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Abstract: The invasion of the golden mussel has resulted in considerable environmental and socioeconomic alterations, which present a considerable threat to the native biodiversity and sustainability of the region. It is of the utmost importance to gain an understanding of the distribution and biological characteristics of this Asian mussel, as well as its interaction with human activities, in order to develop effective strategies for mitigating and preventing its further spread. This study examines the dispersal route and incidence of golden mussels, tracing their movement from initial populations in Argentina to their arrival in the São Francisco River Basin (SFR). The presence of the mussel was confirmed through an integrative assessment, which included shell taxonomic analyses and mitochondrial DNA signatures. This identified populations located 7.5 km from the river's mouth, in proximity to the Atlantic Ocean, in areas such as shrimp farms, artisanal ports, and marinas. The analysis of mitochondrial DNA revealed the presence of South American-specific and shared ancestral haplotypes in the SFR, Grande River, and Argentina. These findings indicate that intracontinental colonisation towards the northeast of South America originated from Asian populations that entered South America via Argentina. The absence of Asian-specific signatures in the RSF, combined with its geomorphological structure, which is unsuitable for large ports or transoceanic vessels, supports the hypothesis of intercontinental dispersal of *Limnoperna fortunei*.

Keywords: bioinvasion; exotic invasive species; watersheds; human facilitation; intercontinental dispersal; bridgehead effects; mitochondrial DNA signatures; invasion genetics; gene flow

Key Contribution: An invasion route was identified in the São Francisco River based on South American (SA) DNA signatures, with no evidence of exclusive Asian signatures supporting the hypothesis of intercontinental dispersal into SA through Argentina

1. Introduction

When introduced to a new area, mussels can become an environmental issue [1,2] these species are often transported via ballast water [3,4], and their introduction can lead to the extinction of local

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species [5]. For instance, *Mytella charruana* (d'Orbigny, 1842) = *Mytella strigata* (Hanley, 1843) has been found in estuarine environments in North America and Asia [6–8], while *Limnoperna fortunei* (Dunker, 1857) has been found in freshwater environments in South America (SA) [9,10]. According to [10], Brazil reportedly spent at least USD 105.53 billion over 35 years (1984-2019), with an average annual expenditure of USD 3.02 (± 9.8) billion. Invaders caused damages and losses amounting to USD USD 104.33 billion, while stakeholders only invested USD 1.19 billion in their prevention, control, or eradication [10] *Limnoperna fortunei*, also known as golden mussel, is native to China and Southeastern Asia and is an invasive species in different ecosystems worldwide [11–13]. It can be found in mussel banks (juveniles and adults) with highly dense populations [14,15] adhering to natural and artificial substrates [16,17] in environments presenting low salinity (< 3PSU) water [18–21] and temperatures ranging from 0° C to 33° C [22–24]. Its sexual maturation happens at 3 to 4 months when the shell length of these individuals reaches 6 mm [25,26].

The dispersion and introduction of the golden mussel are often linked to pelagic larvae in ballast water discharged by ships in SA in 1990 [12]. The presence of *L. fortunei* in SA has resulted in economic and environmental losses [27,28]. The expenses reported for Brazil totaled USD 9.97 million and were solely related to control, damage repair, prevention, and research by *L. fortunei* [10]. Itaipu Hydroelectric Power Plant (HPP) is an example of this impact, having to stop for three days for the mechanical cleaning of golden mussels, resulting in a loss of approximately USD 750,000 [29].

Human activities are a significant factor in the dispersion of this species [30–32]. Additionally, the proliferation of *L. fortunei* severely affects drainage systems and flooded lake reservoirs used for power generation [33] and fishery production [17]. The species has been dispersed successfully upstream in South American rivers so frequently that its current frontier lies on the margin of the Amazon basin [34]. It has already been identified in the São Francisco River (SFR) basin [17,35] and a large portion of the Rio de La Plata basin [36]. The species advances an average of 240 km/year upstream in the middle and lower Paraná River [37] and 30 km/year in the Uruguay River basin [38]. The golden mussel has been shown to cause changes in biological diversity, leading to imbalances in the food chain and alterations in the structure and dynamics of species living in limnic environments [33,39]. [25] state that the impact of golden mussels on native populations is due to their attachment to the structures of other organisms, such as mollusks and crustaceans.

Shell morphology (conchology) and molecular tools are currently utilized to identify golden mussels [40–42]. Molecular tools have become increasingly crucial in bioinvasion studies [43]. They aid mitigation efforts and provide baseline data on the structure of golden mussel populations [29,41,44–49]. Research on the population structure of the golden mussel indicates that the initial *L. fortunei* propagules were introduced to Argentina and Brazil via ballast water from Japan and Korea [45,46]. To analyze the evolutionary processes related to each animal model, *Cox*1 barcode haplotypes are necessary for population genetics [50–52], and fragmentation standards of 600 to 800 bp should be maintained [51]. The absence of such a condition can lead to the emergence of artifacts that affect the comparison or identification of haplotypes within a given species [51]. Haplotypes of *L. fortunei* *Cox*1 barcode have been identified in Asia and SA [29,44–49]. Reports indicate that exclusive haplotypes derived from Asia [48] share ancestral haplotypes (Lfm03) from Asia. Additionally, exclusive haplotypes (Lfm04) from SA populations have been observed [29,45–47].

This study aims to (1) identify and discuss an intercontinental dispersal route for golden mussels, tracing their movement from initial populations in Argentina to their arrival in the estuarine region of the São Francisco River; (2) use integrated conchological and molecular approaches (DNA footprints) to identify populations established in this newly invaded region; and (3) discuss and list the human factors facilitating their spread in this river basin in South America.

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2. Materials and Methods

2.1. São Francisco River

The São Francisco River (SFR) basin spans 2,863 km and covers 639,219 km², equivalent to 7.5% of Brazil's national territory (Figure 1) and located in the Northeast of South America (SA). The basin comprises four physiographic sectors: Upper, Middle, Sub-Middle, and Lower. [53] describes the end portion of the basin as the Lower São Francisco River (LSFR). In the early 1960s, the course of the Piumhi River, a tributary of the Grande River (GR) at the head of the Paraná River (PR) basin, was reversed to the Upper sector of the SFR basin [54]. The LSFR basin covers a total area of 30,337 km² and extends for 274 km (5% of the basin). It includes the area between the power plant complex in Paulo Afonso County (Bahia State) and its mouth in the Atlantic Ocean (see Text S1, Figures S1 and S2). Studies have shown that coastal stretches in the LSFR, specifically the SFR delta, are undergoing erosion (see Text S1). The LSFR has a longitudinal gradient and fauna closer to an estuarine environment than an estuary. The reduction and regulation of the flow, resulting from the various dams along the course of the SFR, especially the Xingó dam located just 180 kilometers from the mouth, probably caused the salt wedge intrusion [55]. The alterations to the ichthyofauna assessed by [55] show the changes caused by river damming in this region. Notably, this region has no reports of intercontinental port activity (see Text S1).

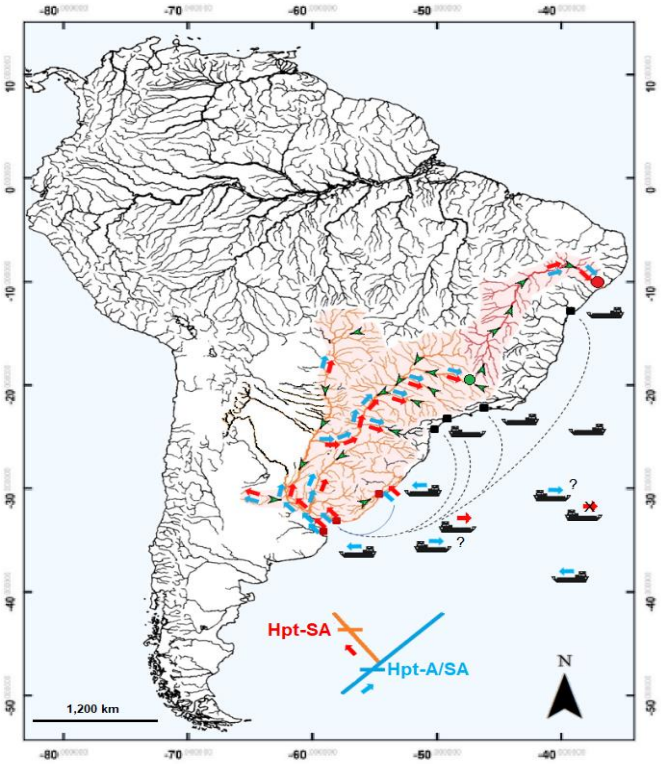

Figure 1. Occurrence of the golden mussel (red shaded areas) in ecoregions (according to [29] and [31], distribution of haplotypes and new occurrence (red circle; see Figure S1) of *Limnoperna fortunei* in South America (SA), with emphasis on its presence in the hydrographic basins of the Paraná River (in orange line) and the São Francisco River (in red line). Blue arrows indicate the occurrence of the Asian ancestral haplotypes (shares of Asia and SA) (Hpt-A/SA); red arrows indicate the South American derived haplotypes (exclusively SA) (Hpt-SA); green arrows indicate the river direction; the green circle indicates the region of the Capitólio dam and the Piumhi watershed transposition, see in [54,77]; vessels: bulk cargo transport; red squares indicate ports with golden mussel occurrence; black squares indicate ports without occurrence; lines indicate cargo cabotage routes between ports with (continuous) and without mussels (dotted); X - doubts about the flow of the common haplotype

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in the sea routes absence of Hpt-SA to Asian populations; ? - Doubts about the flow of Hpt-A/SA in the sea routes.

2.2. Collected Specimens

Golden mussels were first observed in LSFR during ichthyological studies [55]. The mollusks were found in April 2017 and November 2018 as bycatch collected through manual fish trawling (dimension: 30m x 2.8m; 5mm mesh between nodes) carried out every month from May 2017 to April 2018 in shallow areas of LSFR during spring tide. Apnea diving was subsequently used in an active search near specific points at the mouth of SFR in the counties of Brejo Grande and Ilha das Flores (see Figure S1 and Table S1).

Specimens were collected at these locations for molecular analysis on natural and artificial substrates. The points searched were at landing ports for artisanal fishing and marinas (see Figures S2 and S3). The rate of advance (RA) of the golden mussel in the watersheds of SA was calculated using the distance obtained from Google Earth and the number of months between mussel observation dates described in publications, multiplied by 12. The first month of the year is considered to be the month not reported in the references (see Table S1). One hundred specimens were manually collected at each point where the populations had established themselves. Salinity was measured using the Atago® manual refractometer. The specimens were fixed in ethanol and refrigerated at -20°C for preservation. Molecular identification was performed using adductor muscle tissues.

2.3. Identification and Biometry

Ninety specimens were identified using the conchological criteria suggested by [42]. Pictures of the collected specimens were taken using a Leica® M205 C stereomicroscope equipped with an MC170 HD real-time digital camera (Leica®) and LAS 4.8.0 software (Leica®). Specimen size (umbo-ventral axis) was measured using a digital caliper (see Figure S4), modified from [56]. Fifty-six specimens were genetically identified from the three localities sampled.

2.4. Searching for DNA Footprints and Dispersal Route

These fifty-six specimens were analyzed with adaptations using the phenol-chloroform protocol [57]. The mitochondrial gene *Cox*1 fragment was amplified using the primers [58] described. Polymerase Chain Reaction (PCR) was performed according to a protocol defined by [49,50]. The PCR product was purified, and all specimens' fragments (both strands) were sequenced using the automatic *ABI PRISM ™ 377 DNA Sequencer* by *Macrogen* (www.Macrogen.com). After sequencing, all electropherograms were checked manually. Finally, consensus strings were generated and compared with reference *L. fortunei* sequences available in GenBank (www.ncbi.nlm.nih.gov/nuccore/) - the BLASTn tool (www.ncbi.nlm.nih.gov/BLAST/) was used to confirm the identity of haplotypes (Figure 2; see Table S2), according to [45,48].

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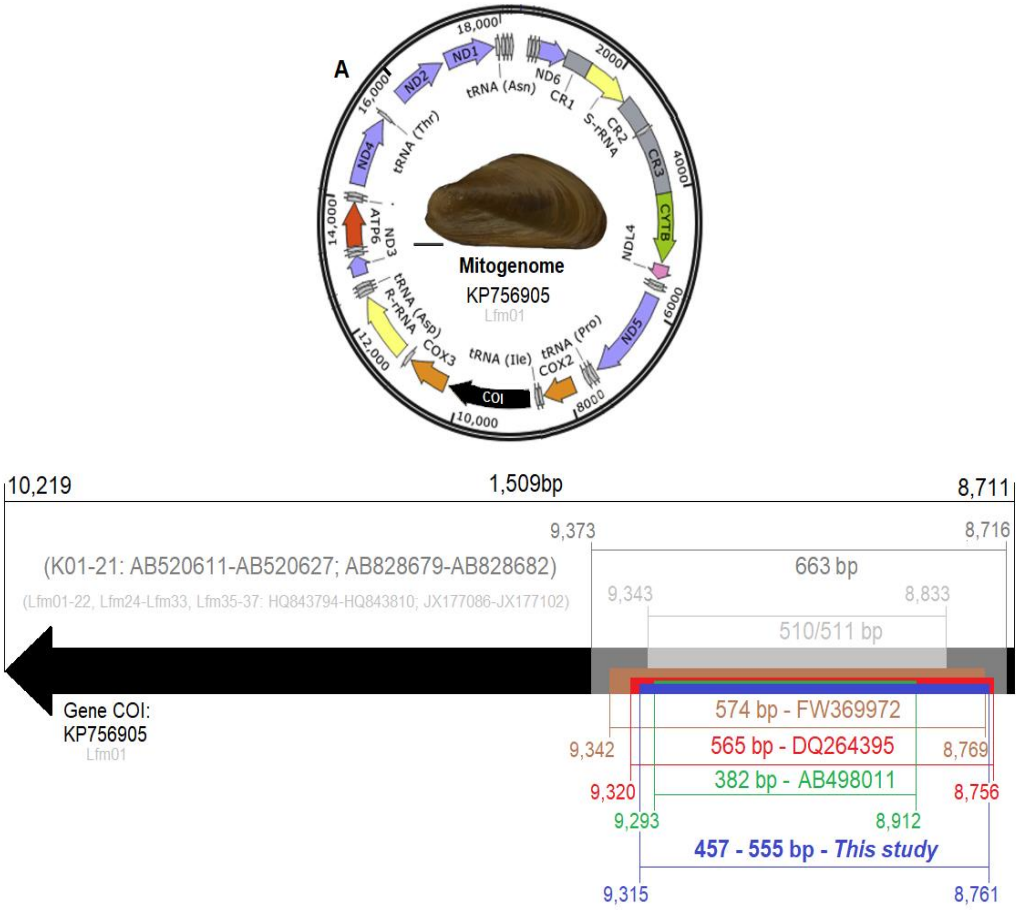

Figure 2. Position of the sample barcode (Cox1) from the Lower São Francisco River and the sequence barcodes available from Genbank and BOLD systems of the *Limnoperna fortunei* mitogenome (KP756905). bp: base pairs; Haplotypes: K01-21 (see 48) and Lfm01-Lf43 (see [29,45–47]; Genbank and BOLD systems accessions: KP756905 [66], AB520611-AB520627, HQ843794 - HQ843810, JX177086 - JX177102 [29,45–47], AB828679-AB828682 [48], FW369972 , DQ264395 [41], AB498011 [60].

To assess the dispersal route of the golden mussel was conducted based on studies that provided or published *Cox*1 sequences, summarizing a total of 2,160 specimens (735 from Asia and 1,425 from SA). By comparing the available sequences and the haplotypes described in the literature, a standardization of the nomenclature was carried out, as presented in Table S2. Thus, the analysis of the golden mussel's dispersal route was traced by comparing the presence of haplotypes and the collection sites of the populations (see Table S2).

3. Results

3.1. Mussel Occurrence

The occurrence of golden mussels was reported at two different times (April 2017 and November 2018) at three other locations in the LSFR basin (VIF - Village Ilha das Flores, BGM - Brejo Grande Marine; MRS - Mouth of the São Francisco River). These sites were the most easterly in SA. The occurrence of the mussel in the LSFR extended its distribution by 500 km in the largest exclusively Brazilian hydrographic basin. The occurrence of golden mussels in nearby estuaries (7.5 km) of the SFR estuary had never been reported in the estuaries of northeastern SA (Figure 1; see Figure S1). This expanded distribution resulted in a species advance of approximately 375 km/year downstream of the LSFR (see Table S1). Living mussels were collected from low salinity collection sites such as estuarine (MRS: 4PSU) and freshwater (VIF and BGM) beaches associated with artisanal fishermen,

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landing ports, and drainage channels used for fish and white shrimp [*Litopeneus vanammei* (Boone, 1931)] farming (see Figures S1 and S2). Live bivalves were collected from the underwater areas of marinas, artisanal fishing ports, and drainage channels used for fish farming. They were attached to unconsolidated (coarse sand and gravel), artificial (bricks and cement fragments), and naturally consolidated substrates (rocks). About the natural consolidated substrate, it was possible to see golden mussels associated with native estuarine species [*Crassotrea* sp. and *Vitta virginea* (Linnaeus, 1758)] able to survive in low salinity estuarine environments associated with river outlets. In Ilha das Flores (VIF), clusters of mussels were recorded, some of which were collected and observed next to aquatic macrophytes (see Figures S1, S2, and S3). Population densities were not assessed at these sites.

3.2. Mussel Identification and DNA Footprints

The shells of the collected specimens were formed by two equal valves with a triangular contour and an elongated base. The subterminal umbo, smooth, simple joint ligament, and very thin, straight, and toothless hinge (see Figure S4) confirm the identification of *L. fortunei* by [40,42]. Voucher specimens were deposited in the mollusk collections of the *Universidade Estadual de Ponta Grossa* in the *Coleção de Moluscos* (UEPG-CMo: CMo 1120; CMo 1121; CMo 1122) and the *Museu de Ciências Naturais* of the *Universidade Federal do Paraná* (MCN-UFPR: MCN.Z 777). Individuals with carapace lengths ranging from 5.25 to 23.79 mm (mean 9.81±4.57 mm) were observed at the site (Table 1) in the VIF, it is possible to observe the presence of the largest individuals, indicating banks with adult specimens and older and better-established population compared to the other two populations (BGM and MSR, see Figure S3).

Table 1. Biometrics of *Limnoperna fortunei* shells found in Brazil. n - number of mussels collected. LA - mean shell length; SD - standard deviation; LR - shell length range.

Location	n	LA ±SD (mm)	LR (mm) [a]	References
São Francisco River ts (-8.54°; -39.456°)	5	- - [c]	15.00 to 30.00	17
Sobradinho reservoir (-9.409; -40.817)	5	- - [c]	15.00 to 30.00	17
Sobradinho hydroelectric (-9.433; -40.828)	5	- - [c]	15.00 to 30.00	17
Ilha da Flores (-10.435°; -36.531°)	30	12.84 ±4.95*	7.44 to 23.79	***This study***
Brejo Grande marine (-10.421°; -36.464°)	30	7.42 ±1.82*	5.36 to 11.75	***This study***
São Francisco River mouth (-10.445°; -36.426°)	30	9.37 ±4.45*	5.25 to 20.14	***This study***
Porto Primavera hydroelectric (-22.474°; -52.474°)	61	- - [c]	4.00 to 27.00	[61] [b]
Itaipu reservoir - Paraná river (-24°; -54°)	- - [c]	- - [c]	6.00 to 35.00	[62]
Paraná River (-25.437°; -54.512°)	800	- - [c]	21.00 to 27.00	[63]
Mirim Lake (-31.799°; -52.382° and -32.116°; - 52.582°)	7,789	- - [c]	3.00 to 32.00	[64] [d]
Mirim Lake (-31.799°; -52.382° and -32.116°; - 52.582°)	147	- - [c]	3.00 to 14.00	[64] [e]
São Gonçalo channel (-32.148°; -52.625°)	7,776	- - [c]	4.00 to 32.00	[65] [d]

ts – transposition system; * total average for the site (9.81±4.57mm); [a] variation in shell length; [b] Inside the stomach of Pterodoras granulosus; [c] not cited by the authors; [d] depth from 3 to 6 m; [e] Inside the stomach of Pimelodus pintado.

Here, we present for the first time the annotation of fragments of the cytochrome oxidase gene (Cox1) gene in the mitogenome of *L. fortunei* (Figure 2). Partial sequences of the Cox1 from the collected samples (VIF-18, BGM-19, and MRS-19; see Table S2 and FS5) resulted in length alignments of 457 (one sequence), 530 (one sequence) and 555 (54 sequences) bp, located between positions 8,761 and 9,315 of the *L. fortunei* mitogenome (KP756905). Four *L. fortunei* haplotypes (99.70% to 100% similarity) were identified for Lfm01, Lfm02, Lfm03, and Lfm4 and deposited in GenBank and BOLDSystem (references XX000000.0 to XX000000.0).

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4. Discussion

4.1. Cox1 Barcode Annotation and Haplotypes in Golden Mussel

The annotation of Cox1 fragments in the mitogenome of *L. fortunei* indicated, that their position affects haplotype identification. The position and length of these Cox1 fragments in golden mussels are not the same as the polymorphic regions observed in haplotypes described in this gene by [45] and [48]. The sequences described in the literature and deposited in Cox1 gene databases range from 382 bp to 1,509 bp in length and are located in different positions in the *L. fortunei* mitogenome (Figure 2). To avoid artifacts in the analysis of the dispersal route, it is essential to verify whether there is overlap across all Cox1 barcode *loci* when describing a haplotype for the specimen referenced in the studies. However, some polymorphic sites that could indicate a specific haplotype were not evaluated (Figure 2; see Table S2 and Figure S5). Therefore, it is crucial to emphasize the importance of accessing reference sequences [41,45–48,60,66] to analyze and identify *L. fortunei* haplotypes. If a new haplotype is described, it must be made available to the scientific community.

4.2. Identified Footprints and Possible Origins

Integrated molecular and morphological identification played a crucial role in confirming the *L. fortunei* in estuarine and freshwater regions of LSFR. The exclusive haplotype found in this site (Lfm01 and Lfm04) is consistent with SA populations [29,46], providing evidence of the species' successful invasion of new hydrographic environments. In the study by [29], it was observed that the exclusive haplotype (Lfm39) was present in SA populations in the SFR and PR basins (see Figure S5 and Tables S2 and S3).

Native distribution of *L. fortunei* is probably limited to the Pearl River basin in China [11,13]. This invasive species can be found in several Asian (Japan and Korea) and SA regions [31,46,48,67]. [46] analyzed the genetic structure of 24 *L. fortunei* populations (ten from Asia and fourteen from SA) living in invaded and native areas based on Cox1 gene sequences (~510bp), and eight microsatellite markers concluded that bivalve populations living in Asia had greater diversity (23 haplotypes) than those living in SA (18 haplotypes), suggesting that Asia has been subject to a more significant number of introduction events for invasive populations. The authors also reported the fine genetic structure of *L. fortunei* on both continents, suggesting strong post-introduction selection and stochastic events inherent to the species' biology. These events contribute to the structure of its population and the occurrence of Cox1 haplotypes exclusive to SA (Lfm01, Lfm04, and Lfm39). In the LSFR, two haplotypes exclusive to SA (Lfm01, Lfm04) were identified (see Figure S5 and Table S2).

The Cox1 haplotypes (457bp to 555bp) observed in specimens collected from LSFR and Sobradinho [29] exhibit features that are characteristic of populations from SA (Lfm02, Lfm03, Lfm11, Lfm15, Lfm36, and Lfm38), Taiwan, Korea, Japan, and China [29,45–48]. Based on the haplotypes analyzed in LSFR, it can be inferred that the observed population in VIF (Lfm02, Lfm03, and Lfm04), BGM (Lfm02, Lfm03, and Lfm04), and MRS (Lfm01, Lfm02, Lfm03, and Lfm04) originated from populations located further south in the American continent. [68] observed similar conditions when comparing mussel populations from five different Brazilian reservoirs using the double digest restriction-site associated DNA sequencing (ddRAD-seq) protocol for the golden mussel. These authors indicated connectivity between basins and absent geographic structure [68]. Same as the data presented here with the Cox1 gene. Exclusive SA haplotypes in SFR and PR basins support the hypothesis of upward colonization towards hydrographic basins in Northeastern Brazil (Figure 1 and see Table S2). It is essential to emphasize the lack of port areas for ships that are not navigable by large vessels at the mouth of the SFR [55,69]. The presence of the golden mussel after thirty years (from 1991 to 2020) in Argentina confirms the theory of the intercontinental spread of the mussel in SA [21]. This fact virtually excludes golden mussel reintroduction due to maritime cabotage (Figure 1), as supported by [36,70,71] (see Figures S1 and S2). The absence of golden mussels in coastal watersheds in Southeast and Northeast Brazil port areas, as confirmed by [29,31], supports the theory of intracontinental dispersal.

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Furthermore, the specimens genotyped by LSFR have haplotypes Lfm02 and Lfm03 (Figure 1; see Figure S5 and Table S2). This data supports the hypothesis that Asian specimens [72] initially invaded SA from Argentina [12,14]. The dispersion process of the golden mussel is indicated by the flow of the targeted gene of haplotypes from Asia to SA (see Table S2). There were no exclusive haplotypes from SA in Asia, as reported by [29,45–49]. This may be related to the export of grains, such as soybeans and sugar, from SA to Asia [73]. According to [74], some grain-loaded ships do not require ballast water to achieve stability. However, vessels arriving from Asia require ballast water to achieve stability; this feature facilitates directional genetic flow from Asia to SA (Figure 1; see Table S2). Studies have shown that *M. strigata*, another invasive mussel in the world, has invaded different areas of America and Asia [7,8]. Notably, *L. fortunei* and *M. strigata* can coexist in low salinities [20,75]. However, it is essential to note that only the golden mussel exhibits directional flow from its native to the invaded area, as demonstrated by various studies [7,8,45–47].

4.3. An Intercontinental Route to Northeastern Brazil

The reversal of the course of the Piumhi River (a tributary of the GR at the head of the PR basin) into the SFR basin in the early 1960s [54] is thought to have facilitated the invasion of the golden mussel, as well as the connectivity of these basins (Figure 1). Invasion risk factors were observed in the studied environment (see Figure S2). This anthropogenic change, which eliminated the natural barrier between these basins, allowed the introduction of species and the genetic flow of fish (*Hypostomus regani* and *Astyanax* "*bimaculatus* group") from the PR basin to the SFR basin [76,77]. The Capitólio dam interfaces the two river basins [54]. The proximity of these two basins, changes in water flow (Moreira Filho, personal communication), and the transit of ships between the basins may have contributed to the invasion of the golden mussel towards the northeastern region of SA. The tributaries of the GR aren´t navigable for commercial vessels, despite other dispersal vectors such as small recreational and fishing boats, fish farming, and the use of sand recovered from mussel-infested areas, such as the Tietê River and other sites in the PR basin [36]. Recently, the presence of the golden mussel in the communication channels of these two interconnected hydrographic basins has been observed in the SFR [35].

In addition, the presence of haplotypes in SA (Lfm01 to Lfm04, Lfm15, and Lfm38) close to the Piumhi River (observed at two sites in the GR tributary by [29] indicates the likely connection between the two basins (Figure 1; see Figure S5 and Table S2). This evidence reinforces the concerns of the expert committee (participants in the National Plan for the Prevention, Control, and Monitoring of the Golden Mussel in Brazil) about the role of water transport from the SFR to the Tocantins River basins as a vector for the invasion of golden mussel larvae and adults [28]. It cannot be excluded that there are other ways of introducing these mussels into the SFR basin, other than the likely dispersal jumps from southeastern to northeastern Brazil in freshwater fish transport tanks [35,68,78].

4.4. Golden Mussel and Human Activities in Northeastern Brazil

Invasion risk factors were observed in the studied environment (see Figures S2C and S3); therefore, they need to be taken into account at this point to implement plans focused on mitigating the occurrence of this invasive species in LSFR. Monitoring and early detection of invasive species is a prerequisite for developing bioinvasion prevention and management plans [79]. The mussel species live in different freshwater environments such as streams, rivers, dams, lakes, coastal lagoons, lagoons in low salinity scenarios, and river deltas [28,80]. Thus, the risk posed by each dispersal vector observed in the watershed is related to economic activities [31], social arrangements, local practices, and habits, which may differ between hydrographic systems and result in different invasion routes or corridors [81].

Confirming golden mussel occurrence in inland regions closer to the coast suggests the vectors of recurrent dispersal in Northeastern Brazil. Similar to the process observed in Japan [48], the introduction of mussels in Northeastern Brazil is also associated with aquaculture [82]. [83] observed golden mussel banks in tilapia (*Oreochromis niloticus*) aquaculture tank nets used in

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southern/southeastern Brazil reservoirs. Curimatã (*Prochilodus argenteus*), tambaqui (*Colossoma macropomum*), tilapia, and common carp (*Ciprinus carpio*) stand out among the fish species farmed in freshwater tanks dug in LSFR. These tanks enable the subsistence of about 2,000 regional families [84].

The LSFR presented small golden mussel beds in low salinity water channels associated with ponds dug to cultivate the grey shrimp species *L. vanammei* (see Figures S2 and S3). The Northeast region represents the central Brazilian pole for grey shrimp farming in marine and freshwater environments, thus contributing to the social and economic development of the region [85,86]. The presence of L. fortunei can affect this productio*n chain*, as mussel fouling can affect pumping systems, reduce water flow in tanks, and intensify disease transmission processes. [87] developed a model to predict the distribution of golden mussels based on air temperature and rainfall. The scenarios generated have shown a high potential for *L. fortunei* to invade aquatic environments in Central America, North America, Europe, Africa, and Oceania, as well as the expansion of golden mussel occurrence areas in Asia and SA. According to the authors, the Amazon, Tocantins, Araguaia, and SFR river basins have the most tremendous potential for invasion in Brazil. [17] confirmed the occurrence of golden mussels in 2015 at two sites near the Sobradinho hydroelectric plant and another at the crossing (transposition system) of the SFR (Table 1). Similar observations were made in the Mato da Onça Reserve, close to the Xingó Hydroelectric Plant [88,89]. The current identification of golden mussel populations in freshwater (VIF and BGM) and in an estuarine area (MRS) of the LSFR (average salinity 14.67 PSU, with a maximum of 26.43PSU and a minimum of 3.59PSU; [90]) in 2017 confirms the predictions of [87] regarding the expanded distribution of golden mussels in the LSFR basin. Similar conditions have been observed for Lagoa dos Patos and the La Plata River [15,20]. However, the results of the present study are 10 years ahead of the projections made by some predictive models [78,87].

The dispersal rate of the golden mussel observed in the SFR basin is higher than that recorded for the Paraná basin (see Table S1), which may be explained by the initial colonization of the golden mussel in the upper SFR. The three populations identified in the LSFR showed a mean shell length of reproductive age specimens (Table 1, see Figure S3). In SA, sexual maturity varies seasonally, starting at 5-6 mm shell length in winter-spring and 7-10 mm in autumn [25]. The downstream dispersal of planktonic larvae [39] or juvenile/adult individuals associated with floating substrates, such as macrophytes, observed in the LSFR ([88], Figure 1, see Figures S1–S3) is a favorable and opposite condition to the upstream distribution of this invasive species in other hydrographic basins, such as the Paraná-Paraguay system, reported in Japan [48].

5. Conclusions

The present study has confirmed the presence of *L. fortunei* populations in different LSFR localities and their range's rapid expansion. These findings indicate route intracontinental colonization towards the Northeast of SA. The data analyzed showed that the populations of golden mussels living in the LSFR originate from populations established in other places in SA, which originated from Asian populations that entered SA via Argentina and reached the study area through human vectors. It´s, therefore, necessary to carry out further studies focusing on monitoring possible vectors of introduction and spread and the occurrence of golden mussels in river basins located in the north and northeast of Brazil. In addition, measures to educate and make people (especially water users) aware of the problem could be considered to allow for the most efficient management. The situation in the Tocantins and Araguaia River basins is similar to that observed in the SFR, and the presence of the gold mussel in these basins is the reality.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. **Table S1.** Rate of advance (RA) of golden mussel in watersheds of South America., and **Table S2.** Sampling details and Haplotypes Codes for mitochondrial cytochrome c oxidase subunit I (Cox1) gene for the golden mussel Limnoperna fortunei. https://docs.google.com/document/d/1_D9itXHi2z90mEK9pIrHiOShhEBKyzz1C4IPzUtqK24/edit; **Figure S1.** Dispersion of the golden mussel (*Limnoperna fortunei)* in the Lower São Francisco River (LSFR) basin., **Figure**

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S2. Example of factors that may have contributed to the dispersion of the golden mussel in the Lower São Francisco River basin., **Figure S3.** *Limnoperna fortunei* present in different substrates in the Lower São Francisco River basin., **Figure S4.** Biometry and Conchiology of *Limnoperna fortunei* obtained in the Lower São Francisco River., and **Figure S5.** Neighbor-Joining (NJ) tree for mitochondrial cytochrome c oxidase subunit I (*Cox1*) gene for the golden mussel**.** https://docs.google.com/document/d/1RO7Wi9TukBv0LRz9M_zBxM7YEPNZi10EKGAjgDClqp0/edit; **Text S1.** The Lower São Francisco River (LSFR) region.; **Text S2.** Methodology. https://docs.google.com/document/d/1lO3uAcnviMT2L-B1tkWBBDLil_BEUyO1JUUdLSinHKA/edit.

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