	88.5
Pseudosuccinea columella	47	24.95	15.7	64
Pseudosuccinea columella	5	26.45	17.55	145.5
Pseudosuccinea columella	474	24.9	14.8	94.5
Pseudosuccinea columella	63	27.15	17.55	103.5
Pseudosuccinea columella	67	26.25	16.25	102.5
Pseudosuccinea columella	258	26.25	16.25	102.5
Pseudosuccinea columella	91	26.8	17	105.5
Pseudosuccinea columella	116	26.8	17	105.5
Pseudosuccinea columella	329	26.25	16.25	102.5
Pseudosuccinea columella	65	25.9	18.2	93.5
Pseudosuccinea columella	24	25.9	18.2	93.5
Pseudosuccinea columella	75	26.9	19.2	106.5
Pseudosuccinea columella	13	26.85	19.35	98.5
Pseudosuccinea columella	7	26.95	18.85	104.5
Pseudosuccinea columella	23	26.85	19.35	98.5
Pseudosuccinea columella	24	25.9	18.2	93.5
Pseudosuccinea columella	65	25.9	18.2	93.5
Pseudosuccinea columella	350	30.25	19.9	66.5
Pseudosuccinea columella	349	30.1	19.7	67.5
Pseudosuccinea columella	349	30.1	19.7	67.5
Pseudosuccinea columella	356	29.95	19.75	66.5
Pseudosuccinea columella	340	30.35	20.15	64.5
Pseudosuccinea columella	334	30.15	20.05	63
Pseudosuccinea columella	342	30.2	20.05	65.5
Pseudosuccinea columella	351	30.1	19.95	66

Pseudosuccinea columella	364	29.95	19.8	64
Pseudosuccinea columella	365	29.65	19.5	67.5
Pseudosuccinea columella	401	29.65	19.6	61.5
Pseudosuccinea columella	296	30.45	20	66.5
Pseudosuccinea columella	341	29.9	19.75	66
Pseudosuccinea columella	343	30.2	20	64.5
Pseudosuccinea columella	370	29.7	19.7	61.5
Pseudosuccinea columella	410	29.75	19.75	60.5
Pseudosuccinea columella	499	28.9	18.9	64.5
Pseudosuccinea columella	457	29.65	19.6	62
Pseudosuccinea columella	337	29.65	19.65	59
Pseudosuccinea columella	446	29.5	19.55	59.5
Pseudosuccinea columella	446	29.6	19.55	63.5
Pseudosuccinea columella	463	29.6	20	71.5
Pseudosuccinea columella	292	30.95	20.05	60
Pseudosuccinea columella	294	30.65	20	62.5
Pseudosuccinea columella	295	30.95	20	59.5
Pseudosuccinea columella	293	30.95	20.1	60.5
Pseudosuccinea columella	299	30.7	19.95	62.5
Pseudosuccinea columella	295	30.7	20.05	63.5
Pseudosuccinea columella	301	30.95	20.05	60
Galba cubensis	425	31.1	19.95	90
Galba cubensis	452	30.85	19.8	83
Galba cubensis	2,052	21.55	10.8	88.5
Galba cubensis	1,037	25.15	15.3	102.5
Galba cubensis	445	30.35	18.8	87
Galba cubensis	13	31.2	23.3	89
Galba cubensis	7	29.95	21.4	22.5
Galba cubensis	6	30.25	21.2	15
Galba cubensis	1,030	24.9	12.75	0
Galba cubensis	19	22.85	16.65	0
Galba cubensis	413	22.85	11.95	0.5
Galba cubensis	24	31.15	23.25	89
Galba cubensis	22	31.45	23.45	117
Galba cubensis	441	30.75	19.3	89
Galba cubensis	507	29	17.95	95
Galba cubensis	143	24.25	13	75
Galba cubensis	40	25.65	14.1	84
Galba cubensis	988	26.05	13.3	21
Galba cubensis	1,629	25.2	8.8	1.5
Galba cubensis	757	27.3	16.6	44
Galba cubensis	1,339	25.95	16.1	212
Galba cubensis	337	22.55	12.75	1
Galba cubensis	1,376	20.8	4.9	21.5
Galba cubensis	1,387	20.75	4.9	21.5
Galba cubensis	1,849	23.05	11.35	96
Galba cubensis	3	24.65	13	45
Galba cubensis	519	30.75	17.55	9.5
Galba cubensis	150	31.3	18.75	4
Galba cubensis	2,396	24.45	10	67
Galba cubensis	1,387	20.75	4.9	21.5
Galba cubensis	1,210	21.65	5.2	19

Galba cubensis	1,245	21.55	5.05	18
Galba cubensis	1,340	21.1	5	26
Galba cubensis	1,351	24.65	15.3	195.5
Galba cubensis	4,384	12.25	-0.95	64.5
Galba cubensis	50	24.8	12.35	38
Galba cubensis	41	24.85	12.4	39.5
Galba cubensis	82	24.65	11.95	43
Galba cubensis	44	24.75	12.3	43
Galba cubensis	55	24.7	12.15	44
Galba cubensis	103	24.5	11.6	42.5
Galba cubensis	10	21.5	14.95	0
Galba cubensis	1,629	25.2	8.8	1.5
Galba schirazensis	2,154	19.95	8.65	103
Galba schirazensis	2,649	17.5	6.2	122
Galba schirazensis	2,107	24.05	14.25	101.5
Galba schirazensis	1,662	26.55	16.65	206
Galba schirazensis	1,779	22.25	11.95	95.5
Galba schirazensis	1,361	27.2	19	148.5
Galba schirazensis	2,987	18.5	7.5	70.5
Galba schirazensis	3,738	10.75	2.45	95.5
Galba schirazensis	1,489	22.05	12.5	153.5
Galba schirazensis	2,646	19	7.7	102.5
Galba schirazensis	1,570	26.7	10.5	0
Galba schirazensis	2,722	15.45	4.7	96
Galba schirazensis	2,059	23.35	13.55	96.5
Galba schirazensis	2,747	22.85	7.65	86
Galba schirazensis	2,799	21.7	6.55	83
Galba schirazensis	2,860	22.5	7.3	83.5
Galba schirazensis	2,880	22.05	6.85	81.5
Galba schirazensis	2,676	23.05	7.6	85
Galba schirazensis	3,532	21.05	6.3	82
Galba schirazensis	2,331	23.75	8.95	75
Galba schirazensis	2,090	24.85	10.55	61.5
Galba schirazensis	2,059	24.45	9.85	61.5
Galba schirazensis	2,013	25.35	11.4	57
Galba schirazensis	1,997	25.35	11.4	57
Galba schirazensis	2,655	19	7.7	102.5
Galba schirazensis	2,824	18.45	6.85	119.5
Galba schirazensis	1,532	22.3	13.5	99.5
Galba schirazensis	1,825	23.9	13.55	108
Galba schirazensis	3,328	16.35	5.75	117.5
Galba schirazensis	2,827	19.45	8.2	92
Galba schirazensis	2,797	20.2	7.75	112
Galba schirazensis	133	22.85	13.65	1
Galba schirazensis	1,552	26.2	13.7	71
Galba schirazensis	1,701	26.25	13.7	63
Galba schirazensis	519	30.75	17.55	9.5
Galba schirazensis	150	31.3	18.75	4
Galba schirazensis	1,613	20.9	12.95	150
Galba schirazensis	1,685	25.85	15.3	226.5
Galba schirazensis	1,581	25.85	15.3	226.5
Galba schirazensis	2,661	23.05	7.6	85

Galba schirazensis	2,107	21.6	13.2	195
Galba schirazensis	2,868	18.45	6.85	119.5
Galba schirazensis	838	27.6	17.6	113.5
Galba schirazensis	1,862	19.85	10.9	87
Galba schirazensis	2,245	21.1	10.45	92.5
Galba schirazensis	3,277	12.9	3.85	82
Galba schirazensis	2,610	22.6	7.2	77
Galba schirazensis	1,805	21.25	11.65	105.5
Galba schirazensis	1,213	25.15	15.9	99
Galba truncatula	1,920	22.4	11.25	107
Galba truncatula	2,893	17.3	6.2	96
Galba truncatula	3,441	12.65	2.15	99.5
Galba truncatula	3,919	11.45	1.05	94
Galba truncatula	1,030	24.9	12.75	0
Galba truncatula	3,781	11.45	1.05	94
Galba truncatula	4,012	11.45	1.05	94
Galba truncatula	2,747	22.85	7.65	86
Galba truncatula	2,700	22.95	7.5	81
Galba truncatula	2,799	21.7	6.55	83
Galba truncatula	2,860	22.5	7.3	83.5
Galba truncatula	2,880	22.05	6.85	81.5
Galba truncatula	2,676	23.05	7.6	85
Galba truncatula	2,736	21.9	6.7	89
Galba truncatula	3,064	21.5	6.55	86.5
Galba truncatula	3,532	21.05	6.3	82
Galba truncatula	2,842	22.05	6.95	90
Galba truncatula	2,090	24.85	10.55	61.5
Galba truncatula	1,892	19.45	4.8	10
Galba truncatula	3,846	15.55	2.95	42
Galba truncatula	3,833	15.6	3.25	50
Galba truncatula	3,853	15.4	2.4	48
Galba truncatula	3,844	16	2.6	44
Galba truncatula	3,864	16	2.6	44
Galba truncatula	3,834	15.95	2.55	42.5
Galba truncatula	3,856	16.15	2.55	41.5
Galba truncatula	3,883	16.35	2.35	38.5
Galba truncatula	3,831	16	2.75	41
Galba truncatula	3,859	15.3	3.15	38
Galba truncatula	3,828	15.8	2.85	39.5
Galba truncatula	3,829	15.9	2.8	39
Galba truncatula	3,862	16.15	2.25	36.5
Galba truncatula	3,902	16.45	2.15	33.5
Galba truncatula	3,834	15.55	2.75	36
Galba truncatula	3,872	16.85	2	32.5
Galba truncatula	3,968	16.15	2.4	32.5
Galba truncatula	3,862	16.15	2.25	36.5
Galba truncatula	3,845	16	2.8	39.5
Galba truncatula	3,863	17.05	2.1	31
Galba truncatula	3,888	16	2.6	44
Galba truncatula	3,896	15.75	2.4	44.5
Galba truncatula	3,841	16	2.7	43
Galba truncatula	3,831	15.8	2.85	39.5

Galba truncatula	3,850	16.3	2.45	38.5
Galba truncatula	3,980	15.05	2.1	50
Galba truncatula	3,974	15.2	2.15	48
Galba truncatula	3,995	15.2	2.15	48
Galba truncatula	3,959	15.25	2.55	39
Galba truncatula	3,895	18.2	1.8	21.5
Galba truncatula	3,886	18.3	1.8	21
Galba truncatula	3,839	18.75	1.55	22
Galba truncatula	3,505	14.25	3.45	85
Galba truncatula	2,280	19.95	9.05	99.5
Galba truncatula	3,721	12.3	1.85	85.5
Galba truncatula	2,760	15.35	7.25	92
Galba truncatula	2,906	15.35	7.25	92
Galba truncatula	2,200	18.8	10.6	77
Galba truncatula	2,922	15.35	7.25	92
Galba truncatula	3,161	20.4	5.5	90.5
Galba truncatula	3,050	21.05	6	88.5
Galba truncatula	2,924	22.05	6.85	81.5
Galba truncatula	2,665	23.1	7.6	80.5
Galba truncatula	2,089	25.15	11	57
Galba truncatula	2,084	16.75	1.9	20.5
Galba truncatula	1,897	17.6	2.3	20.5
Galba truncatula	1,935	18.35	2.25	25.5
Galba truncatula	1,884	18.35	2.25	25.5
Galba truncatula	886	22.25	12.65	99
Galba truncatula	886	21.75	12.65	130.5
Galba truncatula	1,066	25.4	14.9	59
Galba truncatula	13	16.3	6.9	83.5
Galba truncatula	252	20.4	6.55	38.5
Galba truncatula	319	23	10.2	13.5

4. CHAPTER 3 – ECOLOGICAL NICHE AND DISTRIBUTION PATTERNS OF SNAILS THAT HOST *Fasciola hepatica* ACROSS SOUTH AMERICA: UNVEILING THE HIDDEN LANDSCAPES

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ABSTRACT

In South America, *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula* snails serve as vectors of *Fasciola hepatica*, posing major challenges to public and animal health. This study identified environmentally suitable areas for these species using ecological niche models, including Maxent, Random Forest, and Support Vector Machine. Occurrence points (n = 797) were compiled from the international literature (PRISMA protocol) and the malacological collection of FIOCRUZ Institute, Rio de Janeiro, Brazil. *P. columella* (51%), and *G. truncatula* (18%) had the highest records. The distribution of *Pseudosuccinea columella* and *G. cubensis* was influenced by precipitation exhibiting a broad potential across South America. In contrast, the distribution of *G. schirazensis* and *G. truncatula* was influenced by temperature and was limited to high-altitude areas. An ecological niche overlap was observed between *P. columella* and *G. cubensis*; and *G. schirazensis* and *G. truncatula*. Moreover, suitable areas for these species were identified in Argentina, Peru, Bolivia, and Brazil. In Brazil, *P. columella* demonstrated significant environmental suitability in the Cerrado and the Amazon Forest biomes, which have been impacted by land use of pasture expansion and deforestation from unsupervised cattle production. Therefore, predicting the contamination of new areas is critical to mitigate future liver fluke infections in suitable hosts (natives and rural communities, capybaras, etc.) from regions affected by large livestock movements in the continent. As we face climate change threats to vector-borne diseases, these modeling data highlight the need for significant preventive strategies, directed to the welfare and social impact of all South American countries.

Keywords: Lymnaeids, Latin America, Predicted Model, Maxent, Random Forest, and Support Vector Machine.

4.1 INTRODUCTION

Fasciolosis, caused by *Fasciola hepatica* or *F. gigantica* is a global infectious disease that affects domestic and wildlife animals and humans (Alba et al., 2020; Alda et al., 2021). An estimated 180 million people are at risk of infection, with 30 to 70 million individuals currently infected by *Fasciola* spp (Sabourin et al., 2018). Animal infections are highly prevalent, affecting over 600 million cattle worldwide (Toet et al., 2014). The success of this disease is partly explained by the ability of *Fasciola* parasites to infect a wide range of freshwater snails, the intermediate hosts of these parasites (Vazquez et al., 2018; Galavani et al., 2024). Approximately 30 species of snails from the Family Lymnaeidae serve as hosts for Fasciola worldwide (Vazquez et al., 2023; Galavani et al., 2024).

In the Americas, lymnaeids are widely distributed from Canada to Argentina (Vazquez et al., 2018; Alda et al., 2021). In South America, the snail species involved in fasciolosis are *Pseudosuccinea columella* (Say, 1817), *Galba cubensis* (Pfeiffer, 1839), *Galba neotropica* (Bargues, 2007)—considered synonymous of *G. cubensis*—, *Galba schirazensis* (Küster, 1862), *Galba truncatula* (Müller, 1774), *Galba cousini* (Jousseaume, 1887) and Galba meridensis (Bargues, 2011) (Correa et al., 2011; Alda et al., 2021). The last two species have, however, a much more restricted distribution, as they are only present in the northwest region. The role of *G. schirazensis* as a host of *F. hepatica* is still under debate (Caron et al., 2017; Alda et al., 2021; Nasibi et al., 2021).

Even though the distribution of lymnaeid snails can directly influence fasciolosis cases, not all species are equally effective in transmitting the parasite (Alba et al., 2020). Understanding the spatial distribution of lymnaeid snails is crucial for identifying areas at risk of transmitting *F. hepatica* and improving the ability to anticipate and effectively prevent the (re)emergence of the disease. Environmental factors such as water velocity, soil type, evapotranspiration, precipitation, and temperature can affect the presence and abundance of these snails (Vazquez et al., 2018; Alba et al., 2020; Almeida et al., 2024). Climate change, environmental degradation, and changes in land use for pasture also influence snail distribution, leading to the (re)emergence of fasciolosis in some regions (Alba et al., 2020; Almeida et al., 2024). In Brazil, for instance, fasciolosis has recently expanded into regions with few or no previously recorded cases, particularly in the central-western and northern parts of the country, areas affected by deforestation for pastureland (Almeida et al., 2024).

Previous authors have studied the actual distribution of South American lymnaeid species; however, this distribution still requires further clarification and the environmental suitability remains unknown, posing a risk to animal and human health (Alda et al., 2021). The movement of infected mammals, whether through seasonal cattle migration or the introduction of wild mammals, into areas potentially suitable for snail species (capable of transmitting fasciolosis), promotes the establishment of disease transmission. Thus, mapping the distribution of lymnaeid snails and assessing their environmental suitability is essential for understanding the occurrence of *F. hepatica* and the epidemiology of fasciolosis to formulate targeted interventions (Testai et al., 2023; Almeida et al., 2024; An et al., 2024).

This study aimed to use ecological niche modeling (ENM) to predict areas of environmental suitability for lymnaeid species involved in the epidemiology of *F. hepatica* in South America. We developed an ENM for *P. columella*; *G. cubensis*; *G. schirazensis*; and *G. truncatula* which have the largest distribution in South America. Since the role of *G. schirazensis* as a transmitter of *Fasciola* is still debated (Caron et al., 2017; Alba et al., 2020), and mixed populations of *Galba* are found together (Standley et al., 2013; Alda et al., 2018) we have chosen to include this species in our study.

4.2 MATERIAL AND METHODS

4.2.1 Study area

The research area covers all of South America, which exhibits a variety of climate patterns, ranging from tropical, subtropical, and temperate conditions in 14 biomes (Delgado et al., 2022; Jaramillo et al., 2023). The region has a remarkable geographic diversity, ranging from vast deserts like the Atacama and Salar de Uyuni to the world's largest tropical rainforest, the Amazon (Garreaud et al., 2009; Delgado et al., 2022).

The biodiversity of South America is also distinguished by the presence of a rich fauna of non-marine mollusks. Initial estimates suggest the existence of at least 1,400 species of these animals, including mussels, freshwater gastropods, and terrestrial snails. However, this figure represents only a fraction of the total diversity (Miyahira et al., 2022). Certain regions of the continent, such as Lake Titicaca on the

Bolivia-Peru border and the Lower Uruguay River between Argentina and Uruguay, are among the top 20 global hotspots for freshwater gastropods (Strong et al., 2008; Miyahira et al., 2022).

4.2.2 Database of lymnaeids occurrence points

Locations of occurrence for the lymnaeid species were obtained through a literature review, following the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA) guidelines, and by consulting the malacological collection of the Oswaldo Cruz Institute, in Rio de Janeiro, RJ, Brazil. Due to the cryptic nature of *Galba* species, which are not easily morphologically distinguishable, only occurrence points confirmed by molecular biology were included. However, in the case of *P. columella*, we considered studies identifying the species through either molecular biology methods or morphological identification.

The resulting database comprised a total of 797 occurrences, distributed as 403 (51%) occurrences of *P. columella*, 120 (15%) of *G. cubensis*, 130 (16%) of *G. schirazensis*, and 144 (18%) of *G. truncatula*. Records of *G. cubensis* were grouped with *G. neotropica* occurrence points, as these species are considered synonymous. The raw data—including the PRISMA protocol, selected articles, and geographical coordinates of occurrence sites—are on the Mendeley Data repository (https://data.mendeley.com/datasets/r2cc38vz8k/1). Access to the data is provided through the DOI: 10.17632/r2cc38vz8k.1.

4.2.3 Environmental variables database

Environmental variables were categorized into climate and landscape. The 19 bioclimatic variables were obtained from WorldClim (https://worldclim.org/), version 2.0, with a resolution of 30 arc-seconds (~1 km²) in raster format (GeoTIFF). The Land Surface Temperature (LST) and Normalized Difference Vegetation Index (NDVI) variables were acquired through the Google Earth Engine (GEE, https://earthengine.google.com/), also at a resolution of 1 km². The LST was derived using the MODIS/061/MOD11A2 product, while the NDVI was derived using the MODIS/061/MOD13A2 product. The dataset for the four lymnaeid species spans the period from 1974 to 2019. The temporal resolution of the GEE satellite images was determined based on the occurrence data period (Testai et al., 2023).

4.2.4 Ecological Niche Model (ENM)

Initially, the raw occurrence points of the species were filtered based on the modeling area, excluding those outside South America. Then, we applied spatial filters to minimize the influence of variables that are difficult to measure and systematic errors, such as bias caused by sampling effort and its consequent influence on the density of occurrence and environmental characteristics of each capture area (Varela et al., 2014). The following filters were applied to the datasets: i) removal of points with duplicate coordinates; ii) exclusivity of occurrence per pixel; and iii) removal of occurrences in pixels without data.

Variable selection for the models was based on the Pearson correlation, choosing those with a correlation within the range of -0.7 to 0.7. Pearson correlation was calculated considering only the pixels present in the South American area. Thus, nine environmental variables were incorporated into the models: BIO2 (Mean Diurnal Range), BIO3 (Isothermality), BIO5 (Max Temperature of Warmest Month), BIO14 Precipitation of Driest Month, BIO15 (Precipitation Seasonality), BIO18 (Precipitation of Warmest Quarter), BIO19 (Precipitation of Coldest Quarter), LST, and NDVI (Fig. S1).

In the process of generating ENM, some algorithms use only occurrence points, while others require both presence and absence points. In this case, pseudo-absence points were needed for each species in areas with low or absent environmental suitability. For this purpose, an inclusion buffer (defining the maximum limit for sampling pseudo-absences) and an exclusion buffer (a region close to the occurrence points where pseudo-absences were not generated) were considered. Thus, pseudo-absences were generated in the area resulting from the difference between the inclusion buffer and the exclusion buffer. The application of this geographic filter to generate pseudo-absence points around each occurrence leads to a significant improvement in results (Barbet-Massin et al., 2012). The exclusion buffer was defined for all species within a 10 km radius, considering environmentally similar areas near occurrence points. The inclusion buffer was set at 600 km, approximately the average distance between occurrence clusters.

To establish a threshold for identifying regions with limited environmental suitability for creating pseudo-absences, we analyzed the occurrence data of each species using the climatic envelope method (Bioclim), which constructs a multidimensional bounding box within the environmental space. The environmental suitability of the occurrence points in this model was evaluated, considering the 90% of pixels with the highest suitability as occurrence areas and the 10% of pixels with the lowest suitability as absence areas, thus defining an interval for generating pseudo-absences (Barbet-Massin et al., 2012; Testai et al., 2023).

Models were generated using the ModleR platform (Sánchez-Tapia et al., 2020). Three algorithms were employed in this process: Maxent, Random Forest (RF), and Support Vector Machine (SVM). The complete modeling process is outlined in Fig. 1.

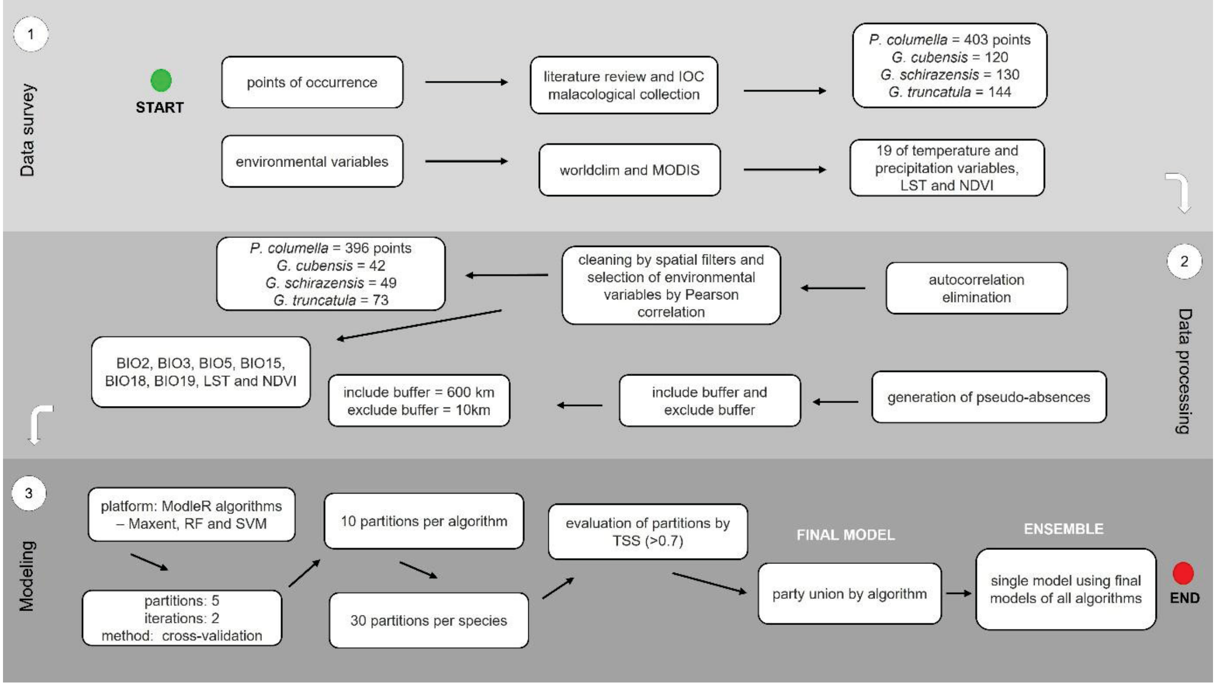


Figure 1. Flowchart of the search stages involved in ecological niche modeling (ENM).

Finally, the occurrence dataset was divided into five partitions. In each partition, we reserved 20% of the occurrences for testing, while we used 80% for training the algorithms. K-fold cross-validation with two iterations was applied for this division, resulting in 10 partitions (models) per algorithm. The performance evaluation of the partitions was conducted using True Skill Statistics (TSS). The TSS value was used as a criterion for selecting partitions with satisfactory results, with those having TSS values above 0.7 considered to be of good quality. Consequently, only partitions with TSS values exceeding 0.7 were included in the construction of the final models for each algorithm.

Final models for each algorithm were then used to build the Ensemble model, which combines the best partitions from each algorithm into a single model. In the final step, variance partitioning was applied to assess the contribution degree (Adj R^2) of each variable in defining the ecological niche for each of the generated Ensemble models. For the variance partitioning analysis, we categorized the components (variables) into four groups: temperature (BIO2, BIO3, and BIO5); precipitation (BIO14, BIO15, BIO18, and BIO19); LST; and NDVI.

4.2.5 Ecological niche overlap

We quantified niche overlap among the four modeled species using Schoener's D index. This index evaluates the degree of resource or habitat characteristic sharing between two species by comparing habitat suitability estimates in each grid cell resulting from the modeling. The index values range from 0 (no match – low niche similarity) to 1 (identical maps – high niche similarity) (Püts et al., 2020; Campos et al., 2023). We calculated the index from the Ensemble model, which consisted of binary models predicting areas of high suitability for the species by integrating predictions from different algorithms in a consensual manner (Sánchez-Tapia et al., 2020).

4.2.6 Environmental suitability of lymnaeids in Brazil, Bolivia, and Peru—countries with high incidences of fasciolosis in South America

Ensemble models for each species were used to analyze areas of environmental suitability. The analysis of the percentage of pixels with low, medium, and high suitability within the federative units of Brazil, Bolivia, and Peru (Bargues et al., 2021; Almeida et al., 2024) (Fig. S2) was conducted using the Google Colab platform (https://colab.research.google.com/) and the Python programming language. To classify the pixel (interpreted as territory) values from the Ensemble models into suitability categories, threshold values were applied: pixels with values < 0.33 were designated as low suitability, those with values between 0.33 and 0.66 as medium suitability, and those > 0.66 as high suitability. Data on the percentages of pixels with medium to high suitability for each federative unit were georeferenced to generate thematic maps using QGIS software (version 3.26).

4.3 RESULTS

4.3.1 Geographic distribution and data filtering of lymnaeid species in South America

After applying spatial filters to the lymnaeids databases, the occurrence points were reduced to 328 out of 403 (81% of the original dataset) for *P. columella*, 43 out of 120 (35%) for *G. cubensis*, 49 out of 130 (37%) for *G. schirazensis*, and 73 out of 144 (50%) for *G. truncatula*. The occurrence points used in the models for each species after cleaning are provided in Table S1. The four lymnaeid species were found to have been collected in multiple countries across South America, with no records in Paraguay, Suriname, or Guyana. *Pseudosuccinea columella* and *G. cubensis* exhibited broad geographic distributions across South America. Brazil had the highest number of records for *P. columella*, while Argentina had the most records for *G. cubensis*, with a single record of this species in Brazil (state of Minas Gerais). *Galba schirazensis* was predominantly found in countries along the Pacific coast including Peru, Ecuador, Colombia, and Venezuela. *Galba truncatula* also had a widespread distribution, mainly in Bolivia, and the only species recorded in Chile (Fig. S3).

For each species, 1,200 pseudo-absence points were generated within low-suitability areas. The created buffers determined that these points were distributed within the inclusion area but outside the exclusion area.

4.3.2 Ecological Niche Modeling (ENM) of lymnaeid species

In evaluating partitions for the construction of the final model for each species, only partitions demonstrating good performance (TSS > 0.7) were considered. For *P. columella*, *G. schirazensis*, and *G. truncatula*, this resulted in constructing the final model using 10 partitions per algorithm. Consequently, in the Ensemble model of these species, all 30 partitions were utilized. In contrast, only 25 partitions were utilized in the Ensemble model construction for *G. cubensis*, where the Maxent and RF algorithms achieved TSS results above the cutoff point; however, they demonstrated considerable variability (0.50 – 0.98 and 0.52 – 0.84, respectively) in the *G. cubensis* models. The three algorithms also presented outliers in TSS values for the partitions of *G. schirazensis* (Maxent 0.85 – 1; RF 0.78 – 0.95; SVM 0.75 – 1) (Fig. S4).

Different algorithms showed varied performances in the Ensemble models: RF achieved the highest performance for *P. columella* (TSS > 0.91), SVM excelled for *G. cubensis* (TSS > 0.93), and also demonstrated superior performance in niche modeling for *G. schirazensis* (TSS > 0.92), while Maxent achieved excellent results for *G. truncatula* (TSS > 0.92).

4.3.3 Contribution of environmental variables to species models

Results from the variance partitioning analysis demonstrated that precipitation variables (BIO14, BIO15, BIO18, and BIO19) represented the largest percentage of contribution to the model for *P. columella*, totaling 29% of the explained variation (Adj $R^2 = 0.29$), followed by temperature variables (BIO2, BIO3, and BIO5), which contributed 18% (Adj $R^2 = 0.18$). For the model of *G. cubensis*, precipitation was also the variable that contributed the most, explaining 12% of the variation (Adj $R^2 = 0.12$) (Fig. 2).

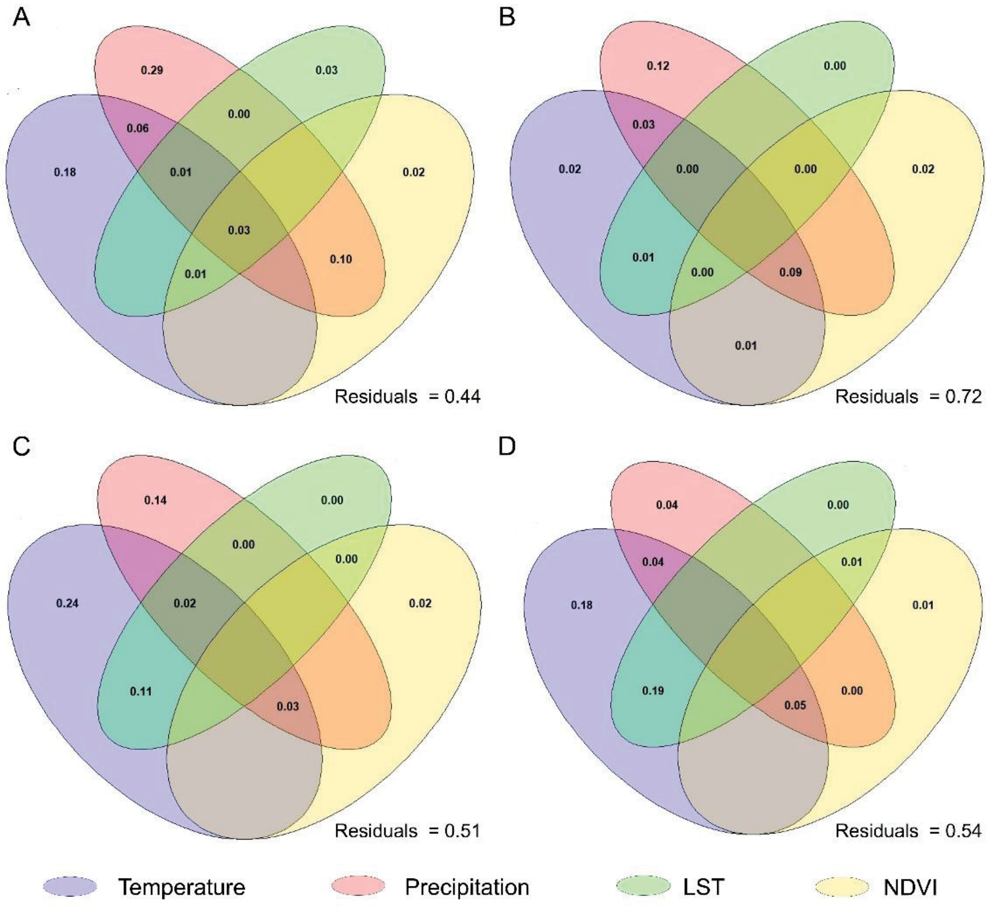


Figure 2. Variance partitioning analysis of the contributions of environmental variables in ecological niche modeling of (A) *Pseudosuccinea columella*; (B) *Galba cubensis*; (C) *Galba schirazensis*; and (D) *Galba truncatula* in South America.
Note. Values less than zero are not shown and are interpreted as zero, corresponding to cases where explanatory variables explain lower variation than normal random variables.

In contrast, temperature was the most important variable in the models of *G. schirazensis* (24%) and *G. truncatula*. For *G. truncatula*, temperature alone contributed 18%, while the relationship between temperature and LST had a higher contribution of 19%. NDVI alone did not significantly contribute to the models of the four species;

however, in the model of *P. columella*, this variable, when interacting with precipitation, contributed 10% to the species model (Adj $R^2 = 0.10$).

After removing correlated variables, the variables used in this model explained 56% of the suitability of areas for *P. columella*, which had the lowest residual in the analysis of variance (0.44). Conversely, the variables influenced 28% of suitability areas for *G. cubensis*. Models for *G. schirazensis* and *G. truncatula* showed similar performance, with variables explaining between 49 and 46% of the models.

4.3.4 Extensive and restricted areas of environmental suitability in South America depending on the lymnaeid species

Considering environmental suitability within the geographical space, the models revealed that *P. columella* and *G. cubensis* have extensive favorable areas in South America, whereas *G. schirazensis* and *G. truncatula* have more restricted areas (Fig. 3). *Pseudosuccinea columella* demonstrated broad environmental suitability across various regions of Brazil, particularly in the southern, southeastern, and midwestern, as well as favorable areas in Bolivia (eastern and central), Argentina (eastern), and Paraguay (southern and eastern). In the northern region of Brazil, within the Amazon Forest biome, the species showed moderate suitability (Fig. 3A).

The model for *G. cubensis* indicated that the species does not show large areas of high suitability in the southern region of Brazil; however, there are considerable areas of high suitability, particularly in the southeast and northeast regions (Fig. 3B) of the country. In the southern and eastern parts of Argentina, extensive areas were also found to be suitable for *G. cubensis*. In the Andean countries, the areas of highest suitability are located closer to the Pacific coast (Fig. 3B), than in the inland regions of South America.

For *G. schirazensis* and *G. truncatula*, the models indicate more restricted and highly suitable areas in the Andes region. In Brazil and Argentina, the models predominantly show areas of low environmental suitability for both species. However, it is noteworthy that areas of medium suitability are more extensive for *G. schirazensis* than for *G. truncatula* across South America (Fig. 3C-D).

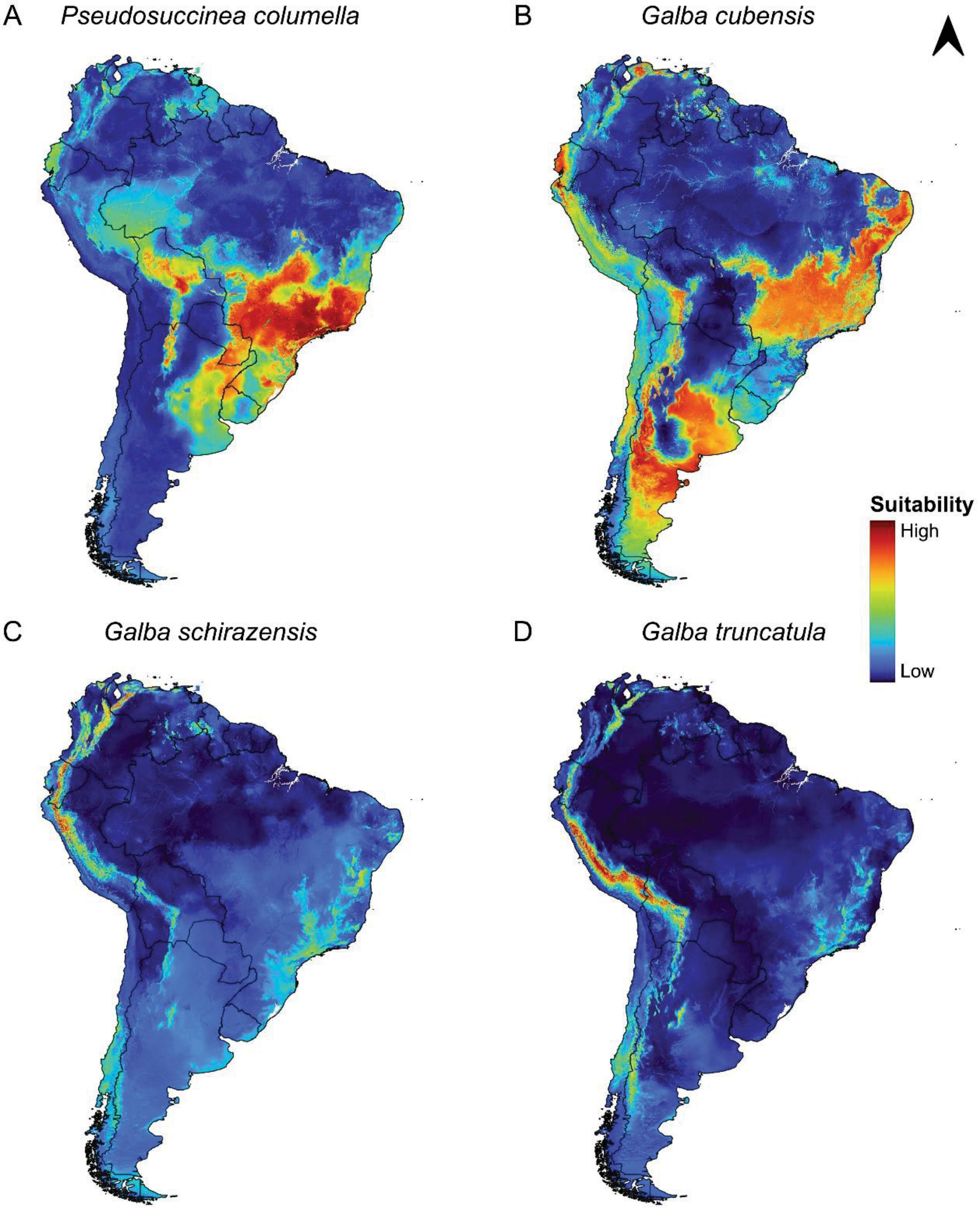


Figure 3. Areas of environmental suitability (high to low) predicted by ecological niche modeling for the occurrence of (A) *Pseudosuccinea columella*, (B) *Galba cubensis*, (C) *Galba schirazensis*, and (D) *Galba truncatula*.

4.3.5 Ecological niche overlap among lymnaeid species

The Schoener D index was used to quantify the overlap of suitable areas among the four modeled species revealing a high niche similarity between the models. *Pseudosuccinea columella* and *G. cubensis* exhibited a strong niche similarity, indicating large areas of overlap with high environmental suitability across various regions, different biomes, and climates of South America. The same occurs between *G. schirazensis* and *G. truncatula* with high similarity between their niches (Fig. 4A). These similarities are maintained when analyzing smaller territorial scales, such as Brazil (Fig. 4B), Peru (Fig. 4D), and Ecuador (Fig. 4E).

In the Andean countries, (i.e., Bolivia), a high similarity was observed between *G. cubensis* and *G. truncatula* (Fig. 4C). This pattern, however, was not identified in Ecuador (Fig. 4E). On the other hand, in Peru (Fig. 4D), there was a high similarity in the niches of the three *Galba* species. In Argentina, niche overlap among the four species was considered low (Fig. 4F).

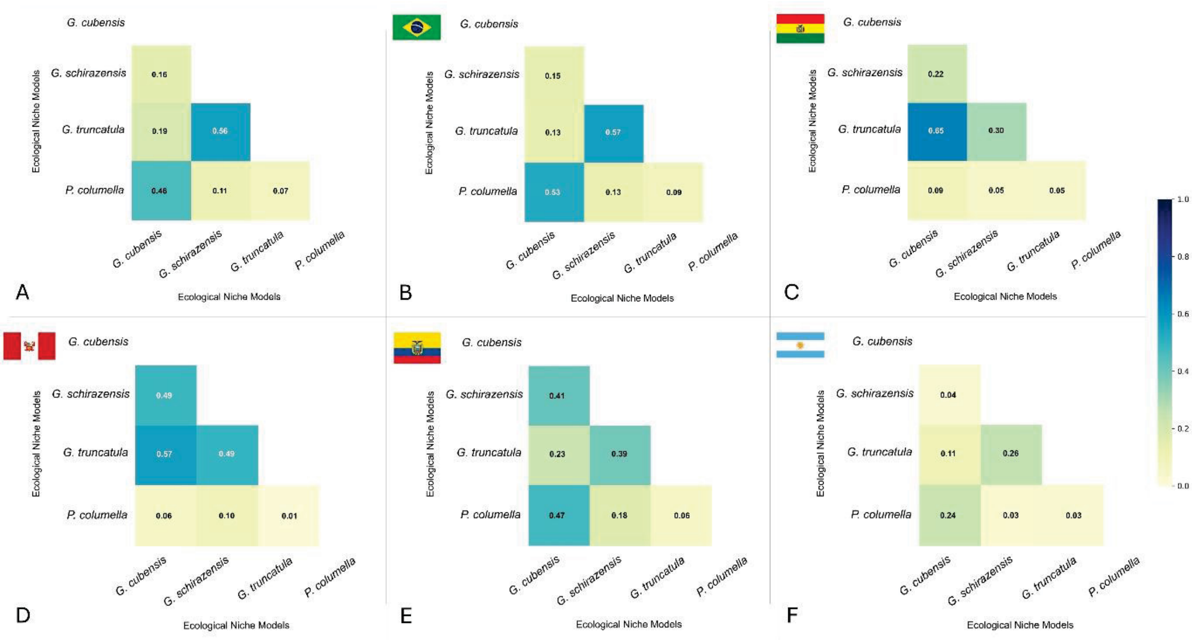


Figure 4. Ecological niche similarity analysis using Schoener's D index for the models of *Pseudesuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula* for (A) South America; (B) Brazil; (C) Bolivia; (D) Peru; (E) Argentina and (F) Ecuador.
Note: An index close to 1 indicates high niche similarity between species, while indexes close to 0 indicate little or no similarity.

4.3.6 Environmental suitability of limneids in countries with a high prevalence of *F. hepatica*

In Brazil, the states of the South and Southeast regions, from Rio Grande do Sul to Minas Gerais, presented extensive areas of suitability (medium to high suitability) for the occurrence of limneid species, mostly for *P. columella*. In the southern region, the state of Paraná had the largest territory with environmental suitability (62%) for *P. columella*, followed by Rio Grande do Sul (41%) and Santa Catarina (42%). In the southeast region, Espírito Santo had the smallest suitable area (16%), but São Paulo and Minas Gerais had 44% of suitable areas. In the northern region, only Acre had 40% of the suitable territory (Fig. 5A). For *G. cubensis*, the states in the southeastern region showed greater suitability than those in the southern region. In the South, Paraná had the largest territory of suitable areas (37%), while in the southeast, Minas Gerais reached 49%. In the center-west, the states of Goiás (43%) and Mato Grosso do Sul (38%) were the states with the highest suitability, while in the northeast, Sergipe has 45% of its territory considered suitable (Fig. 5B). The percentage of the territory of Brazil with medium/high suitability for *G. schirazensis* (9% in São Paulo) and *G. truncatula* (9% in Minas Gerais) was considered low (Fig. 5C-D). The percentages of territory within each suitability class (low, medium, high) for Brazil, Bolivia, and Peru are provided in Table S2.

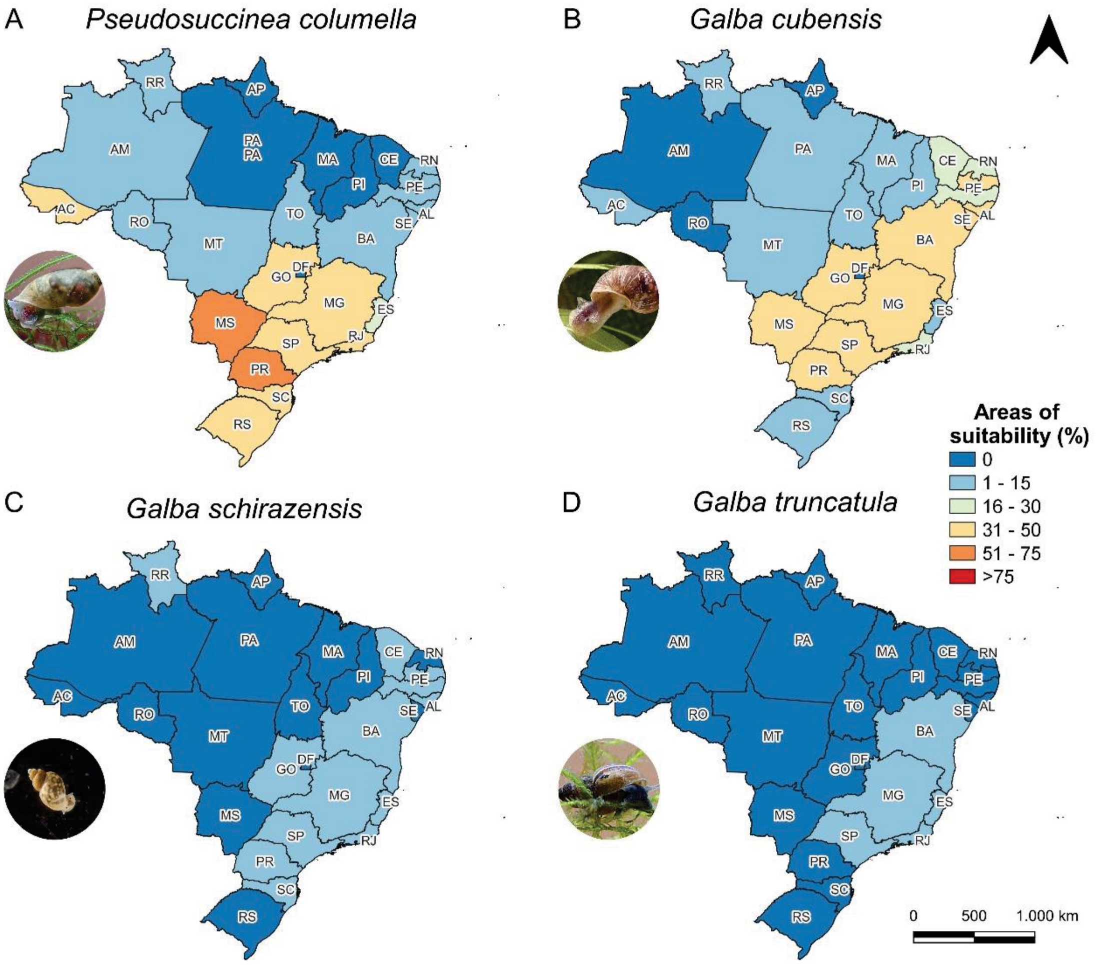


Figure 5. Territories with medium to high environmental suitability, represented as a percentage (%) of pixels, for the occurrence of (A) *Pseudosuccinea columella*, (B) *Galba cubensis*, (C) *Galba schirazensis*, and (D) *Galba truncatula* per state in Brazil.
Note: AC. Acre; AL. Alagoas; AP. Amapá; AM. Amazonas; BA. Bahia; CE. Ceará; DF. Distrito Federal; ES. Espírito Santo; GO. Goiás; MA. Maranhão; MT. Mato Grosso; MS. Mato Grosso do Sul; MG. Minas Gerais; PA. Pará; PB. Paraíba; PR. Paraná; PE. Pernambuco; PI. Piauí; RJ. Rio de Janeiro; RN. Rio Grande do Norte; RS. Rio Grande do Sul; RO. Rondônio; RR. Roraima; SC. Santa Catarina; SP. São Paulo; SE. Sergipe; TO. Tocantins.

In Bolivia, the regions with the highest percentages of territory showing high to medium environmental suitability for *P. columella* were La Paz (33%), Beni (30%), and Santa Cruz de La Sierra (30%) (Fig. 6A). Conversely, no territory in Potosí and Oruro was considered suitable for this species. For *G. cubensis*, *G. schirazensis*, and *G. truncatula*, Beni and Pando exhibited 0% of pixels suitable for these species. Cochabamba (28%) and Chuquisaca (26%) were the departments with the largest territory suitable for the occurrence of *G. cubensis* in Bolivia (Fig. 6B). Additionally, La Paz (only 8%) showed the highest percentage for *G. schirazensis* (Fig. 6C), while

Cochabamba (25%) and La Paz (23%) had the highest percentages for *G. truncatula* (Fig. 6D).

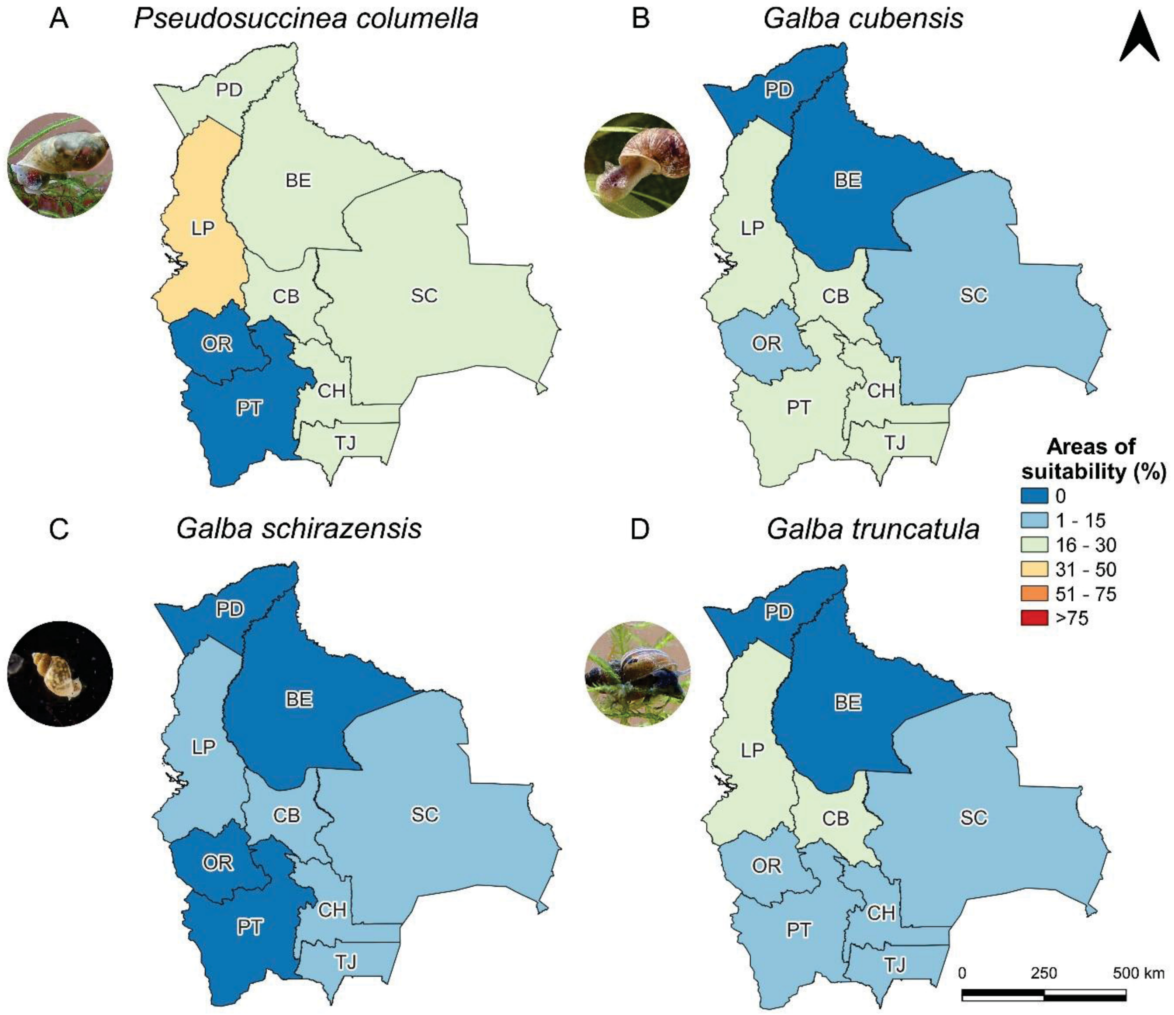


Figure 6. Territories with medium to high environmental suitability, represented as a percentage (%) of pixels, for the occurrence of (A) *Pseudosuccinea columella*, (B) *Galba cubensis*, (C) *Galba schirazensis*, and (D) *Galba truncatula*, per department in Bolivia.
Note: Be. Beni; CH. Chuquisaca; CB. Cochabamba; LP. La Paz; OR. Oruro; PD. Pando; PT. Potosi; SC. Santa Cruz; TJ. Tarija.

In Peru, the highest percentages of medium/high suitability for these species were found in departments near the Pacific Ocean coast, except for *P. columella* (Fig. 7). Madre de Dios was the department with the greatest suitability area (35%) for *P. columella* (Fig. 7A). The departments of Ancash (51%) and Apurimac (51%) and Huanuco (48%) were those with the largest territory suitable for *G. cubensis* (Fig. 7B). *Galba schirazensis* had the highest suitability territory in the department of Cajamarca (41%) (Fig. 7C). Apurimac (57%), Huanuco (47%), and Puno (42%) were the departments with the most suitable areas for *G. truncatula* (Fig. 7D).

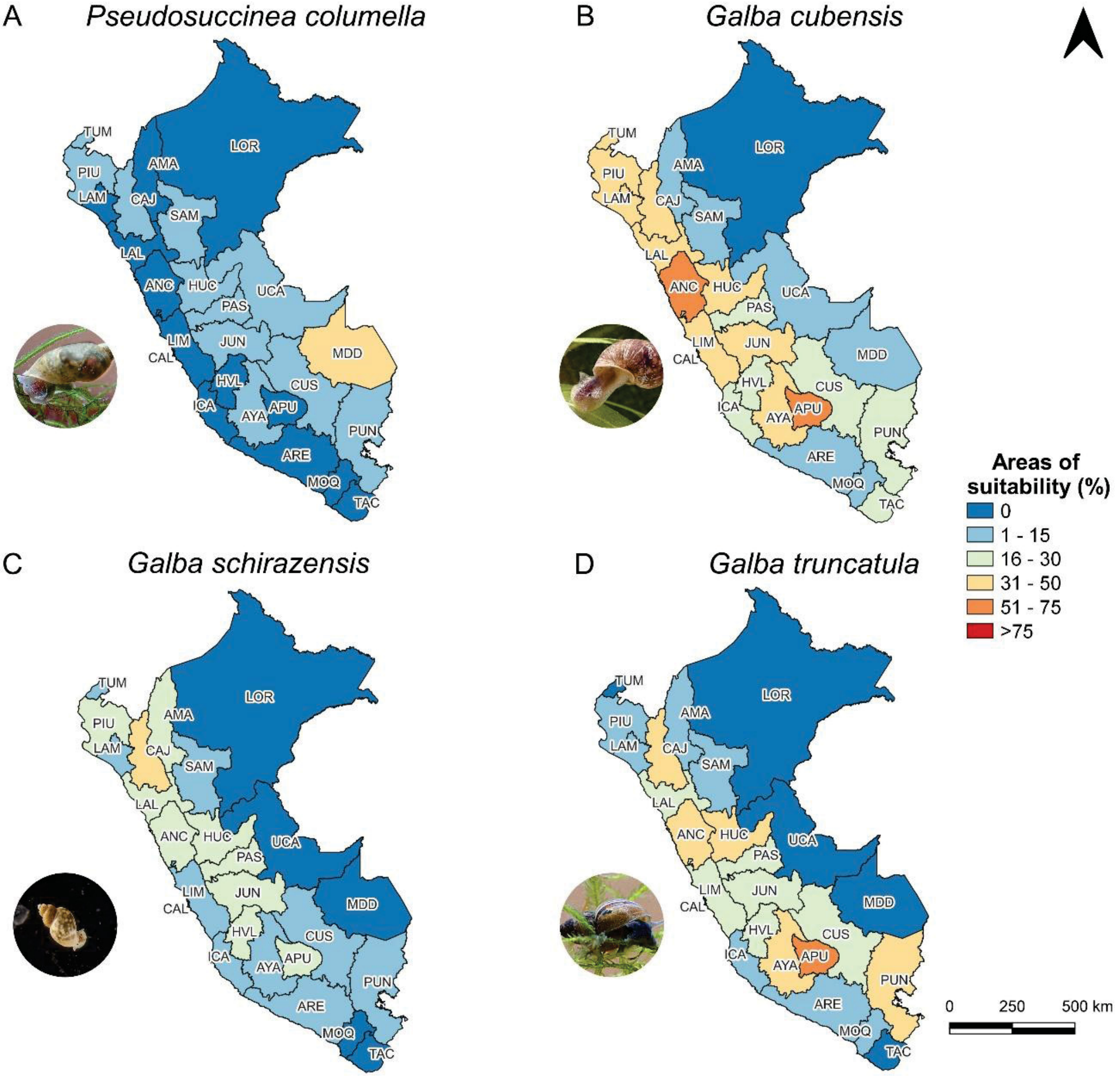


Figure 7. Territories with medium to high environmental suitability, represented as a percentage (%) of pixels, for the occurrence of (A) *Pseudosuccinea columella*, (B) *Galba cubensis*, (C) *Galba schirazensis*, and (D) *Galba truncatula* per department in Peru.
Note: AMA. Amazonas; ANC. Ancash; APU. Apurimac; ARE. Arequipa; AYA. Ayacucho; CAJ. Cajamarca; CAL. Callao; CUS. Cusco; HVL. Huancavelica; HUC. Huanuco; ICA. Ica; JUN. Junin; LAL. La Libertad; LAM. Lambayeque; LIM. Lima; LOR. Loreto; MDD. Madre de Dios; MOQ. Moquegua; PAS. Pasco; PIU. Piura; PUN. Puno; SAM. San Martin; TAC. Tacna; TUM. Tumbes; UCA. Ucayali.

4.4 DISCUSSION

This is the first ecological niche modeling study covering species of lymnaeid snails for South America. *Pseudosuccinea columella*, *G. cubensis*, *G. schirazensis*, and *G. truncatula* were found to be distributed across several countries, with suitable habitats varying among species and showing some overlap in ecological niches.

4.4.1 Climatic and geographic factors influence lymnaeid distribution and habitat suitability

The geographic distribution of *P. columella* and *G. cubensis* is broader than that of *G. schirazensis* and *G. truncatula*. This pattern can be explained by the influence of climatic variables. Precipitation had the greatest impact on the distribution models of *P. columella* (29%) and *G. cubensis* (12%). The availability of habitats with higher humidity, even across varying altitudes, likely accounts for the wide distribution of these two species in South America.

Population of *P. columella* and *G. cubensis* are predominantly found at lower altitudes compared to *G. schirazensis* and *G. truncatula* (Celi-Erazo et al., 2020; Bargues et al., 2021). For example, *P. columella* has not been reported above 2,000 m.a.s.l. in Colombia (Pereira et al., 2020) and in Argentina, it is most abundant between 200 and 500 m.a.s.l (Martín et al., 2016). However, *P. columella* has also been documented at higher altitudes, including 4,200 and 4,300 m.a.s.l. in Peru (Londoñe et al., 2009) and 3,000 m.a.s.l. in Guatemala (Lepe-López et al., 2020). This ability to establish populations across diverse regions is attributed to its strong invasive potential (Martín et al., 2016).

In contrast, *G. schirazensis* and *G. truncatula* are more geographically restricted due to their narrower temperature tolerances. Temperature was the most influential variable in the distribution models of *G. schirazensis* (24%) and *G. truncatula* (18%). Both species exhibited high environmental suitability in the Altiplano regions of Bolivia and Peru, characterized by high altitudes and low temperatures (Segura et al., 2022). For instance, Bargues et al., (2021) analyzed 33 specimens from 11 populations in the Bolivian Altiplano, identifying all as *G. truncatula*. Similarly, *G. schirazensis* populations have been recorded at Ecuadorian sites above 3,000 m.a.s.l (Bargues et al., 2012).

Although the variance analysis in this study showed that NDVI contributed only 10% to the *P. columella* model when interacting with precipitation and had limited influence overall, NDVI still highlights areas that can support snail host proliferation. Such areas include ponds and irrigation channels, which are particularly prevalent in regions with highly suitable climates. These conditions facilitate interactions between parasites, mammal hosts, and snail hosts (Vazquez et al., 2014; Alba et al., 2020). Moreover, the low influence of NDVI on the models can be explained by the fact that, unlike climate, which is influenced by factors such as altitude, NDVI is not geographically limited. However, these two variables complement each other: the climate creates suitable areas, while the landscape helps identify where NDVI indices are lower and associated with pasture areas (Testai et al., 2023).

4.4.2 Invasive potential and risk of transmission of *F. hepatica* in South America

Our data suggest that *P. columella* and *G. cubensis* exhibit greater ecological flexibility and invasive potential in South America, enabling them to thrive in diverse environments across both temperate and arid regions, including parts of Brazil and Argentina. In contrast, *G. schirazensis* and *G. truncatula* have a distribution more restricted to specific areas, such as the Andean Altiplano and high-altitude regions in Ecuador and Colombia.

All of these species—with the exception of *G. schirazensis*, whose origin is in the Middle East—are native to the Americas (Schniebs et al., 2018; Alda et al., 2021). The origin of *G. truncatula*, however, remains under debate: while Mas-Coma (2011) suggests it is a European species that invaded the Americas, Correa et al. (2010) argue that it belongs to an American clade that subsequently spread to other regions. Genetic population analyses have shown that these species have expanded their global distribution due to recent, human-mediated introductions (Lounnas et al., 2017; Lounnas et al., 2018). These studies also indicate that, as hermaphroditic organisms, these species tend to self-fertilize—a reproductive strategy that greatly facilitates the colonization of new habitats (Bonel et al., 2023).

Introducing these species outside their native regions facilitates the spread of *F. hepatica* in both humans and animals (Alba et al., 2020). Therefore, identifying suitable regions for *P. columella*, *G. cubensis*, *G. schirazensis*, and *G. truncatula* is crucial for assessing the risk of fasciolosis. Our study identified high-suitability areas that overlap with regions where bovine fasciolosis has been increasing, primarily due to inadequate control and insufficient monitoring of cattle transport (Pritsch et al., 2019; Almeida et al., 2024). Effective inspection and quarantine measures before animal transport are essential to prevent the spread of *F. hepatica* from endemic to low- or non-endemic areas, particularly in regions with high environmental suitability for snail hosts.

We suggest that particular attention should be given to certain regions of South America. For instance, southeastern Brazil, and northeastern and southern Argentina, where the highly transmissive snail host *G. cubensis* shows high environmental suitability. Although the variables in the analysis of variance explained only 28% of the variability (residual = 0.72), these regions remain at high risk for fasciolosis and should be prioritized for surveillance. The relatively low explanatory power of the variables may be influenced by several factors, such as environmental diversity in the calibration region and the density and spatial distribution of these species (Cobos et al., 2019). Only 43 confirmed molecular occurrence points have been recorded in these regions for *G. cubensis*, including just one confirmed record in Minas Gerais, Brazil. Our analysis suggests that these regions may be suitable for the species' presence. However, due to limited sampling or geographic barriers, populations have yet to be documented in these areas (Testai et al., 2023).

4.4.3 Mapping suitable areas for lymnaeid species: implications for parasite expansion, disease transmission, and health planning

Due to the morphological similarity among *Galba* species (Alda et al., 2021), mapping high-probability occurrence areas using suitability pixels from Brazil, Bolivia, and Peru is essential. These data provide valuable guidance for epidemiological work and the development of human and veterinary health plans, clarifying the role of these species in the Fasciola life cycle and disease transmission. In southern Brazil, *P. columella* showed a higher percentage of suitable areas compared to *G. cubensis*, suggesting that *P. columella* may be the main snail host for *F. hepatica* transmission. Conversely, in the southeastern and midwestern parts of Brazil, overlapping suitable areas for *P. columella* and *G. cubensis* suggest that both species could contribute to parasite transmission.

The analysis identifies suitable areas for *P. columella* in Brazil's midwest and northern regions, while *G. cubensis* shows potential distribution in the southeastern and northeastern regions. These findings emphasize the importance of targeted surveillance policies, which are essential to mitigating the risk of fasciolosis spread through the introduction of infected animals into areas where these mollusks are present, enabling the parasite's life cycle to continue (Kelley et al., 2020). Cases of fasciolosis have been reported in regions where *P. columella*, the main intermediate host in the country, or species of the genus *Galba* have not yet been documented (Medeiros et al., 2014; Almeida et al., 2024). Recent observations indicate a geographic spread of the disease in cattle, beyond the traditionally endemic southern region of Brazil, now affecting sensitive biomes such as the Pantanal (wetland) and the Amazon Forest (Almeida et al., 2024). This expansion is closely linked to land use, particularly deforestation in the Pantanal, Cerrado, and Amazon (McManus et al., 2016; Almeida et al., 2024)

In Peru, Madre de Dios had 38% of its area suitable for *P. columella*, while Apurimac had 51% of its territory suitable for *G. cubensis* and *G. truncatula* (57%), and Cajamarca had 41% for *G. schirazensis*. *Galba cubensis*, *G. schirazensis*, and *G. truncatula* were reported in Cajamarca (Bargues et al., 2012), where our model indicated only 2% suitability for *P. columella* and over 30% for the other species. Additionally, the models indicated that Loreto (0%) and Ucayali (0%) did not show high suitability for any of the four species. Infection in animals is reported sporadically in the departments of Loreto and Ucayali, where climatic and ecological conditions do not appear to favor snail hosts of *F. hepatica* (Diaz-Quevedo et al., 2021).

Our approach enables the evaluation of environmental suitability for specific species or the cryptic *Galba* species, considering their potential co-occurrence in certain locations. Cryptic species present a significant challenge, often leading to under- or overestimations of transmission risk (Alda et al., 2021; Soler et al., 2023). Given the epidemiological and transmission heterogeneities in endemic areas, which are closely linked to the lymnaeid species, accurate identification of these snails is critical. Understanding the geographic distribution and role of these snails as hosts of *F. hepatica*, as well as the dynamics of displacement of other hosts, is essential to improve control and surveillance strategies aimed at preventing the spread of diseases such as fasciolosis (Machado et al., 2024; Dadar et al., 2022; Soler et al., 2023)

4.5 REFERENCES

Alba, A., Vazquez, A.A., Hurtrez-Boussès, S., 2020. Towards the comprehension of fasciolosis (re)emergence: An integrative overview. Parasitology 148, 385–407.

Alda, P., Lounnas, M., Vázquez, A.A., Ayaqui, R., Calvopiña, M., Celi-Erazo, M., Dillon, R.T., Jarne, P., Loker, E.S., Muñiz Pareja, F.C., Muzzio-Aroca, J., Nárvaez, A.O., Noya, O., Robles, L.M., Rodríguez-Hidalgo, R., Uribe, N., David, P., Pointier, J.P., Hurtrez-Boussès, S., 2018. A new multiplex PCR assay to distinguish among three cryptic *Galba* species, intermediate hosts of *Fasciola hepatica*. Vet. Parasitol. 251, 101–105.

Alda, P., Lounnas, M., Vázquez, A.A., Ayaqui, R., Calvopiña, M., Celi-Erazo, M., Dillon, R.T., González Ramírez, L.C., Loker, E.S., Muzzio-Aroca, J., Nárvaez, A.O., Noya, O., Pereira, A.E., Robles, L.M., Rodríguez-Hidalgo, R., Uribe, N., David, P., Jarne, P., Pointier, J.P., Hurtrez-Boussès, S., 2021. Systematics and geographical distribution of Galba species, a group of cryptic and worldwide freshwater snails. Mol. Phylogenet. Evol. 157.

Almeida, T.M., Neto, I.R., Oliveira Brandão, Y., Molento, M.B., 2024. Geographic expansion of *Fasciola hepatica* (Linnaeus, 1758) due to changes in land use and cover in Brazil. Int. J. Parasitol. 54, 201–212.

An, Q., Li, Y., Sun, Z., Gao, X., Wang, H., 2024. Seasonal prediction of the distribution of three major malaria vectors in China: Based on an ecological niche model. PLoS Negl. Trop. Dis. 18(1).

Barbet-Massin, M., Jiguet, F., Albert, C.H., Thuiller, W., 2012. Selecting pseudo-absences for species distribution models: How, where and how many? Methods Ecol. Evol. 3(2), 327–338.

Bargues, D.M., Artigas, P., Khoubbane, M., Ortiz, P., Naquira, C., Mas-Coma, S., 2012. Molecular characterization of *Galba truncatula*, *Lymnaea neotropica*, and *L. schirazensis* from Cajamarca, Peru and their potential role in transmission of human and animal fascioliasis. Parasites Vectors. 5(174).

Bargues, M.D., Angles, R., Coello, J., Artigas, P., Funatsu, I.R., Cuervo, P.F., Buchon, P., Mas-Coma, S., 2021. One Health initiative in the Bolivian Altiplano human fascioliasis hyperendemic area: Lymnaeid biology, population dynamics, microecology and climatic factor influences. Rev. Bras. Parasitol. Vet. 30(2).

Bonel, N., Nakadera, Y., Pizá, J., Vázquez, A.A., Koene, J.M., David, P., Jarne, P., Alda, P., 2023. Reproductive strategies, genetic diversity, and invasive ability in Lymnaeidae. In: The Lymnaeidae; 265–284.

Campos, J.C., Garcia, N., Alírio, J., Arenas-Castro, S., Teodoro, A.C., Sillero, N., 2023. Ecological Niche Models using MaxEnt in Google Earth Engine: Evaluation, guidelines and recommendations. Ecol. Inform. 76(8).

Caron, Y., Celi-Erazo, M., Hurtrez-Boussès, S., Lounnas, M., Pointier, J.P., Saegerman, C., Losson, B., Benítez-Ortíz, W., 2017. Is *Galba schirazensis* (Mollusca, Gastropoda) an intermediate host of *Fasciola hepatica* (Trematoda, Digenea) in Ecuador? Parasite 24, 24.

Celi-Erazo, M., Alda, P., Montenegro-Franco, M., Pavon, D., Minda-Aluisa, E., Calvopiña, M., Pointier, J.P., Hurtrez-Boussès, S., Cevallos, W., Benítez-Ortíz, W., Rodríguez-Hidalgo, R., 2020. Prevalence of *Fasciola hepatica* infection in *Galba cousini* and *Galba schirazensis* from an Andean region of Ecuador. Vet. Parasitol. Reg. Stud. Reports 20.

Cobos, M.E., Osorio-Olvera, L., Peterson, A.T., 2019. Assessment and representation of variability in ecological niche model predictions. BioRxiv.

Correa, A.C., Escobar, J.S., Noya, O., Velásquez, L.E., González-Ramírez, C., Hurtrez-Boussès, S., Pointier, J.P., 2011. Morphological and molecular characterization of Neotropic Lymnaeidae (Gastropoda: Lymnaeoidea), vectors of fasciolosis. Infect. Genet. Evol. 11, 1978–1988.

Dadar, M., Omar, S.S., Shahali, Y., Fakhri, Y., Godfroid, J., Khaneghah, A.M., 2022. The prevalence of camel brucellosis and associated risk factors: A global meta-epidemiological study. Qual Assur Saf Crop Foods. 14(3), 55–93.

Delgado, R.C., Santana, R.O., Gelsleichter, Y.A., Pereira, M.G., 2022. Degradation of South American biomes: What to expect for the future? Environ. Impact Assess. Rev. 96.

Diaz-Quevedo, C., Frias, H., Cahuana, G.M., Tapia-Limonchi, R., Chenet, S.M., Tejedo, J.R., 2021. High prevalence and risk factors of fascioliasis in cattle in Amazonas, Peru. Parasitol Int. 85.

Galavani, H., Haniloo, A., Raeghi, S., Ghatee, M.A., Karamian, M., 2024. Bioclimatic analysis and spatial distribution of fascioliasis causative agents by assessment of Lymnaeidae snails in northwestern provinces of Iran. Parasit. Vectors 17(1), 244.

Garreaud, R.D., Vuille, M., Compagnucci, R., Marengo, J., 2009. Present-day South American climate. Palaeogeogr. Palaeoclimatol. Palaeoecol. 281(3-4), 180–195.[

Jaramillo, C., 2023. The evolution of extant South American tropical biomes. New Phytologist 239(2), 477–493.

Kelley, J.M., Rathinasamy, V., Elliott, T.P., Rawlin, G., Beddoe, T., Stevenson, M.A., Spithill, T.W., 2020. Determination of the prevalence and intensity of *Fasciola hepatica* infection in dairy cattle from six irrigation regions of Victoria, Southeastern Australia, further identifying significant triclabendazole resistance on three properties. Vet Parasitol. 277.

Lepe-López, M., Villatoro-Paz, F., Valdez-Sandoval, C., Rios, L., Díaz-Rodríguez, M., Guerra-Centeno, D., 2020. Reporte de *Pseudosuccinea columella* infectados con *Fasciola hepatica* en Sierra de los Cuchumatanes, Guatemala. Rev MVZ Cordoba. 25(1), 94–97.

Londoñe, P.B., Chávez, A.V., Li, O.E., Suárez, F.A., Pezo, D.C., 2009. Presence of lymnaeidae snails with larvae of *Fasciola hepatica* in altitudes over 4000 m above sea level in the southern highlands of Peru. Rev Investig Vet Peru. 20(1), 58–65.

Lounnas, M., Correa, A.C., Vázquez, A.A., Dia, A., Escobar, J.S., Nicot, A., Arenas, J., Ayaqui, R., Dubois, M.P., Gimenez, T., Gutiérrez, A., González-Ramírez, C., Noya, O., Prepelitchi, L., Uribe, N., Wisnivesky-Colli, C., Yong, M., David, P., Loker, E.S., Jarne, P., Pointier, J.P., Hurtrez-Boussès, S., 2017. Self-fertilization, long-distance flash invasion and biogeography shape the population structure of *Pseudosuccinea columella* at the worldwide scale. Mol Ecol. 26(3), 887–903.

Lounnas, M., Correa, A.C., Alda, P., David, P., Dubois, M.P., Calvopiña, M., Caron, Y., Celi-Erazo, M., Dung, B.T., Jarne, P., Loker, E.S., Noya, O., Rodríguez-Hidalgo, R., Toty, C., Uribe, N., Pointier, J.P., Hurtrez-Boussès, S., 2018. Population structure and genetic diversity in the invasive freshwater snail *Galba schirazensis* (Lymnaeidae). Can J Zool. 96, 425–435.

Machado, G., Vilalta, C., Recamonde-Mendoza, M., Corzo, C., Torremorell, M., Perez, A., VanderWaal, K., 2019. Identifying outbreaks of Porcine Epidemic Diarrhea virus through animal movements and spatial neighborhoods. Sci Rep. 9(457).

Martín, P.R., Ovando, X.M.C., Seuffert, M.E., 2016. First record of the freshwater snail *Pseudosuccinea columella* (Gastropoda: Lymnaeidae) in southern Pampas (Argentina) and assessment of future spread. Molluscan Res. 36, 213–221.

McManus, C., Barcellos, J.O.J., Formenton, B.K., Hermuche, P.M., Carvalho, O.A.de, Guimarães, R., Gianezini, M., Dias, E.A., Nascimento Lampert, V.Do, Zago, D., Neto, J.B., 2016. Dynamics of cattle production in Brazil. PLoS One. 11(1).

Medeiros, C., Scholte, R.G.C., D'Ávila, S., Caldeira, R.L., Carvalho, O.d.S., 2014. Spatial distribution of Lymnaeidae (Mollusca, Basommatophora), intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematoda, Digenea) in Brazil. Rev Inst Med Trop Sao Paulo. 56(3), 235–252.

Miyahira, I.C., Clavijo, C., Callil, C.T., Cuezzo, M.G., Darrigran, G., Gomes, S.R., Lasso, C.A., Mansur, M.C.D., Pena, M.S., Ramírez, R., Santos, R.C.L. dos, Santos, S.B., Scarabino, F., Torres, S.H., Vogler, R.E., Cowie, R.H., 2022. The conservation of non-marine molluscs in South America: Where we are and how to move forward. Biodivers. Conserv. 31(4), 1–32.

Nasibi, S., Salahi Moghaddam, A., Ziaali, N., Akhlaghi, E., Mohammadi, M.A., Hanafi-Bojd, A.A., Fasihi Harandi, M., 2021. Molecular, morphological, and spatial study of *Galba schirazensis* (Pulmonata, Lymnaeidae) from southeastern Iran. Iran. J. Parasitol. 16(1), 52–53.

Pereira, A.E., Uribe, N., Pointier, J.P., 2020. Lymnaeidae from Santander and bordering departments of Colombia: Morphological characterization, molecular identification and natural infection with *Fasciola hepatica*. Vet. Parasitol. Reg. Stud. Reports 20.

Pritsch, I.C., Stanula, E.C.A., Anjos, A., Bertot, J.A., Molento, M.B., 2019. Fascioliasis in buffaloes: A 5-year forecast analysis of the disease based on a 15-year survey in Brazil. Rev Bras Parasitol Vet. 28(3), 410–415.

Püts, M., Taylor, M., Núñez-Riboni, I., Steenbeek, J., Stäbler, M., Möllmann, C., Kempf, A., 2020. Insights on integrating habitat preferences in process-oriented ecological models – A case study of the southern North Sea. Ecol. Modell. 431(6).

Sabourin, E., Alda, P., Vázquez, A., Hurtrez-Boussès, S., Vittecoq, M., 2018. Impact of human activities on fasciolosis transmission. Trends Parasitol. 34, 891–903.

Sánchez-Tapia, A., Mortara, S.R., Souza, D., Rocha, B., Mendes Barros, F.S., Gall, G., Ferreira De Siqueira, M., 2020. modleR: A modular workflow to perform ecological niche modeling in R.

Schniebs, K., Glöer, P., Quiñonero-Salgado, S., Lopez-Soriano, J., Hundsdoerfer, A.K., 2018. The first record of *Galba cubensis* (L. Pfeiffer, 1839) (Mollusca: Gastropoda: Lymnaeidae) from open fields of Europe. Folia Malacol. 26(1), 3–15.

Segura, H., Espinoza, J.C., Junquas, C., Lebel, T., Vuille, M., Condom, T., 2022. Extreme austral winter precipitation events over the South-American Altiplano: regional atmospheric features. Clim Dyn. 59, 3069–3086.

Soler, P., Abdala, A.M., Larroza, M., 2023. Genetic characterization and regional distribution of lymnaeid snails in northern Patagonia, Argentina. Vet Parasitol Reg Stud Reports. 44(77).

Standley, C.J., Prepelitchi, L., Pietrokovsky, S.M., Issia, L., Stothard, J.R., Wisnivesky-Colli, C., 2013. Molecular characterization of cryptic and sympatric lymnaeid species from the Galba/Fossaria group in Mendoza Province, Northern Patagonia, Argentina. Parasit. Vectors 6(1).

Strong, E.E., Olivier, G., Ponder, W., Bouchet, P., 2008. Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. Hydrobiologia 595(1), 149–166.

Testai, R., Siqueira, M.F. de, Rocha, D.S.B., Roque, A.L.R., Jansen, A.M., Xavier, S.C. das C., 2023. Space-environment relationship in the identification of potential areas of expansion of *Trypanosoma cruzi* infection in *Didelphis aurita* in the Atlantic Rainforest. PLoS One 18(7).

Toet, H., Piedrafita, D.M., Spithill, T.W., 2014. Liver fluke vaccines in ruminants: Strategies, progress and future opportunities. Int. J. Parasitol. 44, 915–927.

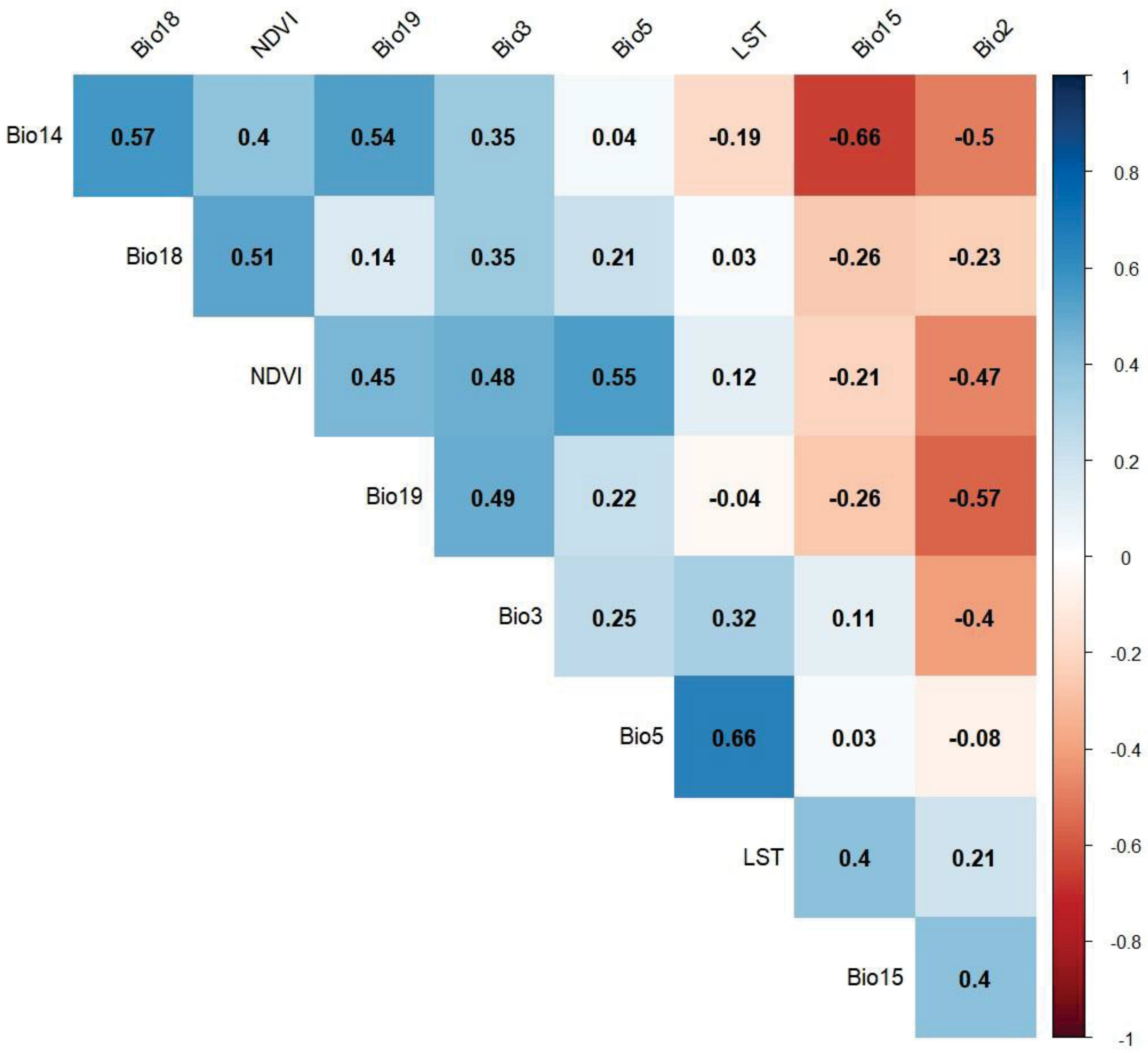
Varela, S., Anderson, R.P., García-Valdés, R., Fernández-González, F., 2014. Environmental filters reduce the effects of sampling bias and improve predictions of ecological niche models. Ecography 37(11), 1084–1091.

Vázquez, A.A., Sánchez, J., Pointier, J.P., Théron, A., Hurtrez-Boussès, S., 2014. *Fasciola hepatica* in Cuba: Compatibility of different isolates with two intermediate snail hosts, *Galba cubensis* and *Pseudosuccinea columella*. J Helminthol. 88(4), 434–440.

Vázquez, A.A., Alda, P., Lounnas, M., Sabourin, E., Alba, A., Pointier, J.P., Hurtrez-Boussès, S., 2018. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: Digenea): A worldwide review. CAB Rev. 13(62), 1–15.

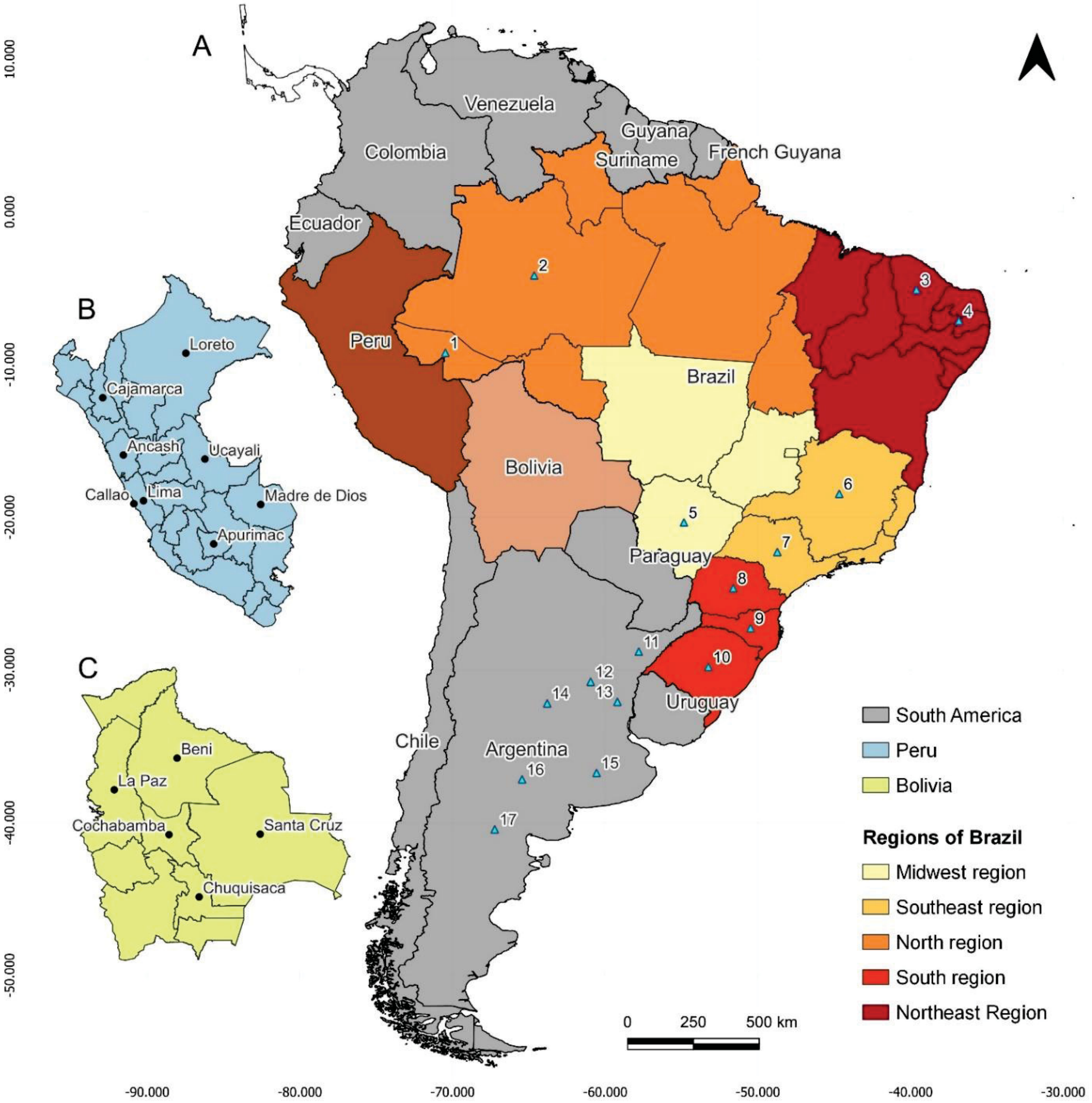
Vázquez, A.A., Alba, A., Alda, P., Vittecoq, M., Chapuis, E., Faugere, D., Pointier, J.P., Hurtrez-Boussès, S., 2023. Lymnaeid snails and the transmission of fasciolosis: Understanding the differential risks from local to global scale. In: Vinarski, M.V., Vázquez, A.A. (Eds.), The Lymnaeidae. Zoological Monographs 7, 359–394.

5.1 SUPPLEMENTARY MATERIAL

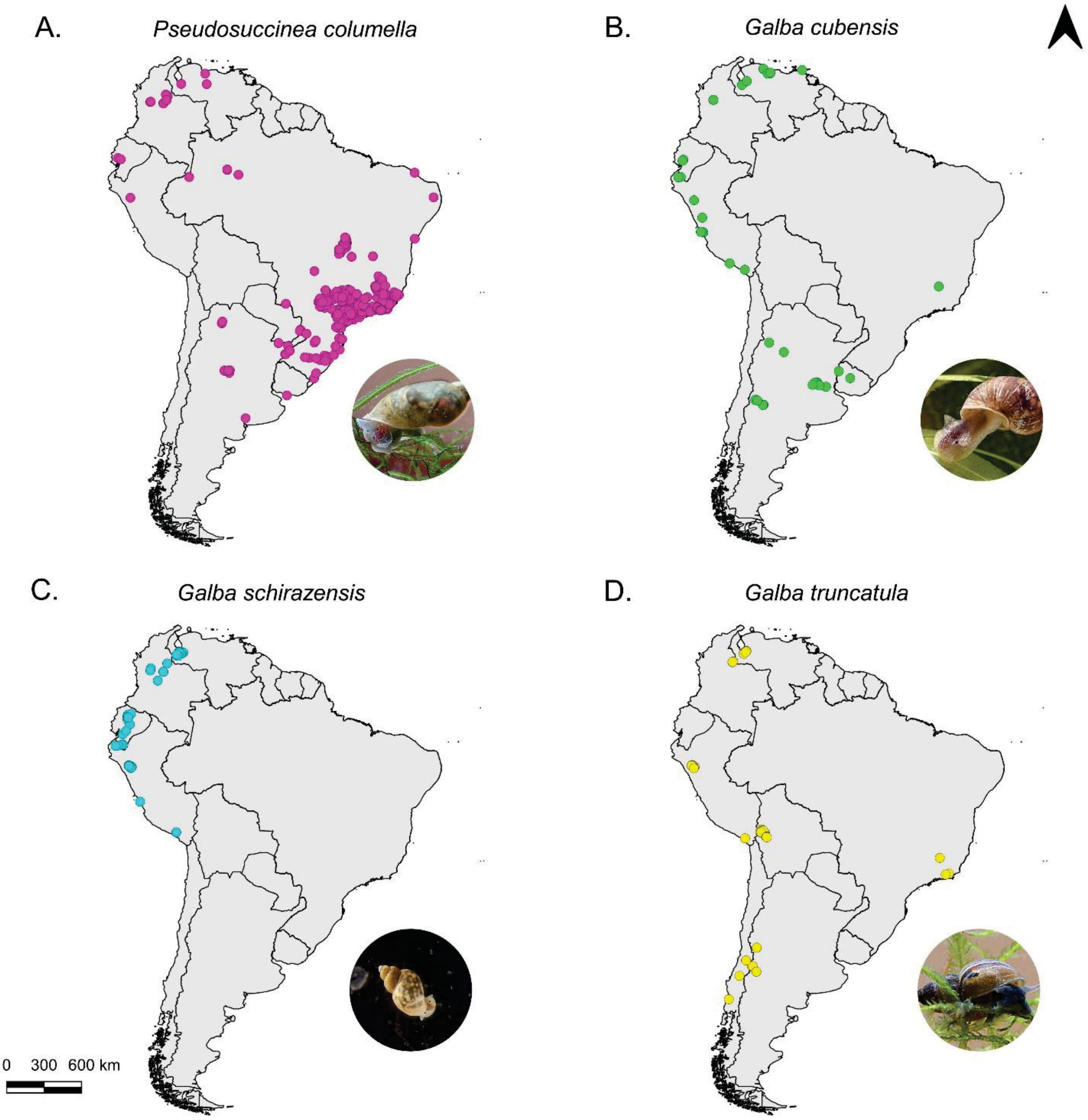


	Bio18	NDVI	Bio19	Bio3	Bio5	LST	Bio15	Bio2
Bio14	0.57	0.4	0.54	0.35	0.04	-0.19	-0.66	-0.5
Bio18		0.51	0.14	0.35	0.21	0.03	-0.26	-0.23
NDVI			0.45	0.48	0.55	0.12	-0.21	-0.47
Bio19				0.49	0.22	-0.04	-0.26	-0.57
Bio3					0.25	0.32	0.11	-0.4
Bio5						0.66	0.03	-0.08
LST							0.4	0.21
Bio15								0.4

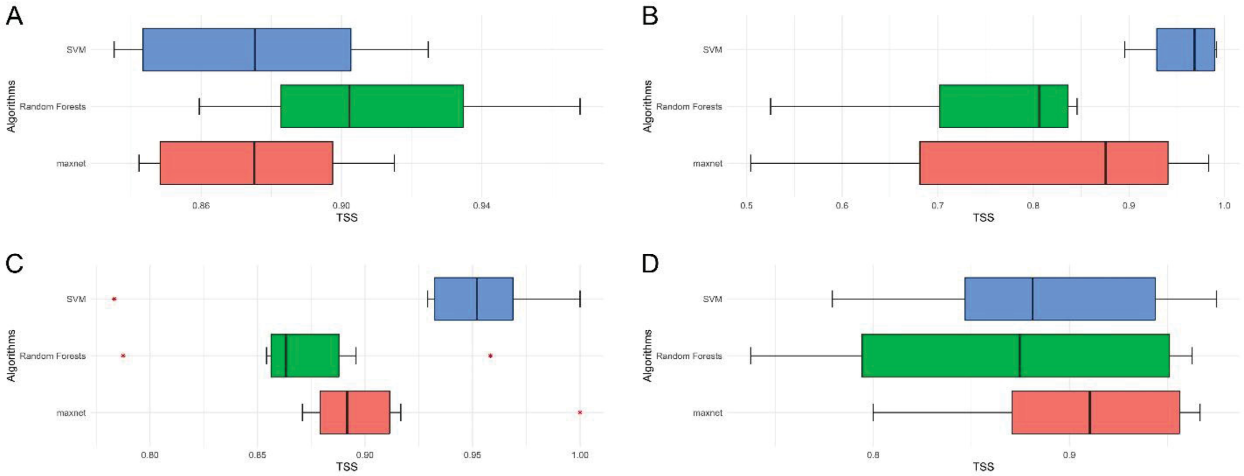
Supplementary Figure S1. Pearson correlation analysis between selected environmental variables to construct an ecological niche model of *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis* and *Galba truncatula*.



Supplementary Figure S2. Political location map: A. Countries of South America; B. Departments of Peru; C. Departments of Bolivia. The numbers indicate the states of Brazil, and the triangles indicate the provinces of Argentina. 1. Acre; 2. Amazonas; 3. Ceará; 4. Paraíba; 5. Mato Grosso do Sul; 6. Minas Gerais; 7. São Paulo; 8. Paraná; 9. Santa Catarina; 10. Rio Grande do Sul; 11. Corrientes; 12. Santa Fé; 13. Entre Ríos; 14. Córdoba; 15. Buenos Aires; 16. La Pampa; and 17. Río Negro.



Supplementary Figure S3. Occurrence points of (A) *Pseudosuccinea columella*, (B) *Galba cubensis*, (C) *Galba schirazensis*, and (D) *Galba truncatula* in South used in ecological niche modeling after spatial filtering of occurrence data.



Supplementary Figure S4. Boxplot of True Skill Statistics (TSS) values for the partitions (models) generated by the Support Vector Machine, Random Forest, and Maxent algorithms, used in the construction of the ensemble model for *Pseudosuccinea columella* (A), *Galba cubensis* (B), *Galba schirazensis* (C), and *Galba truncatula* (D).

Supplementary Table S1. Occurrence points of (A) *Pseudosuccinea columella*, (B) *Galba cubensis*, (C) *Galba schirazensis*, and (D) *Galba truncatula* in South used in ecological niche modeling after spatial filtering of occurrence data.

Species	**Geographic coordinate**	
	Longitude	**Latitude**
Pseudosuccinea columella	-67.7875	10.0814
Pseudosuccinea columella	-71.1244	8.6445
Pseudosuccinea columella	-75.4494	6.1219
Pseudosuccinea columella	-78.2224	-7.2597
Pseudosuccinea columella	-67.5658	8.6173
Pseudosuccinea columella	-43.9732	-19.6807
Pseudosuccinea columella	-57.5475	-27.5592
Pseudosuccinea columella	-75.3833	6.1500
Pseudosuccinea columella	-65.4678	-24.7947
Pseudosuccinea columella	-62.1410	-38.0470
Pseudosuccinea columella	-64.4300	-31.7250
Pseudosuccinea columella	-65.3780	-24.5500
Pseudosuccinea columella	-65.1000	-31.4000
Pseudosuccinea columella	-54.4760	-25.6890
Pseudosuccinea columella	-56.2070	-27.9750
Pseudosuccinea columella	-56.3740	-34.8620
Pseudosuccinea columella	-79.9852	-1.6932
Pseudosuccinea columella	-79.5606	-1.8840
Pseudosuccinea columella	-57.5333	-27.5500
Pseudosuccinea columella	-54.5639	-25.8783
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Galba truncatula	-70.9231	8.7450
Galba truncatula	-70.8592	8.7967
Galba truncatula	-70.8114	8.8447
Galba truncatula	-70.9914	-17.3234
Galba truncatula	-70.8081	8.8678
Galba truncatula	-70.8302	8.8370
Galba truncatula	-78.2642	-7.2413

Galba truncatula	-78.4469	-7.1834
Galba truncatula	-78.4484	-7.1285
Galba truncatula	-78.2224	-7.2597
Galba truncatula	-78.3931	-7.2043
Galba truncatula	-78.4615	-7.1515
Galba truncatula	-78.3211	-7.2016
Galba truncatula	-78.2021	-7.2589
Galba truncatula	-78.0263	-7.3351
Galba truncatula	-78.2298	-7.2256
Galba truncatula	-78.1813	-7.4383
Galba truncatula	-69.3514	-32.5903
Galba truncatula	-68.6778	-16.0642
Galba truncatula	-68.8036	-16.1758
Galba truncatula	-68.5744	-16.2411
Galba truncatula	-68.5397	-16.2958
Galba truncatula	-68.5169	-16.2961
Galba truncatula	-68.5644	-16.3353
Galba truncatula	-68.4889	-16.3844
Galba truncatula	-68.3889	-16.4308
Galba truncatula	-68.5986	-16.3878
Galba truncatula	-68.8803	-16.4369
Galba truncatula	-68.7386	-16.4408
Galba truncatula	-68.6983	-16.4408
Galba truncatula	-68.5083	-16.5747
Galba truncatula	-68.3061	-16.5247
Galba truncatula	-68.7642	-16.5208
Galba truncatula	-68.2797	-16.6328
Galba truncatula	-68.1961	-16.5786
Galba truncatula	-68.5075	-16.5700
Galba truncatula	-68.6653	-16.4436
Galba truncatula	-68.2728	-16.6961
Galba truncatula	-68.5064	-16.2989
Galba truncatula	-68.4986	-16.2997
Galba truncatula	-68.5450	-16.3103
Galba truncatula	-68.7433	-16.4414
Galba truncatula	-68.4564	-16.4244
Galba truncatula	-68.5028	-16.2311
Galba truncatula	-68.4725	-16.2686
Galba truncatula	-68.4586	-16.2569
Galba truncatula	-68.7550	-16.4781
Galba truncatula	-68.0439	-17.0875
Galba truncatula	-68.0203	-17.0953
Galba truncatula	-67.9428	-17.1775
Galba truncatula	-70.8100	8.8200
Galba truncatula	-71.1800	8.4600
Galba truncatula	-70.9100	8.6900
Galba truncatula	-72.7433	7.2928
Galba truncatula	-72.7379	7.2981

Galba truncatula	-72.6321	7.3774
Galba truncatula	-72.7325	7.3142
Galba truncatula	-78.3447	-7.0892
Galba truncatula	-78.3517	-7.1300
Galba truncatula	-78.4072	-7.1719
Galba truncatula	-78.4664	-7.1706
Galba truncatula	-78.1494	-7.5353
Galba truncatula	-70.0391	-35.1967
Galba truncatula	-69.9363	-35.1654
Galba truncatula	-69.4153	-35.9550
Galba truncatula	-69.4000	-35.9500
Galba truncatula	-42.5167	-22.2500
Galba truncatula	-42.9667	-22.3833
Galba truncatula	-43.7833	-20.0500
Galba truncatula	-73.2514	-39.8029
Galba truncatula	-71.7734	-36.5452
Galba truncatula	-70.8587	-34.3764

Supplementary Table S2. Percentage of pixels within the low, medium, and high environmental suitability classes in the niche models for *Pseudosuccinea columella*, *Galba cubensis*, *Galba schirazensis*, and *Galba truncatula* across Brazil, Bolivia, and Peru.

Country	Federative unit	Label	Low (%)	Medium (%)	High (%)	Medium + High (%)	Specie
Bolivia	Beni	BE	71	24	6	30	*P. columella*
Bolivia	Chuquisaca	CH	84	16	0	16	*P. columella*
Bolivia	Cochabamba	CB	77	22	1	23	*P. columella*
Bolivia	La Paz	LP	67	29	4	33	*P. columella*
Bolivia	Oruro	OR	100	0	0	0	*P. columella*
Bolivia	Pando	PD	77	23	0	23	*P. columella*
Bolivia	Potosí	PT	100	0	0	0	*P. columella*
Bolivia	Santa Cruz	SC	70	23	8	30	*P. columella*
Bolivia	Tarija	TJ	84	12	4	16	*P. columella*
Bolivia	Beni	BE	100	0	0	0	*G. cubensis*
Bolivia	Chuquisaca	CH	74	18	8	26	*G. cubensis*
Bolivia	Cochabamba	CB	72	19	8	28	*G. cubensis*
Bolivia	La Paz	LP	78	21	1	22	*G. cubensis*
Bolivia	Oruro	OR	98	2	0	2	*G. cubensis*
Bolivia	Pando	PD	100	0	0	0	*G. cubensis*
Bolivia	Potosí	PT	84	16	1	16	*G. cubensis*
Bolivia	Santa Cruz	SC	95	4	1	5	*G. cubensis*
Bolivia	Tarija	TJ	82	13	5	18	*G. cubensis*
Bolivia	Beni	BE	100	0	0	0	*G. schirazensis*
Bolivia	Chuquisaca	CH	93	7	0	7	*G. schirazensis*
Bolivia	Cochabamba	CB	99	1	0	1	*G. schirazensis*
Bolivia	La Paz	LP	92	8	0	8	*G. schirazensis*
Bolivia	Oruro	OR	100	0	0	0	*G. schirazensis*
Bolivia	Pando	PD	100	0	0	0	*G. schirazensis*
Bolivia	Potosí	PT	100	0	0	0	*G. schirazensis*
Bolivia	Santa Cruz	SC	99	1	0	1	*G. schirazensis*
Bolivia	Tarija	TJ	99	1	0	1	*G. schirazensis*
Bolivia	Beni	BE	100	0	0	0	*G. truncatula*

Bolivia	Chuquisaca	CH	83	13	3	17	*G. truncatula*
Bolivia	Cochabamba	CB	75	16	8	25	*G. truncatula*
Bolivia	La Paz	LP	77	13	10	23	*G. truncatula*
Bolivia	Oruro	OR	92	8	0	8	*G. truncatula*
Bolivia	Pando	PD	100	0	0	0	*G. truncatula*
Bolivia	Potosí	PT	92	8	0	8	*G. truncatula*
Bolivia	Santa Cruz	SC	99	1	0	1	*G. truncatula*
Bolivia	Tarija	TJ	92	8	0	8	*G. truncatula*
Brazil	Acre	AC	60	40	0	40	*P. columella*
Brazil	Alagoas	AL	98	2	0	2	*P. columella*
Brazil	Amazonas	AM	97	3	0	3	*P. columella*
Brazil	Amapá	AP	100	0	0	0	*P. columella*
Brazil	Bahia	BA	89	11	0	11	*P. columella*
Brazil	Ceará	CE	100	0	0	0	*P. columella*
Brazil	Distrito Federal	DF	100	0	0	0	*P. columella*
Brazil	Espírito Santo	ES	84	4	12	16	*P. columella*
Brazil	Goiás	GO	50	20	30	50	*P. columella*
Brazil	Maranhão	MA	100	0	0	0	*P. columella*
Brazil	Minas Gerais	MG	56	16	28	44	*P. columella*
Brazil	Mato Grosso do Sul	MS	48	6	46	52	*P. columella*
Brazil	Mato Grosso	MT	87	10	2	13	*P. columella*
Brazil	Pará	PA	100	0	0	0	*P. columella*
Brazil	Paraíba	PB	98	2	0	2	*P. columella*
Brazil	Pernambuco	PE	99	1	0	1	*P. columella*
Brazil	Piauí	PI	100	0	0	0	*P. columella*
Brazil	Paraná	PR	38	19	43	62	*P. columella*
Brazil	Rio de Janeiro	RJ	64	5	31	36	*P. columella*
Brazil	Rio Grande do Norte	RN	99	1	0	1	*P. columella*
Brazil	Rondônia	RO	92	8	0	8	*P. columella*
Brazil	Roraima	RR	99	1	0	1	*P. columella*
Brazil	Rio Grande do Sul	RS	59	32	8	41	*P. columella*

Brazil	Santa Catarina	SC	58	30	12	42	*P. columella*
Brazil	Sergipe	SE	98	2	0	2	*P. columella*
Brazil	São Paulo	SP	56	2	42	44	*P. columella*
Brazil	Tocantins	TO	93	5	2	7	*P. columella*
Brazil	Acre	AC	98	2	0	2	*G. cubensis*
Brazil	Alagoas	AL	59	30	11	41	*G. cubensis*
Brazil	Amazonas	AM	100	0	0	0	*G. cubensis*
Brazil	Amapá	AP	100	0	0	0	*G. cubensis*
Brazil	Bahia	BA	67	13	20	33	*G. cubensis*
Brazil	Ceará	CE	73	19	9	27	*G. cubensis*
Brazil	Distrito Federal	DF	100	0	0	0	*G. cubensis*
Brazil	Espírito Santo	ES	86	12	2	14	*G. cubensis*
Brazil	Goiás	GO	57	11	33	43	*G. cubensis*
Brazil	Maranhão	MA	99	1	0	1	*G. cubensis*
Brazil	Minas Gerais	MG	51	21	28	49	*G. cubensis*
Brazil	Mato Grosso do Sul	MS	62	26	12	38	*G. cubensis*
Brazil	Mato Grosso	MT	88	10	2	12	*G. cubensis*
Brazil	Pará	PA	99	1	0	1	*G. cubensis*
Brazil	Paraíba	PB	61	12	27	39	*G. cubensis*
Brazil	Pernambuco	PE	82	7	11	18	*G. cubensis*
Brazil	Piauí	PI	98	1	1	2	*G. cubensis*
Brazil	Paraná	PR	63	36	1	37	*G. cubensis*
Brazil	Rio de Janeiro	RJ	72	21	7	28	*G. cubensis*
Brazil	Rio Grande do Norte	RN	81	10	9	19	*G. cubensis*
Brazil	Rondônia	RO	100	0	0	0	*G. cubensis*
Brazil	Roraima	RR	98	2	0	2	*G. cubensis*
Brazil	Rio Grande do Sul	RS	96	4	0	4	*G. cubensis*
Brazil	Santa Catarina	SC	94	6	0	6	*G. cubensis*
Brazil	Sergipe	SE	55	28	18	45	*G. cubensis*
Brazil	São Paulo	SP	63	14	23	37	*G. cubensis*
Brazil	Tocantins	TO	98	1	0	2	*G. cubensis*

Brazil	Acre	AC	100	0	0	0	*G. schirazensis*
Brazil	Alagoas	AL	99	1	0	1	*G. schirazensis*
Brazil	Amazonas	AM	100	0	0	0	*G. schirazensis*
Brazil	Amapá	AP	100	0	0	0	*G. schirazensis*
Brazil	Bahia	BA	95	5	0	5	*G. schirazensis*
Brazil	Ceará	CE	99	1	0	1	*G. schirazensis*
Brazil	Distrito Federal	DF	100	0	0	0	*G. schirazensis*
Brazil	Espírito Santo	ES	98	2	0	2	*G. schirazensis*
Brazil	Goiás	GO	99	1	0	1	*G. schirazensis*
Brazil	Maranhão	MA	100	0	0	0	*G. schirazensis*
Brazil	Minas Gerais	MG	92	8	0	8	*G. schirazensis*
Brazil	Mato Grosso do Sul	MS	100	0	0	0	*G. schirazensis*
Brazil	Mato Grosso	MT	100	0	0	0	*G. schirazensis*
Brazil	Pará	PA	100	0	0	0	*G. schirazensis*
Brazil	Paraíba	PB	99	1	0	1	*G. schirazensis*
Brazil	Pernambuco	PE	98	2	0	2	*G. schirazensis*
Brazil	Piauí	PI	100	0	0	0	*G. schirazensis*
Brazil	Paraná	PR	96	4	0	4	*G. schirazensis*
Brazil	Rio de Janeiro	RJ	95	5	0	5	*G. schirazensis*
Brazil	Rio Grande do Norte	RN	100	0	0	0	*G. schirazensis*
Brazil	Rondônia	RO	100	0	0	0	*G. schirazensis*
Brazil	Roraima	RR	99	1	0	1	*G. schirazensis*
Brazil	Rio Grande do Sul	RS	100	0	0	0	*G. schirazensis*
Brazil	Santa Catarina	SC	98	2	0	2	*G. schirazensis*
Brazil	Sergipe	SE	100	0	0	0	*G. schirazensis*
Brazil	São Paulo	SP	91	9	0	9	*G. schirazensis*
Brazil	Tocantins	TO	100	0	0	0	*G. schirazensis*
Brazil	Acre	AC	100	0	0	0	*G. truncatula*
Brazil	Alagoas	AL	100	0	0	0	*G. truncatula*
Brazil	Amazonas	AM	100	0	0	0	*G. truncatula*
Brazil	Amapá	AP	100	0	0	0	*G. truncatula*

Brazil	Bahia	BA	98	2	0	2	*G. truncatula*
Brazil	Ceará	CE	100	0	0	0	*G. truncatula*
Brazil	Distrito Federal	DF	100	0	0	0	*G. truncatula*
Brazil	Espírito Santo	ES	99	1	0	1	*G. truncatula*
Brazil	Goiás	GO	100	0	0	0	*G. truncatula*
Brazil	Maranhão	MA	100	0	0	0	*G. truncatula*
Brazil	Minas Gerais	MG	96	4	0	4	*G. truncatula*
Brazil	Mato Grosso do Sul	MS	100	0	0	0	*G. truncatula*
Brazil	Mato Grosso	MT	100	0	0	0	*G. truncatula*
Brazil	Pará	PA	100	0	0	0	*G. truncatula*
Brazil	Paraíba	PB	100	0	0	0	*G. truncatula*
Brazil	Pernambuco	PE	100	0	0	0	*G. truncatula*
Brazil	Piauí	PI	100	0	0	0	*G. truncatula*
Brazil	Paraná	PR	100	0	0	0	*G. truncatula*
Brazil	Rio de Janeiro	RJ	97	3	0	3	*G. truncatula*
Brazil	Rio Grande do Norte	RN	100	0	0	0	*G. truncatula*
Brazil	Rondônia	RO	100	0	0	0	*G. truncatula*
Brazil	Roraima	RR	100	0	0	0	*G. truncatula*
Brazil	Rio Grande do Sul	RS	100	0	0	0	*G. truncatula*
Brazil	Santa Catarina	SC	100	0	0	0	*G. truncatula*
Brazil	Sergipe	SE	100	0	0	0	*G. truncatula*
Brazil	São Paulo	SP	97	3	0	3	*G. truncatula*
Brazil	Tocantins	TO	100	0	0	0	*G. truncatula*
Peru	Amazonas	AMA	100	0	0	0	*P. columella*
Peru	Áncash	ANC	100	0	0	0	*P. columella*
Peru	Apurímac	APU	100	0	0	0	*P. columella*
Peru	Arequipa	ARE	100	0	0	0	*P. columella*
Peru	Ayacucho	AYA	99	1	0	1	*P. columella*
Peru	Cajamarca	CAJ	98	2	0	2	*P. columella*
Peru	Callao	CAL	100	0	0	0	*P. columella*
Peru	Cusco	CUS	97	3	0	3	*P. columella*

Peru	Huánuco	HUC	96	4	0	4	*P. columella*
Peru	Huancavelica	HVL	100	0	0	0	*P. columella*
Peru	Ica	ICA	100	0	0	0	*P. columella*
Peru	Junín	JUN	88	12	0	12	*P. columella*
Peru	La Libertad	LAL	100	0	0	0	*P. columella*
Peru	Lambayeque	LAM	100	0	0	0	*P. columella*
Peru	Lima	LIM	100	0	0	0	*P. columella*
Peru	Loreto	LOR	100	0	0	0	*P. columella*
Peru	Madre de Dios	MDD	65	35	0	35	*P. columella*
Peru	Moquegua	MOQ	100	0	0	0	*P. columella*
Peru	Pasco	PAS	90	10	0	10	*P. columella*
Peru	Piura	PIU	98	2	0	2	*P. columella*
Peru	Puno	PUN	93	7	0	7	*P. columella*
Peru	San Martín	SAM	97	3	0	3	*P. columella*
Peru	Tacna	TAC	100	0	0	0	*P. columella*
Peru	Tumbes	TUM	99	1	0	1	*P. columella*
Peru	Ucayali	UCA	88	12	0	12	*P. columella*
Peru	Amazonas	AMA	85	14	1	15	*G. cubensis*
Peru	Áncash	ANC	49	50	1	51	*G. cubensis*
Peru	Apurímac	APU	49	49	1	51	*G. cubensis*
Peru	Arequipa	ARE	85	14	1	15	*G. cubensis*
Peru	Ayacucho	AYA	63	37	1	37	*G. cubensis*
Peru	Cajamarca	CAJ	55	39	6	45	*G. cubensis*
Peru	Callao	CAL	67	16	18	33	*G. cubensis*
Peru	Cusco	CUS	82	18	0	18	*G. cubensis*
Peru	Huánuco	HUC	52	46	2	48	*G. cubensis*
Peru	Huancavelica	HVL	72	27	1	28	*G. cubensis*
Peru	Ica	ICA	71	27	2	29	*G. cubensis*
Peru	Junín	JUN	64	36	0	36	*G. cubensis*
Peru	La Libertad	LAL	66	31	3	34	*G. cubensis*
Peru	Lambayeque	LAM	67	24	9	33	*G. cubensis*

Peru	Lima	LIM	66	31	3	34	*G. cubensis*
Peru	Loreto	LOR	100	0	0	0	*G. cubensis*
Peru	Madre de Dios	MDD	99	1	0	1	*G. cubensis*
Peru	Moquegua	MOQ	88	12	0	12	*G. cubensis*
Peru	Pasco	PAS	78	22	0	22	*G. cubensis*
Peru	Piura	PIU	56	29	15	44	*G. cubensis*
Peru	Puno	PUN	70	30	0	30	*G. cubensis*
Peru	San Martín	SAM	89	11	0	11	*G. cubensis*
Peru	Tacna	TAC	73	26	1	27	*G. cubensis*
Peru	Tumbes	TUM	54	27	19	46	*G. cubensis*
Peru	Ucayali	UCA	99	1	0	1	*G. cubensis*
Peru	Amazonas	AMA	84	12	3	16	*G. schirazensis*
Peru	Áncash	ANC	71	22	6	29	*G. schirazensis*
Peru	Apurímac	APU	82	18	0	18	*G. schirazensis*
Peru	Arequipa	ARE	99	1	0	1	*G. schirazensis*
Peru	Ayacucho	AYA	89	11	0	11	*G. schirazensis*
Peru	Cajamarca	CAJ	59	16	25	41	*G. schirazensis*
Peru	Callao	CAL	99	1	0	1	*G. schirazensis*
Peru	Cusco	CUS	92	8	0	8	*G. schirazensis*
Peru	Huánuco	HUC	78	22	0	22	*G. schirazensis*
Peru	Huancavelica	HVL	77	22	1	23	*G. schirazensis*
Peru	Ica	ICA	99	1	0	1	*G. schirazensis*
Peru	Junín	JUN	76	24	0	24	*G. schirazensis*
Peru	La Libertad	LAL	79	12	10	21	*G. schirazensis*
Peru	Lambayeque	LAM	94	3	3	6	*G. schirazensis*
Peru	Lima	LIM	95	5	0	5	*G. schirazensis*
Peru	Loreto	LOR	100	0	0	0	*G. schirazensis*
Peru	Madre de Dios	MDD	100	0	0	0	*G. schirazensis*
Peru	Moquegua	MOQ	100	0	0	0	*G. schirazensis*
Peru	Pasco	PAS	83	17	0	17	*G. schirazensis*
Peru	Piura	PIU	83	8	9	17	*G. schirazensis*

Peru	Puno	PUN	96	4	0	4	*G. schirazensis*
Peru	San Martín	SAM	89	11	0	11	*G. schirazensis*
Peru	Tacna	TAC	100	0	0	0	*G. schirazensis*
Peru	Tumbes	TUM	93	7	0	7	*G. schirazensis*
Peru	Ucayali	UCA	100	0	0	0	*G. schirazensis*
Peru	Amazonas	AMA	96	4	0	4	*G. truncatula*
Peru	Áncash	ANC	66	11	23	34	*G. truncatula*
Peru	Apurímac	APU	43	18	39	57	*G. truncatula*
Peru	Arequipa	ARE	92	8	0	8	*G. truncatula*
Peru	Ayacucho	AYA	67	18	15	33	*G. truncatula*
Peru	Cajamarca	CAJ	69	16	15	31	*G. truncatula*
Peru	Callao	CAL	100	0	0	0	*G. truncatula*
Peru	Cusco	CUS	78	12	10	22	*G. truncatula*
Peru	Huánuco	HUC	53	18	29	47	*G. truncatula*
Peru	Huancavelica	HVL	78	11	11	22	*G. truncatula*
Peru	Ica	ICA	99	1	0	1	*G. truncatula*
Peru	Junín	JUN	72	9	19	28	*G. truncatula*
Peru	La Libertad	LAL	80	7	13	20	*G. truncatula*
Peru	Lambayeque	LAM	97	2	1	3	*G. truncatula*
Peru	Lima	LIM	83	11	6	17	*G. truncatula*
Peru	Loreto	LOR	100	0	0	0	*G. truncatula*
Peru	Madre de Dios	MDD	100	0	0	0	*G. truncatula*
Peru	Moquegua	MOQ	96	4	0	4	*G. truncatula*
Peru	Pasco	PAS	84	7	9	16	*G. truncatula*
Peru	Piura	PIU	90	7	3	10	*G. truncatula*
Peru	Puno	PUN	58	17	25	42	*G. truncatula*
Peru	San Martín	SAM	96	4	0	4	*G. truncatula*
Peru	Tacna	TAC	100	0	0	0	*G. truncatula*
Peru	Tumbes	TUM	100	0	0	0	*G. truncatula*
Peru	Ucayali	UCA	100	0	0	0	*G. truncatula*

6. CONCLUDING REMARKS

The geographic expansion of *Fasciola hepatica* in Brazil represents an increasing concern for public health and the environment. The occurrence of this parasitosis in previously unaffected regions can be attributed, among other factors, to changes in land use and land cover patterns, as well as to climatic variations that alter the environmental conditions favorable to the development of intermediate hosts. Areas vulnerable to *F. hepatica* are characterized by environments that support the parasite's biological cycle, such as bodies of water with aquatic vegetation, the presence of animals susceptible to infection, and environmental conditions suitable for the intermediate hosts.

The implications of this expansion are not limited to veterinary health, directly affecting human health, especially in areas where transmission occurs through the consumption of contaminated food. The increase in the incidence of fascioliasis can have economic repercussions due to livestock production losses and increased demands for treatment and disease control. In this context, control strategies should include measures to restrict livestock access to flooded or marshy areas, especially during seasons of higher transmission, as well as the monitoring and management of intermediate host mollusks in areas identified as environmentally suitable.

In addition to the effects on public health and the economy, the expansion of *F. hepatica* highlights the urgency of effective prevention policies, which should consider the environmental dynamics and human practices that favor the spread of the parasite. Continuous monitoring of at-risk areas and the implementation of integrated control strategies are essential to mitigate the impacts of the expansion and ensure the health of ecosystems and affected human populations.

This study contributes to the understanding of the spatial and environmental dynamics of *F. hepatica*, providing a solid foundation for future research and public policies for the control and prevention of fascioliasis in Brazil. Furthermore, the ecological niche modeling used in this work proved to be an effective tool for identifying at-risk areas and vulnerabilities to the spread of *F. hepatica*, allowing for a more detailed and accurate analysis of the environmental factors influencing its distribution. Understanding these factors can support land management policies aimed at controlling disease spread.

For the next steps, it is crucial to develop continuous monitoring methods and validate the areas identified as vulnerable. The integration of environmental, ecological, and epidemiological data will improve the accuracy of disease distribution forecasts, facilitating the implementation of targeted and effective control measures. Collaboration between public health, environmental, and agricultural sectors will be key to addressing the challenges posed by the expansion of fascioliasis. Raising awareness among local communities about the risks of the disease and promoting prevention practices, such as controlling host mollusks and proper animal management, are also essential to mitigate the disease.

Therefore, the results of this study reinforce the importance of an integrated and sustainable approach to control *F. hepatica*, considering the ecological, social, and economic dynamics of the affected regions. The increasing reports of human fascioliasis in South America, especially in areas with recent environmental changes and expansion of livestock activities, emphasize the need to frame fascioliasis as a One Health issue, given its interface between animal health, human health, and the environment. Combating fasciolosis requires continuous and collaborative efforts to mitigate the impacts of the expansion of this parasite, protect human and animal health, and ensure ecosystem sustainability. Finally, the data presented here represent the most up-to-date overview of fasciolosis in Brazil; however, knowledge remains limited by the lack of publicly available data on infected cattle from state and municipal slaughterhouses, including those from illegal or unregulated facilities.

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7. REFERENCES

ALBA, A.; VAZQUEZ, A. A.; HURTREZ-BOUSSÈS, S. Towards the comprehension of fasciolosis (re-) emergence: an integrative overview. **Parasitology**, v. 148, p. 385-407, 2020. DOI: 10.1017/S0031182020002255.

ALDA, P.; LOUNNAS, M.; VÁZQUEZ, A. A.; AYAQUI, R.; CALVOPIÑA, M.; CELI-ERAZO, M.; DILLON, R. T.; GONZÁLEZ RAMÍREZ, L. C.; LOKER, E. S.; MUZZIO-AROCA, J.; NÁRVEZ, A. O.; NOYA, O.; PEREIRA, A. E.; ROBLES, L. M.; RODRÍGUEZ-HIDALGO, R.; URIBE, N.; DAVID, P.; JARNE, P.; POINTIER, J. P.; HURTREZ-BOUSSÈS, S. Systematics and geographical distribution of *Galba* species, a group of cryptic and worldwide freshwater snails. **Molecular Phylogenetics and Evolution**, v. 157, 2021.

BARGUES, M. D.; ANGLES, R.; COELLO, J.; ARTIGAS, P.; FUNATSU, I. R.; CUERVO, P. F.; BUCHON, P.; MAS-COMA, S. One Health initiative in the Bolivian Altiplano human fasciolosis hyperendemic area: Lymnaeid biology, population dynamics, microecology and climatic factor influences. **Revista Brasileira de Parasitologia Veterinária**, v. 30, n. 2, 2021.

BARGUES, M. D.; HALAJIAN, A.; ARTIGAS, P.; LUUS-POWELL, W. J.; VALERO, M. A.; MAS-COMA, S. Paleobiogeographical origins of *Fasciola hepatica* and *F. gigantica* in light of new DNA sequence characteristics of *F. nyanzae* from hippopotamus. **Frontiers in Veterinary Science**, v. 9, p. 990872, 2022. DOI: 10.3389/fvets.2022.990872.

BENNEMA, S. C.; LÓPEZ-ABÁN, J. P.; GOMES, A. S.; GOMES, R. R.; SILVA, A. S.; ZANELLA, R. D. Modelling the spatial distribution of *Fasciola hepatica* in bovines using decision tree, logistic regression and GIS query approaches for Brazil. **Parasitology**, v. 144, n. 13, p. 1677–1685, nov. 2017. DOI: 10.1017/S0031182017000786.

GALAVANI, H.; HANILOO, A.; RAEGHI, S.; GHATEE, M. A.; KARAMIAN, M. Bioclimatic analysis and spatial distribution of fasciolosis causative agents by assessment of Lymnaeidae snails in northwestern provinces of Iran. **Parasites & Vectors**, v. 17, n. 1, p. 244, 2024.

MAS-COMA, M. S.; VALERO, M. A.; BARGUES, M. D. Fasciola, lymnaeids and human fascioliosis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology, and control. **Advances in Parasitology**, v. 69, p. 41-146, 2009. DOI: 10.1016/S0065-308X(09)69002-3.

MINISTÉRIO DA AGRICULTURA E PECUÁRIA - MAPA. Carne bovina e milho são destaques na exportação brasileira. Disponível em: https://www.gov.br/agricultura/pt-br/assuntos/noticias/carne-bovina-e-milho-sao-destaques-na-exportacao-brasileira. Acesso em: 1 fev. 2025.

McMANNUS, C.; BARCELLOS, J. O. J.; FORMENTON, B. K.; HERMUCHE, P. M.; CARVALHO, O. A.; GUIMARÃES, R.; GIANEZINI, M.; DIAS, E. A.; LAMPERT, V. N.; ZAGO, D.; NETO, J. B. Dynamics of cattle production in Brazil. **PLOS One**, v. 11, p. e0147138, 2016. DOI: 10.1371/journal.pone.0147138.

MEDEIROS, C.; SCHOLTE, R. G. C.; D'ÁVILA, S.; CALDEIRA, R. L.; CARVALHO, O. S. Spatial distribution of Lymnaeidae (Mollusca, Basommatophora), intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematoda, Digenea) in Brazil. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 56, p. 235-252, 2014. DOI: 10.1590/S0036-46652014000300010.

MOLENTO, M. B.; DUTRA, L. H.; PRITSCH, I. C.; GARBIN, V. P.; PEREIRA, A. M.; GAVIÃO, A.; GABRIEL, A. L.; DE SOUSA, R. S.; VIANA, J. G. A. *Fasciola hepatica* infection in cattle and the use of simulation models for endemic areas. J**ournal of Helminthology**, v. 94, p. e185, 2020. DOI: 10.1017/S0022149X2000067X.

SABOURIN, E.; ALDA, P.; VÁZQUEZ, A.; HURTREZ-BOUSSÈS, S.; VITTECOQ, M. Impact of human activities on fasciolosis transmission. **Trends in Parasitology**, v. 34, p. 891-903, 2018. DOI: 10.1016/j.pt.2018.08.004

VÁZQUEZ-PERERA, A. A.; ALDA, P.; LOUNNAS, M.; SABOURIN, E.; ALBA, A.; POINTIER, J. P.; HURTREZ-BOUSSÈS, S. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: Digenea): a worldwide review. **CABI Reviews**, v. 13, p. 1-15, 2018. DOI: 10.1079/PAVSNNR201813062.