Hydrobiologia (2024) 851:1203–1217
https://doi.org/10.1007/s10750-023-05378-1

PRIMARY RESEARCH PAPER

Opening the black box of the invasion of *Corbicula* clams (Bivalvia, Corbiculidae) in South America: a genetic and morphological evaluation

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Received: 19 October 2022 / Revised: 21 June 2023 / Accepted: 6 September 2023 / Published online: 11 October 2023

Abstract Among species of *Corbicula*, only the hermaphroditic androgenetic lineages are invasive while sexual species are restricted to the native range (mainly Asia). Four androgenetic lineages of *Corbicula* spp. have been identified in American and European freshwater systems, with the *Corbicula* sp. form A/R lineage being abundant on both continents. This lineage is considered an important invasive species because of its impact on aquatic ecosystems and industrial cooling systems. The present study identified the invasive lineages of *Corbicula* spp. in freshwater environments of South America based on morphometric data and used genetic marker (mtDNA COI gene) to investigate their genetic relatedness to the invasive lineages from Europe and North America. Four important results arise: (1) there are two invasive lineages of *Corbicula* in South America, *Corbicula* sp. form C/S and *Corbicula* sp. form A/R, and they are clonally expanding their distribution in continental aquatic systems of South America; (2) populations with intermediate morphotype were detected in three distinct sites of Brazil; (3) one population with individuals presenting a new COI haplotype was recovered with an intermediate morphotype; (4) the morphotype of the invasive lineages of *Corbicula* spp. in South America presents high correlation with their COI haplotypes.

Keywords Invasion genetics · Geometric morphometrics · Clonal lineages · Phylogeny · COI

Introduction

Species of *Corbicula* clams are native to Asia, Australia, Africa, and the Middle East, inhabiting both estuarine and freshwater environments (Araújo et al., 1993; Park & Kim, 2003). The genus includes dioecious sexual species, which seem mainly restricted to the native region in Asia, and the hermaphroditic androgenetic lineages that were genetically recognized invasive lineages were detected in America, Europe (Pigneur et al., 2014a), and Russia (Bespalaya et al., 2018).

Androgenetic reproduction is a peculiar asexual mode of reproduction in which the offspring are paternal clones, inheriting only the nuclear chromosomes from the sperm, while all the maternal nuclear

Handling editor: Manuel Lopes-Lima

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10750-023-05378-1.

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chromosomes from the oocyte are extruded as polar bodies after fertilization (Komaru et al., 2006; Houki et al., 2011; Pigneur et al., 2012; Vastrade et al., 2022). In the species of *Corbicula,* this reproductive mode is characterized by the presence of biflagellate and unreduced sperm (Komaru et al., 1997; Konishi et al., 1998). Moreover, the sperm from an androgenetic lineage of a species of *Corbicula* can fertilize the egg of a different lineage, combining its nuclear genome with the mitochondrial genome of the other, resulting in a cytonuclear mismatch (Komaru et al., 2006; Hedtke et al., 2008; Vastrade et al., 2022). When the maternal nuclear genome is incompletely extruded, an admixture with the paternal genome can occur—event known as androgenetic parasitism—and a hybrid lineage is formed (e.g., Komaru et al., 2006; Hedtke et al., 2008; Bespalaya et al., 2018).

The invasive *Corbicula* spp. presently identified in America and Europe were divided into four lineages (Pigneur et al., 2014a; Tiemann et al., 2017; Vastrade et al., 2022) and appear to reproduce through androgenesis. First, *Corbicula* sp. form A/R is abundant and widely distributed in Europe and America (Lee et al., 2005; Hedtke et al., 2008; Pigneur et al., 2014a). Second, *Corbicula* sp. form C/S is present in South America and Europe (Pigneur et al., 2014a; Santos et al., 2016). Third, *Corbicula* sp. form B is known only in North and South America (Lee et al., 2005; Hedtke et al., 2008). Four, *Corbicula* sp. form Rlc is reported only in Europe (Pigneur et al., 2011b; 2014a). Recently, *Corbicula* sp. form D was recovered in Illinois, USA (Tiemann et al., 2017). However, form D appears to be a hybrid at the nuclear and morphological level, with a COI haplotype identical to that of *Corbicula* sp. form A/R, as observed in several *Corbicula* sp. populations of form A/R (Hedtke et al., 2008; Pigneur et al., 2014a; Tiemann et al., 2017; Vastrade et al., 2022).

These invasive *Corbicula* spp., especially the form A/R lineage, are considered one of the most invasive mollusks worldwide (Darrigran, 2002; Sousa et al., 2008; Crespo et al., 2015; Darrigran et al., 2020) due to their large geographic distribution in freshwater ecosystems, the high densities they can reach (5000 individuals/m^2) (Mansur et al., 2012; Ferreira-Rodriguez et al., 2018), their high fecundity (e.g., 90.000 descendants in one reproductive period) (McMahon, 1999; Paschoal et al., 2013), their impact on phytoplankton abundance (Pigneur et al., 2014b), and their invasive behavior (Darrigran, 2002). As is common for most invasive species, *Corbicula* sp. form A/R has a high reproductive capacity (Cao et al., 2017). Cao et al. (2017) demonstrated, in populations of *Corbicula* sp. from Argentina, that neither the oocyte incubation nor the gamete spawning was affected by low temperatures (8.2–9 °C) and low salinities (0–3‰). *Corbicula* spp., therefore, appear capable of locally adapting during temporal water level fluctuations, surviving low temperatures (2 °C) (Castañeda et al., 2018), and promoting morphological changes in Neotropical regions (Paschoal et al., 2015) with distinct incubation pattern (Ludwig et al., 2014). In temperate climates, immature individuals are released from gill chambers being almost juvenile, while in subtropical climates, the release occurs at a pediveliger stage (Cao et al., 2017). These characteristics likely maximize their success of invasion in a wide range of environments. Additionally, a single hermaphroditic androgen is enough to initiate a new population owing to its ability to self-reproduce (Pigneur et al., 2014a).

Compared to the native species, the invasive *Corbicula* spp. present a much lower genetic diversity in their introduced range (Pigneur et al., 2014a; Gomes et al., 2016). This is probably the consequence of their androgenetic mode of reproduction (Siripattrawan et al., 2000; Lee et al., 2005; Hedtke et al., 2008; Pigneur et al., 2011b; 2012). Indeed, according to Pigneur et al. (2014a), a few individuals recently invaded America and Europe and, through their clonal mode of reproduction, the established clonal diversity was maintained. Within the native range, however, where sexual and androgenetic species co-occur, high genetic diversity is observed (Pigneur et al., 2014a; Vastrade et al., 2022).

In South America, the first report of *Corbicula* spp. was in Argentina around 1980 (Ituarte, 1981). Since then, the number of published records of *Corbicula* spp. has increased (Santos et al., 2012) and their current distribution encompasses the northern (39°5’S, 68°34’W) and southern (43°21′18.28"S 65°39′34.93"W) Argentine region of Patagonia (Semenas & Flores, 2005; Labaut et al., 2021a, b; Perez et al., 2022), the Negro River in Northern Brazil (Pimpão & Martins, 2008), the Orinoco basin in Venezuela (Lasso et al., 2009), and the island of Salamanca in Colombia (Aristizábal, 2008). According to

Castañeda et al. (2018), their distribution is usually limited to temperatures between 2 °C and 34 °C.

Four species of *Corbicula* were reported from South America and identified solely on basis of morphological characteristics, are *Corbicula fluminea* (O.F. Müller, 1774), *Corbicula largillierti* (R.A. Philippi, 1844), *Corbicula* cf. *fluminalis* (O.F. Müller, 1774), and *Corbicula* sp. (detected only by Mansur et al., 2004) (Mansur et al., 2004; Santos et al., 2016). In 2005, three distinct morphotypes of *Corbicula* were genetically identified, each one corresponding to a distinct mitochondrial COI haplotype and nuclear 28S genotype with no genetic variability observed within each form (Lee et al., 2005) and, years later, were assigned to the invasive lineages *Corbicula* sp. forms A/R, *Corbicula* sp. forms B, and *Corbicula* sp. forms C/S (Pigneur et al., 2014a). Lee et al. (2005) also detected hybrid specimens in the Iguazu Falls (Iguaçu River, Paraná state in Brazil), which presented 'mixed' genotypes between form C/S lineage and form B lineage associated with C/S morphotype. Besides, based on ISSR markers, Bagatini et al. (2005) demonstrated that there are three distinct morphotypes of *Corbicula* in the Rosana reservoir (Paraná state, Brazil) which shared a single multilocus genotype (MLG) and were assigned to *Corbicula* sp. form A/R lineage. In 2014, Pigneur et al. (2014a) performed a world phylogeographic survey on specimens of *Corbicula* spp. and identified in two localities in South America the presence of the invasive lineages *Corbicula* sp. forms A/R and C/S, with no genetic variability observed within each form using both COI haplotype and 10 microsatellite markers. These two invasive lineages are widely distributed in South American rivers (Santos et al., 2012; Santana, 2013) that were linked to *C. fluminea* which corresponds to *Corbicula* sp. form A/R, and *C. largillierti* corresponding to *Corbicula* sp. form C/S (Hedtke et al., 2008; Pigneur et al., 2014a). On the other hand, *C.* cf. *fluminalis* is apparently restricted to Patos Lagoon and Guaiba Lake (Southern Brazil) (Mansur et al., 2012; Santos et al., 2012), which is genetically recognized as *Corbicula* sp. form B lineage (Lee et al., 2005; Hedtke et al., 2008). However, there are projection studies attesting suitable areas to *Corbicula* cf. *fluminalis* be present in South America (Reyna et al., 2018).

In the present study, a geographically wide sampling effort across South American basins was performed to determine which invasive lineages of *Corbicula* are present in South America using geometric morphometrics and genetic analyses of the mtDNA COI gene. Besides, we evaluated if there are individuals with cytonuclear mismatches as suggested by Pigneur et al. (2012).

Materials and methods

Sample collection

From 2011 to 2014, 507 specimens of *Corbicula* spp. of 22 putative populations were collected from rivers of Argentina, Brazil, and Colombia (Table 1). All specimens were preserved in 96% ethanol. In the Laboratory of Molecular Ecology and Evolutionary Parasitology at the Universidade Federal do Paraná, Brazil (LEMPE), the shells were separated from the soft tissue and the right shell of each specimen was photographed with a Canon Rebel EOS T3 digital camera for morphological analysis. The preserved tissues (in 96% ethanol) were used for molecular analyses.

COI gene amplification

A fragment of 650 bp of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified for each sampled specimen of *Corbicula* clams by Polymerase Chain Reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al., 1994). Amplifications were performed in 25 µl total volume including 0.5 µl of gDNA, 1×Reaction buffer, 200 µM of dNTPs, 0.5 µM of both primers, and 0.1 µl of Taq DNA polymerase (Life Technologies). PCR conditions were 5 min at 95 °C followed by 35 cycles of the 30 s at 94 °C, 30 s at 44 °C, and 40 s at 72 °C, and then a final extension of 5 min at 72 °C. Amplified fragments were sequenced in both directions in an Applied Biosystems 3130 automatic sequencer. Sequences were assembled and edited, and a consensus was generated using Geneious® 6.1.2 (Biomatters). We compiled 40 COI haplotypes that were available on GenBank (Table S1) and added those sequences into our COI alignment to further be analyzed.

Table 1 Characterization of the invasive *Corbicula* spp. from South America

Country	Number in Fig. 1	Site code	Location	Coordinates	*N*	M (f)	H(f)	Lineage
Argentine	1,2	ARG1	Río de la Plata	34° 55′58.11"S 57° 49′21.78"W	32	A/R (12); C/S (20);	FW5 (12); FW17 (20)	A/R; C/S
	3	ARG2	Río Negro River	38° 56′54.6"S 68° 01′05.8"W	12	C/S	FW17 (12)	C/S
Brazil	4	PP	Praia da Prata	10° 13′29"S 48° 22′3"W	32	C/S	FW5 (18); FW17 (14)	A/R; C/S
	5	CLM	Capitão Leônidas Marques	25° 32′36"S 53° 29′33"W	12	A/R	FW5 (12)	A/R
	6	IGU	Rio Paraná River	25° 26′48"S 54° 30′16"W	15	A/R	FW5 (15)	A/R
	7	GUA	Rio Paraná River	24° 04’S 54° 15’W	31	A/R	FW5 (31)	A/R
	8	PU	Rio Iguaçu River	26° 14′7.85"S 51° 04′28.17"W	23	Intermediate	FWBra (23)	FWBra*
	9	JAC	Rio Jacuí River	29° 37′24″S 53° 17′26″W	31	Intermediate	FW5 (31)	A/R
	10	BAR	Lago Guaíba	30° 25’S 51° 12’W	26	A/R	FW5 (26)	A/R
	11	GO	Rio Claro River	17° 55′47.49"S 51° 43′31.58"W	19	A/R	FW5 (19)	A/R
	12	RJ	Rio Guandu River	22° 48′23.08"S 43° 37′25.67"W	17	A/R	FW5 (17)	A/R
	13	MAT	RioArroio Tovroraipi	29° 34′46"S 54° 25′16"W	39	A/R	FW5 (39)	A/R
	14	IMI	Rio Iguatemi River	23° 44’S 54° 33’W	23	A/R	FW5 (23)	A/R
	15	ITU	Tocantins RiverPedral do Tauri	05° 07′46.31S 49° 18′46.54W	40	A/R	FW5 (40)	A/R
	16	ITA	UHE Itá/Uruguai River	27° 16′39.91S 52° 23′13.32W	30	A/R	FW5 (30)	A/R
	17	LAG	Lagoa Santa	19° 38′12.1S 43° 53′18.09W	12	A/R	FW5 (12)	A/R
	18	SOB	UHE Sobradinho/São Francisco River	09° 25’S 40° 51’W	20	A/R; C/S	FW5 (13); FW17 (7)	A/R; C/S
	19	AMA	Reserva Amanã Negro River	03° 012′2544.6"S 60° 15′9.558.6"W	10	A/R	FW5 (10)	A/R
	20	BRA	Paranoá Lake	NA	32	A/R; C/S; Intermediate	FW5 (21); FW17 (11)	A/R; C/S
	21	ROS	Paranádo Corvo River	22° 21′0.92S 52° 42′58.03W	30	A/R	FW5 (30)	A/R
Colombia	22	COL	Magdalena River	11° 01’ N 74° 46’ W	21	A/R; C/S	FW5 (8); FW17 (13)	A/R; C/S

UHE: Hydroelectric power plants. *N*: sampling size. M(f): morphotype form and its frequencies. H(f): name of the COI haplotype and its frequency. *: new lineage. NA: information not available

COI haplotypes identification and phylogenetic analysis

The COI haplotypes (H) of each specimen from South America were compared to reference sequences from GenBank (Table S1), and frequencies (f) were individually counted. The evolutionary relationships among the COI haplotypes for all *Corbicula* spp. were investigated by reconstructing a Bayesian phylogeny with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) implemented by the CIPRES Science Gateway (available at http://www.phylo.org/news/mrbayes). The best evolutionary model was assessed by jModelTest, and based on Akaike information criteria (AIC) (Posada, 2008), the $TrN+I+\Gamma$ model for all codon positions was used. In each analysis, we ran four independent Markov Chain Monte Carlo (MCMC) for 10 million generations. We evaluated burn-in by plotting the log-likelihood scores for each sampling point using TRACER v1.5 (Rambaut & Drummond, 2007), all ESS values in the final runs were >900, and the first 25% of the trees were discarded as burn-in for each run. The residual trees were used to build a consensus tree using LogCombiner v.1.5.4 (part of the BEAST package, Drummond & Rambaut, 2007) and estimate Bayesian posterior probabilities (PP). For all analyses, a published COI sequence of *Neocorbicula limosa* (Maton, 1811) was used as an outgroup. Phylogenetic trees were visualized and edited using FigTree v1.3.1 (Rambaut, 2009).

Morphological data analysis

Using our dataset, we tested the hypothesis of Pigneur et al. (2012) suggesting a correlation between the COI lineage with the morphotype of invasive *Corbicula* lineages. The presence of intermediate morphotypes—i.e., hybrids between lineages—was also investigated as suggested by Lee et al. (2005) and Pigneur et al. (2014a).

For the morphological analysis, we obtained the length (L), width (W), and height (H) ratios (L/W, L/H, and H/W) for each right shell of the 507 individuals, corresponding to quantitative characteristics. In addition, geometric morphometric analyses were performed using two-dimensional anatomical landmarks from 11 internal homologous points (Fig. S1) using tpsDig2 software (Rohlf, 2006), following the protocol of Sousa et al. (2007). The ratios and each landmark were taken three times for each individual and a mean was calculated for each individual. The shape variables (quantitative characteristics and landmarks compiled) generated by the x and y coordinates of each landmark were adjusted to minimize any effect in translation, rotation, and scale, as suggested by Sousa et al. (2007), and used to construct a matrix for subsequent analysis. The morphological variation (ratios and landmarks compiled) among the COI haplotypes, within and between populations, was assessed by PCA. For both analyses, lineages of *Corbicula* were assigned according to their respective COI haplotypes. Furthermore, the generalized Procrustes ANOVA analysis (GPA) algorithm (Dryden & Mardia, 1998) was performed to test the morphological differences by Wilk's λ, in which significant P values were accessed for 1000 replications between the lineages assigned by COI. All morphological analyses were performed in the software MorphoJ (Klingenberg, 2011).

Results

COI haplotypes and phylogenetic relationships

We identified three distinct COI haplotypes in our entire dataset ($N=507$) (Table 1): haplotypes FW5 and FW17, and a third one named herein as FWBra (GenBank accession number: MH460421; Fig. S2), which are distinctly distributed within the South American hydrographic basins (Table 1; Fig. 1). The haplotype FW5 was predominant in our sampling, being detected in 19 out of the 22 South American sites (Table 1; Fig. 1). The haplotype FW17 was identified in six South American sites (ARG1, ARG2, PP, SOB, BRA, and COL) (Table 1; Fig. 1). The FWBra haplotype was identified only in the PU site (Table 1; Fig. 1).

Based on our phylogeny, the FW5 haplotype matches with the invasive lineage *Corbicula* sp. form A/R and the FW17 haplotype matches with the invasive lineage *Corbicula* sp. form C/S (Fig. 2). The new FWBra haplotype was separately clustered from the others within the freshwater clade (Fig. 2) and, comparatively, it presents 94,67%, and 98,54% of identity with FW17 and FW5, respectively (Table S1; Fig. S2); therefore, we assign it as a putative new lineage called *Corbicula* sp. FWBra. The other two invasive lineages, European *Corbicula* sp. form Rlc (haplotype FW4) and *Corbicula* sp. form B (haplotype FW1), were not identified in our sampling.

Shells morphotype

We identified 327 individuals belonging to *Corbicula* sp. form A/R lineage, 115 individuals of *Corbicula* sp. form C/S lineage, and 65 individuals with an intermediate morphotype based on

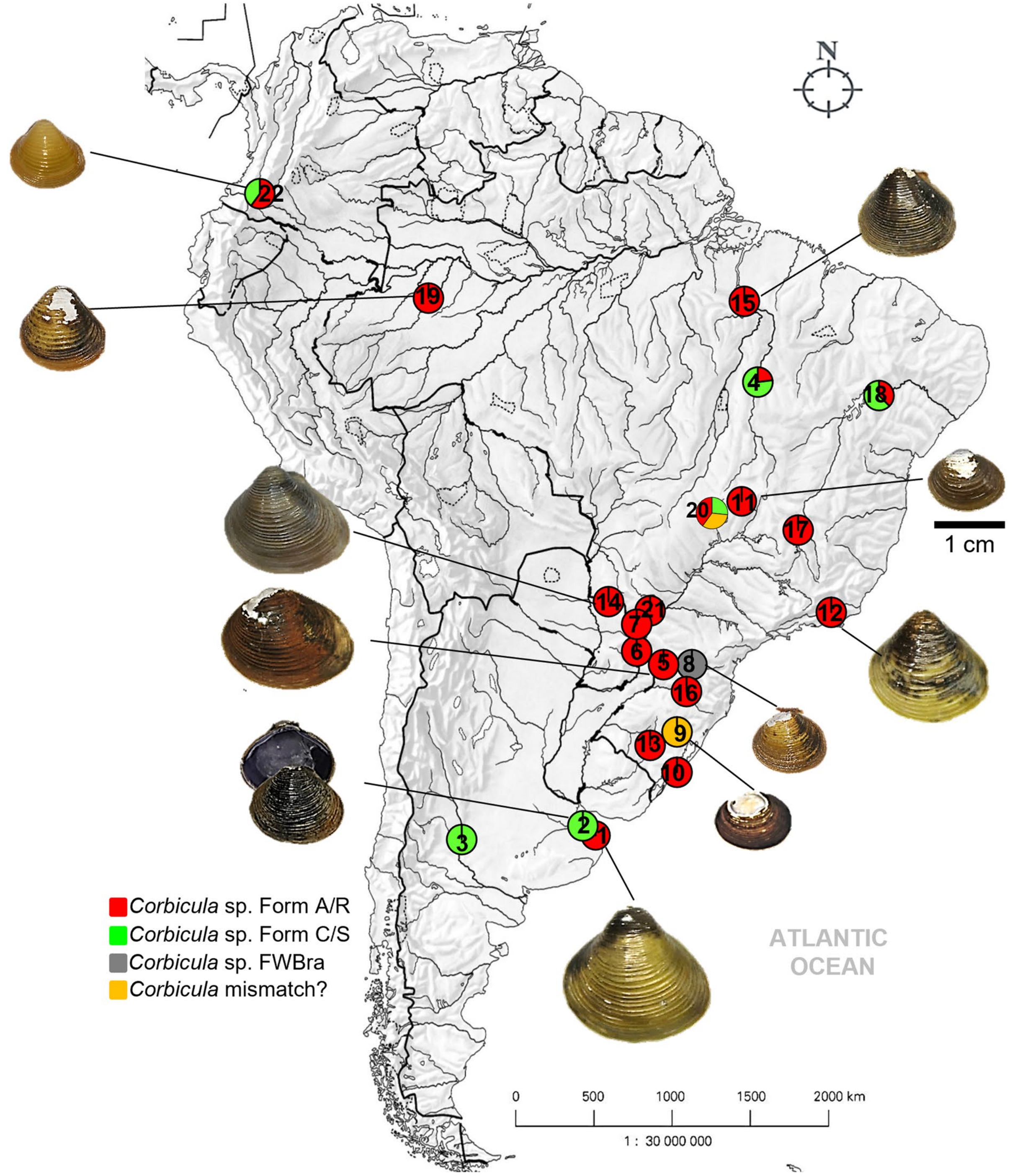

Fig. 1 Distribution of the invasive *Corbicula* lineages detected in the South America hydrographic basins and its respective COI haplotype, and morphotypes. The pie charts correspond to the distribution of each lineage and the colors (as indicated in the legend) and their respective frequency proportions for each site (details are in Table 1). The *Corbicula* mismatches are also shown

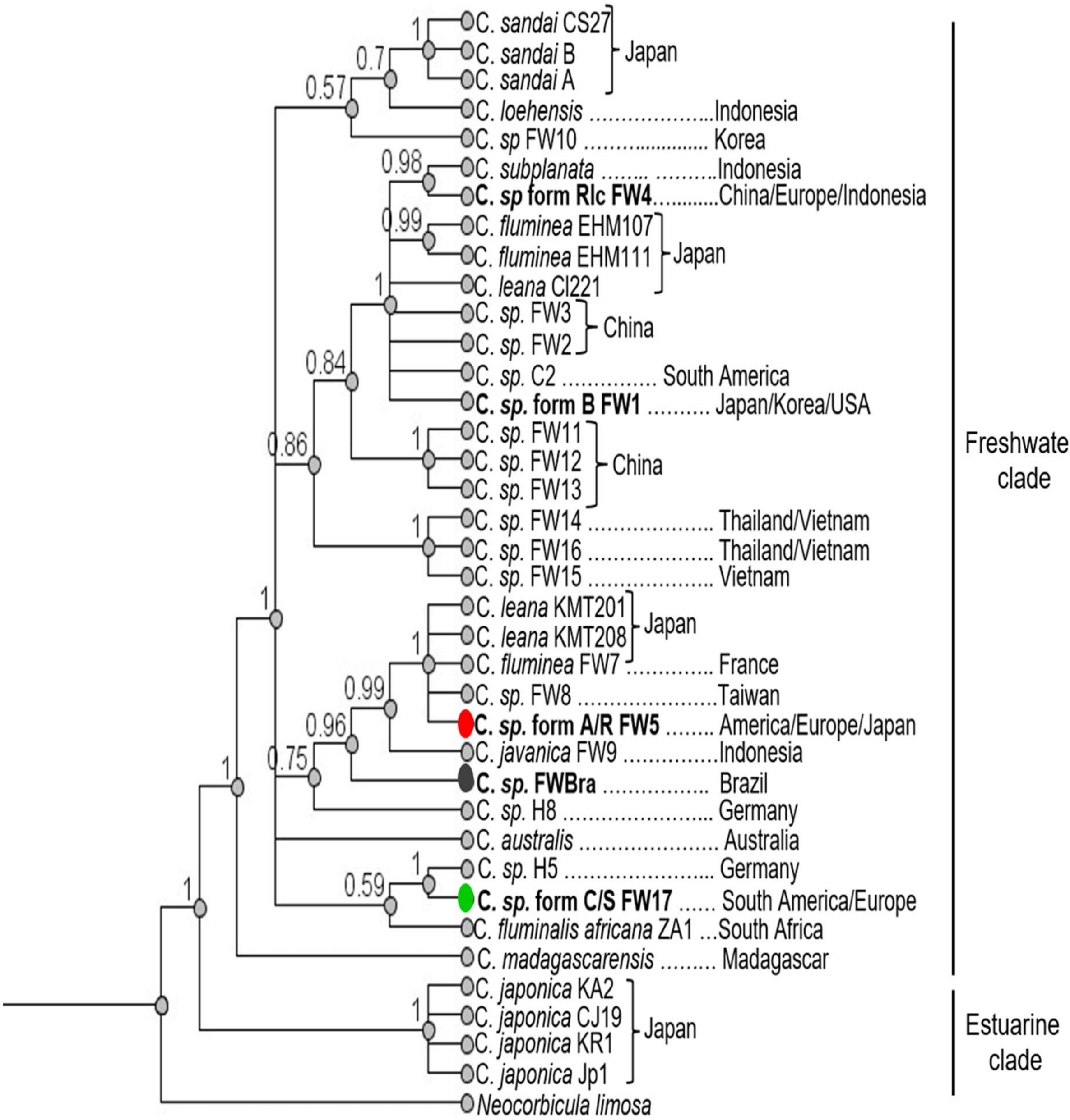

Fig. 2 Bayesian phylogenetic relationship of the COI haplotypes for estuarine and freshwater *Corbicula* spp. with their respective occurrence. Names in bold indicate the four invasive *Corbicula* lineages with androgenetic reproduction. The three lineages detected in this study are highlighted following red for *Corbicula* sp. form A/R, green for *Corbicula* sp. form C/S, and gray for *Corbicula* sp. FWBra. The posterior probabilities are indicated above each branch

the morphological analyses (Table 1). Based on quantitative characteristics, *Corbicula* sp. form A/R presents high morphological variability but is distinguished from the remaining morphotypes by presenting a robust shell with a high umbo with spaced concentric rings (Fig. 1; Table S2). The *Corbicula* sp. form C/S presents a triangular, ovate shell with juxtaposed concentric rings and a low and round umbo; the internal color can be dark purple and the pallial line is evident (Fig. 1). The specimens with intermediate morphotypes were characterized by ovoid shells and flat often eroded umbo, closely juxtaposed concentric lines, light-purple internal color, and pallial lines scarcely evident. This intermediate morphotype was found in PU and JAC populations and some individuals in BRA (Fig. 1; Table 1).

The PCA described 99,7% of the total variability between the lineages, which PC1 axis described 77,8% retrieving the L/H and L/W ratios and PC2 described 21,9% retrieving the H/W ratio (Fig. 3), and demonstrated more morphological variations within *Corbicula* sp. form A/R than between *Corbicula* sp. form A/R and form C/S. The PCA with COI haplotypes assigned showed that the morphotype of C/S, A/R, and FWBra strongly correlated with their lineages (Wilk's $\lambda = 0.082$, $F = 33.93$, $P \leq 0.001$), except for few specimens of *Corbicula* sp. form C/S and *Corbicula* sp. form A/R that overlapped with *Corbicula* sp. FWBra (Fig. 3).

Discussion

Clonal invasion of *Corbicula clams* in South America

The present study confirms the existence of two invasive lineages of *Corbicula* well-established and widespread in South American hydrographic systems: *Corbicula* sp. form C/S and *Corbicula* sp. form A/R. Moreover, an additional lineage with a distinct COI haplotype (FWBra) and intermediate morphotype was retrieved in one population in Brazil. This genetic homogeneity within each population suggests clonal propagation, as previously observed in Europe and North America (Hedtke et al., 2008; Pigneur et al., 2011a, b; 2014a; Vastrade et al., 2022).

The *Corbicula* sp. form C/S was detected in populations ARG1, and ARG2 from Argentina, in populations PP, SOB, and BRA from Brazil, and in populations COL in Colombia. All South American individuals that shared the COI FW17 haplotype, also shared the morphotype C/S with a similar description to *Corbicula* sp. form C/S lineage from Pigneur et al. (2014a). The *Corbicula* sp. form C/S was apparently

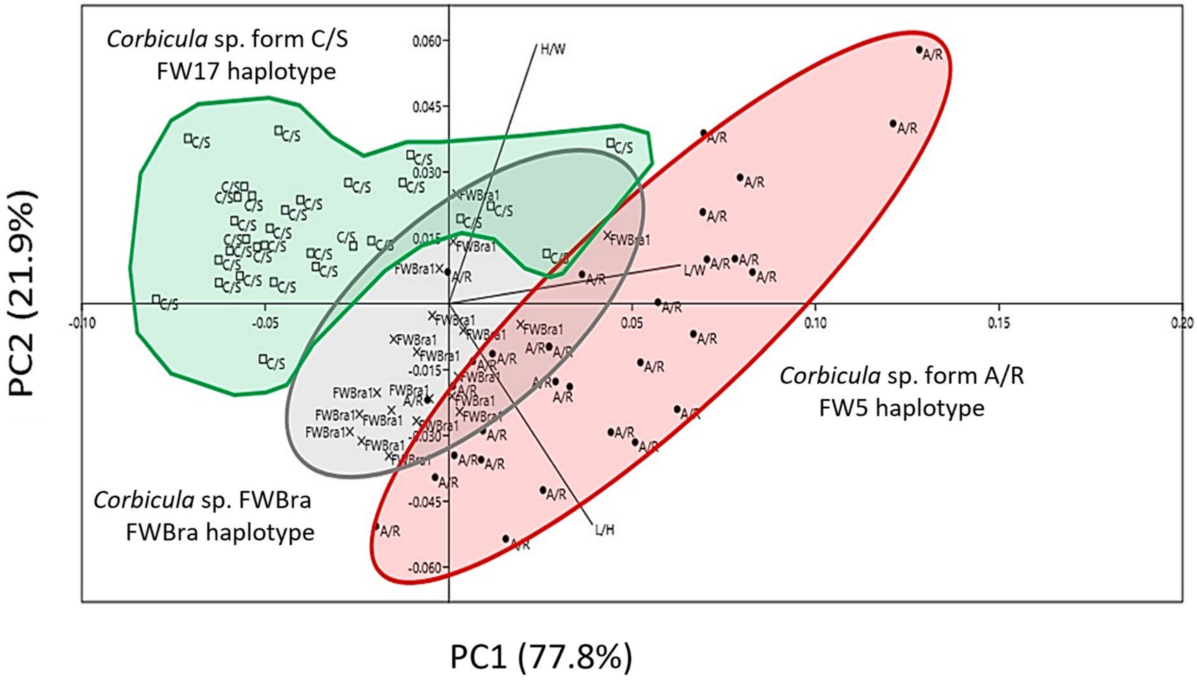

Fig. 3 Principal components analysis of morphological variation of three lineages of *Corbicula* sp. detected in this study in South American hydrographic basins, with their respective COI haplotypes. The colors are following red for *Corbicula* sp. form A/R, green for *Corbicula* sp. form C/S, and gray for *Corbicula* sp. FWBra

introduced around 1980 in Europe (Haeslopp, 1992) and Argentina/Brazil because it was then first reported on both continents (Ituarte, 1994); its propagules were likely accidentally introduced in both continents (Pigneur et al., 2014a). A recent study by Vastrade et al. (2022) showed a biogeographic origin of *Corbicula* sp. form C/S in South Africa, clustering with *C. fluminalis africana* (Krauss,1848), which ancestor could therefore have colonized both continents independently or sequentially around the same time, from the same pool of South Africa. The morphotype of *Corbicula* sp. form C/S found in South America diverges morphologically from clams from other regions (Table S2) and suggests to us phenotypic plasticity within the *Corbicula* whose shell shape likely depends on habitat patterns, despite low genetic differences (for the marker studied, Pigneur et al., 2014a; López-Soriano et al., 2018; Bespalaya et al., 2018).

The individuals of *Corbicula* sp. form A/R detected in this study share the same COI FW5 haplotypes with the *Corbicula* sp. form A/R from other parts of the world (Hedteke et al., 2008; Pigneur et al., 2014a; Bespalaya et al., 2021), but they presented a wide morphological variability across South America. The first report of *Corbicula* sp. form A/R in North America was around 1930, in Europe in 1983 (McMahon, 1983), and in South America around 1980 (Ituarte, 1981). The origin of *Corbicula* sp. form A/R points to Japan, lake Biwa where the sexual *C. sandai* is found (Vastrade et al., 2022). Since identical clones of *Corbicula* sp. form A/R are widely distributed across Europe and America, one clone must have started the colonization in North America and has been maintained through androgenesis (Pigneur et al., 2014a), and based on our results, they depict the same pattern of geographic expansion in South America. Moreover, since there was no genetic distinction among specimens within the studied populations, we advocate that *Corbicula* sp. form A/R and *Corbicula* sp. form C/S from South America are composed of clones, each lineage is strictly correlated with a single COI haplotype but both lineages presented high phenotypic variability even in other regions of the world (Table S2).

Gradual changes in shell morphology have been observed in other invasive mollusks such as *Melanoides tuberculatus* (Müller, 1774) (e.g., Peso et al., 2011), and *Dreissena polymorpha* (Pallas, 1771) (Lajtner et al., 2004), but also to another *Corbicula* spp. worldwide (Table S2). We presume that the wide phenotypic variability detected herein is likely influenced by spatial and local abiotic characteristics of the distinct Brazilian hydrographic basins (Mansur et al., 2012)—the same is likely true also for the European populations of *Corbicula* (Pfenninger et al., 2002; Sousa et al., 2007; Pigneur et al. 2011b) (Table S2). Information about the influence of how local abiotic characteristics of the newly invaded environment influence the phenotype of the shells of invasive *Corbicula* spp. is still scarce, but according to Crespo et al. (2015), *Corbicula* sp. form A/R populations presents a broad environmental tolerance especially influenced by changing temperatures. Hence, this lineage appears more plastic and generalized, reaching a wider range in geographical and ecological distribution (Pigneur et al., 2014a; Crespo et al., 2015; this study). In addition, the recognition that *Corbicula* spp. in South American rivers do not always incubate early larval stages in their gills—but instead, release the first D-larvae stages in the environment or are released from gill chambers being almost juvenile,—favors new pools of clones in a short period, resulting in high dispersion rates during the reproductive seasons (Ludwig et al., 2014). These novel discoveries indicate the invasiveness of clonal *Corbicula* spp. is not affected by the reduction in genetic variability and natural barriers do not appear to be a limiting factor in the new environment. Nevertheless, further studies should attempt to correlate the clonal fitness and invasiveness in South American *Corbicula* sp. form A/R to evaluate if they are associated with the phenotypic plasticity of this lineage influenced by local environmental conditions.

Populations with intermediate morphotypes

We observed discrepancies between the shell morphotypes and COI haplotypes in two populations: JAC and BRA from Brazil. The JAC and some BRA individuals shared the same FW5 haplotype with *Corbicula* sp. form A/R but exhibited an intermediate morphotype, which might indicate local adaptation and phenotype plasticity. The intermediate morphotype found in this study is like those found in Iguazu Falls by Lee et al. (2005), which described as “the shell resembles *Corbicula.* sp. form C/S but visibly less triangular and lightly coarse and with spaced external co-marginal rings" (Table S2). But also resemble the morphotypes found in Indonesia for *Corbicula tobae Martens*, 1900 (Fig. 3a of Bespalaya et al., 2021).

Based on our results, 23 individuals from the population PU site might represent a distinct lineage since they shared the FWBra haplotype and their shells have flat umbos, contrasting to the traditional description of any other *Corbicula* spp. (e.g., Ituarte, 1981; Lee et al., 2005; Sousa et al., 2007; Lee et al., 2005; Bespalaya et al. 2018; López-Soriano et al., 2018). The PCA demonstrated that the PU individuals exhibit an intermediate morphotype that fits between the *Corbicula*. sp. C/S and A/R forms. Interestingly, Lee et al. (2005) detected 'mixed' populations of *Corbicula* at Iguassu Falls—at the mouth of Iguaçu River—which is the same river where the PU site is located and reported genetic similarity between *Corbicula*. sp. form B (mtDNA) and mixed 28S (nDNA) probably between *Corbicula*. sp. form B and form C/S lineages. The detection of a single maternally inherited COI haplotype (FWBra) across 23 specimens from the PU site and presently restricted to this population suggests that these individuals could have resulted from cytonuclear mismatches (Hedtke et al., 2008; Pigneur et al., 2012). On the other hand, the fact that the FWBra haplotype is a sister clade of the subclade *C. leana*/*C*. sp. form A/R—FW5/*C*. sp. *fluminea*—FW7/*C*. sp.—FW8/*C. javanica*—FW9 reinforces our assumption that it is a distinct lineage. Another restricted invasive lineage is observed in Europe, the *Corbicula* sp. form Rlc, which also presents a unique COI haplotype (FW4) and distinct multilocus genotype pattern from the other invasive *Corbicula* lineages (Pigneur et al., 2012). We do not know for sure if it has an invasive behavior or if it is a lineage originating in South America such as *Corbicula*. sp. form C/S (Vastrade et al., 2022). Hence, we tentatively propose that these PU individuals should be treated as a distinct lineage as *Corbicula* sp. FWBra with a cryptic status, since we do not identify it in other sampling did not cover all South American hydrographic basins.

The intermediate morphotypes could represent hybrid specimens between distinct invasive lineages *Corbicula* sp. form C/S and form A/R that live in sympatry in some sites as we detected, following androgenetic parasitism with incomplete maternal nuclear expulsion (Hedtke et al., 2008; Pigneur et al., 2012), which was also detected in Russia and Indonesia (Bespalaya et al., 2018). Moreover, according to Pigneur et al. (2011b), a *Corbicula* individual exhibiting a mitochondrial/nuclear mismatch should have the morphotype of the nuclear genotype combined with the mitochondrial haplotype of another lineage. According to the "hybridization-invasion" hypothesis of Ellstrand & Schierenbeck (2000), interspecific hybridization may promote invasiveness because nuclear/mitochondrial hybrids and mismatches can create intermediate phenotypes relative to the parental taxa, which may increase the likelihood of survival and the success of establishment in novel habitats (Facon et al., 2005; Rius & Darling, 2014). Besides, these populations may be the result of a *cytonuclear mismatch* between the other two invasive lineages (Komaru et al., 2006; Hedtke et al., 2008; Pigneur et al., 2012), due to the maternal nuclear genome extruded, and an admixture with the paternal genome with intermediate morphotype (e.g., Komaru et al., 2006; Hedtke et al., 2008; Bespalaya et al., 2018). Thus, we know that tracing back the origin of clonal individuals is challenging due to the low population resolution level, as detected in this study for *Corbicula* spp., but further genomic studies may circumvent such limitation.

Invasion process in South America

The introduction process of an exotic population into a new environment can give us a unique opportunity to evaluate invasion dynamics in expanding populations (e.g., Darrigran, 1992; Borges et al., 2017; Ludwig et al., 2021; Carranza et al., 2023). The dispersal of lineages of *Corbicula* in South America, as it happens in general with other non-native species in the world (Seebens et al., 2017), is characterized by the increase in the rate of first geographical records over time but does not show any sign of saturation. Herein, we postulate that, based on our results, the invasion of the *Corbicula* sp. forms C/S and A/R lineages occurred through multiple introductions (and/or multiple propagules) which could explain their wide geographic distribution in South America, as also observed for *Limnoperna fortunei* (Dunker, 1857) (Ludwig et al., 2021). Based on the first records, the first introduction in South America likely occurred in the Río de la Plata River in Argentina (Ituarte, 1981); a second in the Patos Lagoon, in Southern Brazil (Mansur et al., 2004); the third in the Amazon estuary, in Northern Brazil (Beasley et al., 2003; Pimpão & Martins, 2008); and a fourth through the river systems surrounding of the Caribbean Sea (Martínez, 1987). The Río de la Plata River in Argentina is an international commercial port and one of the largest rivers in the world, into which flows Argentinian, Brazilian, and Uruguayan rivers (Ituarte, 1994). The specific geographic position of this basin in South America and the connectivity with tributaries of neighborhood countries favor the dissemination of new propagules of *Corbicula* into other continental waters in South America (Ghabooli et al., 2013; Darrigran et al., 2020). This hypothesis is supported by the detection of *Corbicula* sp. form A/R in ARG1 and of *Corbicula* sp. form C/S detected in ARG2. Thus, based on our data, the Rio del Plata could represent the genetic pool source for IGU, GUA, CLM, RJ, GO, MAT, IMI, ROS, and ITA populations, like the proposal for *L. fortunei* (Ludwig et al., 2021).

In the Patos Lagoon (city of Porto Alegre, Southern Brazil), a distinct morphotype was detected in the JAC population. There are four *Corbicula* spp. living sympatrically in the Patos Lagoon: *Corbicula* sp. form A/R, form C/S, form B, and *Corbicula* sp. (Mansur et al., 2012; Santos et al., 2012). In addition, the JAC population is situated in the city of Agudo on the Jacuí River, which flows directly into the Patos Lagoon, which is less than 19 km from it, respectively. Based on the proximity of those sites to the Patos Lagoon, it seems probable that the propagules *Corbicula* originated from the Patos Lagoon.

The Amazon estuary (Belém city, Pará state, Northern Brazil), which is a port region, is connected by water with ITU and PP sites in the Tocantins River. These two locations are located 572,6 km far from each other. The Tocantins River is a very important river located in Northern Brazil, it crosses four Brazilian states (Goiás, Tocantins, Pará, and Maranhão), and there are six hydroelectric power plants installed in its waters. According to Belz et al. (2012), a vector of dissemination of *Corbicula* in South American basins is sand transport, which is dredged from hydropower reservoirs. Thus, we postulate that there is an exchange of propagules of those rivers and between the PP and ITU sites, in the Praia da Prata site we identified the *Corbicula* sp. form C/S and form A/R lineages living in sympatry. The rapid spread of *C. fluminea* through North American freshwater has been the result of human activities (e.g., as fish bait) (Darrigran, 2002). Although no data are available on the dispersal of *C. fluminea* in South America, similar spread mechanisms to those observed in North America could occur (e.g., *C. fluminea* for live bait fishing) (Labaut et al., 2021a, b).

Our ability to identify the dispersion mechanisms during the invasion process remains limited by the clonal genetic pattern of *Corbicula* lineages detected herein, thus, we presume that human activities have indirectly aided the propagation of these invasive clams in South America via sand transportation in Brazilian hydrographic basins, as also detected by *L. fortunei* (Belz et al., 2012; Ludwig et al., 2021; Carranza et al., 2023). Nevertheless, we cannot rule out the fact that free-swimming *Corbicula* larvae can also disperse naturally (at least 1.2 km/year upstream) without human aid as suggested by Voelz et al. (1998).

Acknowledgements Special thanks to Damminsky PB (Institutos Lactec), S.B. dos Santos (UERJ), H. Tagliaro (UFPE), M.C.D. Mansur (UFRS), K. Kotzian (UFSM), T.D.H.A. Vidigal (UFMG), O. M. Netto (MAxClean), and D. Pimpão (INPA), for providing samples for this study. We also thank the Instituto Lactec, UNLP (11/N927), CONICET (PIP 1017), PICT-2019-01417 and Conselho de Desenvolvimento Científico e Tecnológico (CNPq) for supporting this study, and for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarship assistance of SL.

Data availability The data of this study are included as an electronic supplementary file.

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