UNIVERSIDADE FEDERAL DO PARANÁ

SANDRA LUDWIG

CORBICULA SPP. (BIVALVIA, CORBICULIDAE) NA AMÉRICA DO SUL: HISTÓRICO DE INTRODUÇÃO, LINHAGENS ANDROGÊNICAS E GENÉTICA DA INVASÃO

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas – Zoologia, Setor de Ciências Biológicas da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas área de concentração Zoologia.

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"CORBICULA (BIVALVIA, CORBICULIDAE) SPP. NA AMÉRICA DO SUL: HISTÓRICO DE INTRODUÇÃO, LINHAGENS ANDROGÊNICAS E GENÉTICA DA INVASÃO"

Tese aprovada como requisito parcial para obtenção do grau de Doutora em Zoologia, do Setor de Ciências Biológicas da Universidade Federal do Paraná, pela seguinte Comissão Examinadora:

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A persistência é o menor caminho para o êxito - Charles Chaplin

RESUMO

O gênero *Corbicula* (Bivalvia, Corbiculidae) Megerle von Mühlfeld, 1811 possui linhagens sexuais e hermafroditas androgênicas que habitam ambientes estuarinos e de água-doce ao redor do mundo. As linhagens sexuadas de *Corbicula* estão restritas à região natural (Ásia, Austrália, Oriente Médio e África), enquanto que as linhagens invasoras são encontradas nos continentes Americano e Europeu. Dessa forma, acredita-se que reprodução androgênica pode ter um papel importante durante o processo de estabelecimento das linhagens invasoras em um novo ambiente. As linhagens androgênicas invasoras de *Corbicula* são entituladas como: *Corbicula* sp. forma A/R, *Corbicula* sp. forma C/S, *Corbicula* sp. forma B e *Corbicula* sp. forma Rlc, e quando estabelecidas em um novo ambiente, geram impactos ecológicos e econômicos significativos. Devido à alta variabilidade morfológica de suas conchas, a determinação específica das espécies de *Corbicula* utilizando somente dados morfológicos resulta frequentemente em uma determinação taxonômica errônea. Assim, esta tese de doutorado tem como objetivo: (i) identificar as linhagens Sul-Americanas de *Corbicula* utilizando dados de morfometria geométrica e dados moleculares do mtDNA citocromo oxidase subunidade I (COI) e de dez marcadores nucleares de microssatélites; (ii) inferir o relacionamento filogenético das linhagens invasoras de *Corbicula* da América do Sul com seus relativos da América do Norte e Europa, assim como, com as linhagens sexuadas da região natural; (iii) propor um método molecular para a detecção de larvas de *Corbicula* spp. em amostras de plâncton com o intuito de monitorar esses moluscos invasores e fornecer informações sobre o ciclo de vida e processo de dispersão no ambiente invadido. Baseando-se nos resultados obtidos, foi possível detectar duas linhagens invasoras de *Corbicula* na América do Sul, *C.* sp. forma A/R e *C.* sp. forma C/S. Além disso, espécimes com morfotipos e genótipos intermediários também foram detectados, que aqui foram considerados como consequência do *mismatch* citonuclear entre duas linhagens simpátricas de *Corbicula.* Os espécimes de Porto União, Santa Catarina, apresentaram ainda um haplótipo único (FWBra1) e que está presente unicamente nessa população. Ainda, foi detectada uma extensa variação morfológica nos espécimes da linhagem *C.* sp. forma A/R, mas que está restritamente associada à presença de um único genótipo e ao haplótipo mtDNA FW5; ou seja, apresentam um padrão genético clonal para a América do Sul. O mesmo padrão clonal se repetiu para a linhagem *C.* sp. forma C/S, cuja apresentou um único genótipo e a presença do único haplótipo mtDNA FW17. Adicionalmente, provavelmente, múltiplas introduções e *admixture* de novos propágulos de diferentes regiões invadidas podem estar propiciando a manutenção da diversidade clonal desses moluscos na América do Sul, caracterizando-se em uma metapopulação de clones entre os continentes Americano e Europeu, para cada uma das linhagens detectadas. Assim, o método de monitoramento molecular desenvolvido a partir do mtDNA COI se mostrou eficiente na detecção de larvas de *Corbicula* spp. invasoras, sendo possível detectar até mesmo uma única larva em 1000 mL de amostra de plâncton. Assim, o método molecular desenvolvido pode realizar o monitoramento/prospecção dos primeiros estágios larvais do ciclo de vida de *Corbicula* spp. em corpos d'água que foram invadidos e/ou que são considerados em risco de invasão eminente por esses moluscos.

ABSTRACT

The genus *Corbicula* (Bivalvia, Corbiculidae) Megerle von Mühlfeld, 1811 has sexual and hermaphroditic androgenetic lineages which habitat estuarine and freshwater domains around the world. The sexual lineages of *Corbicula* are restricted to natural range (Asia, Australia, Middle East and Africa), while the invasive lineages are found in American and European continent and, are characterized by hermaphroditic androgenetic reproduction. In this way, it is believed that the androgenetic reproduction can play an important role during the stablishment process of invasive lineages into new environment. The invasive androgenetic lineages of *Corbicula* are known as: *Corbicula* sp. form A/R, *Corbicula* sp. form C/S, *Corbicula* sp. form B and *Corbicula* sp. form Rlc and, when stablished into new environment they cause significative ecological and economic impacts. Besides that, they present high morphological variability in their shells, thus, the specific determination of species of *Corbicula* using only morphological data often results in erroneous taxonomic determination. For those reasons, this doctoral thesis aims to: (i) identify the South American lineages of *Corbicula* using morphometric geometric data and molecular data from mtDNA cytochrome oxidase subunit I (COI) and ten nuclear markers of microsatellites; (ii) infer the phylogenetic relationships of invasive lineages of *Corbicula* of South America with their counterparts of North America and Europe, as, sexual lineages from natural range and; (iii) propose a molecular method to larvae detection of *Corbicula* spp. in plankton samples, in order to monitoring these invasive molluscs and provide information about their life cycle and dispersion process into invaded environment. Based on obtained results of this thesis, we detected two invasive lineages of *Corbicula* in South America: *C*. sp. form A/R e *C*. sp. form C/S. Besides that, specimens with intermediate morphotype and genotype were also detected, which were considered as consequence of cytonuclear mismatch between sympatric invasive lineages of *Corbicula*. Specimens of Porto União, Santa Catarina state, presented intermediate genotype but unique mtDNA COI haplotype (FWBra1) and, until now, it is presented only in this population. Also, it was detected an extensive morphological variation in *C*. sp. form A/R specimens but it is restricted associated to the presence solely of one genotype and one mtDNA COI haplotype FW5; in other words, those specimens presented a clonal genetic pattern to South America. The same clonal pattern repeated to *C*. sp. form C/S, which presented solely one genotype and one mtDNA COI haplotype FW17. Additionally, most probably, multiple introductions and admixture of new propagules from distinct invaded regions are propitiating the maintenance of clonal diversity of these molluscs into South America, characterizing themselves in metapopulation of clones between American and European continents, to both lineages detected. Finally, the molecular method developed through mtDNA COI was efficient in detection of larvae of invasive *Corbicula* spp., which could detect even one larva in 1000 mL of plankton sample. In this way, the molecular method developed can monitoring and prospects the early stages of larvae of *Corbicula* spp.' life cycle in invaded watersheds and/or those that are considered in risk to eminent invasion by these molluscs.

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PRÓLOGO

Invasões biológicas provem ótimas oportunidades para investigar processos microevolutivos através de escalas temporais contemporâneas. Um fator chave para tais estudos é a quantidade de variação genética das populações invasoras dentro da área introduzida. No entanto, o modo de reprodução da espécie/linhagem tem um papel importante e/ou determinístico no padrão da variabilidade genética da população invasora; como foi detectado recentemente para os moluscos bivalves do gênero *Corbicula*. O gênero *Corbicula* tem como origem a Ásia, África, Austrália e o Oriente Médio mas há, também, linhagens que foram introduzidas no continente Americano e Europeu e, se tornaram invasoras. As linhagens de *Corbicula* podem apresentar reprodução sexuada ou assexuada e, estão distribuídas nos ambientes estuarinos e de água doce. As espécies estuarinas apresentam reprodução sexuada e esperma reduzido com um flagelo. Por outro lado, as espécies de água doce (que incluem as linhagens invasoras) apresentam reprodução assexuada androgênica e esperma não reduzido com dois flagelos. A reprodução androgênica nas linhagens invasoras de *Corbicula*, de acordo com estudos recentes, tem um papel importante durante o processo de introdução e invasão em um novo ambiente. Dessa forma, as linhagens invasoras androgênicas de *Corbicula* caracterizam-se por um padrão genético clonal, ou seja, as populações invasoras apresentam um mesmo genótipo e não há (ou há pouca) variabilidade genética entre os indivíduos.

Partindo da problemática exibida acima, esta tese está dividida em quatro capítulos, os quais apresentam detalhadamente quais são as linhagens androgênicas invasoras de *Corbicula* presentes na América do Sul, e como estas são caracterizadas morfologicamente e geneticamente. Além disso, essa tese traz informações de qual é a linhagem de *Corbicula* mais amplamente distribuída (*Corbicula* sp. form A/R) e qual sua distribuição atual. Adicionalmente, esta tese contribui com informações sobre o histórico de introdução e invasão de *Corbicula* sp. form A/R e fornece *insights* sobre a genética de invasão de tal linhagem na América do Sul. A partir dos resultados obtidos, ainda, é proposto um método molecular de detecção de larvas de *Corbicula* spp. em amostras de plâncton, com o objetivo de sugerir um método rápido, barato e eficaz de monitoramento destes moluscos invasores que já estão amplamente distribuídos e estabelecidos na América do Sul.

CAPÍTULO I

***Corbicula* sp. form A/R (Mollusca: Bivalvia), a silent invader in South America**[§]

ABSTRACT

The genus *Corbicula* incorporates sexual and hermaphroditic androgenetic lineages that inhabit estuaries and freshwater habitats across the globe. The invasive representatives of *Corbicula* in the American and European continents are androgenetic lineages. The Asian clam, *Corbicula* sp. form A/R has been introduced around the world and also in South American rivers, which introduction dates 1980s from the River Río de La Plata estuary in Argentina and subsequently in the same year in Patos Lagoon, Southern Brazil. Subsequently to these dates, the number of occurrences in several hydrographic basins increased and it is possible to find these clams in almost all hydrographic basins of South America. Thus, this study synthetizes the knowledge about invasive *Corbicula* lineages around world and, especially in South American range. Also, discuss how some *Corbicula* features can potentiate the invasion success of *Corbicula* form A/R in South American rivers.

Keywords: alien species; distribution; invasion history; review; Neotropical; Asian clam.

Running head: SILENT INVADER IN SOUTH AMERICA

[§]Artigo formatado conforme à revista Journal of Molluscan Studies

INTRODUCTION

Corbiculidae is a family of Mollusca Bivalvia, subclass Heterodonta, order Veneroida, superfamily Corbiculoidae (Graf, 2013). Corbiculidae is a widespread family of moderate-sized clams, often tinged or colored with violet on the interior. Müller described in 1774 three species of *Tellina* Linnaeus, 1758: *T. fluminea*, *T. fluvialitis* and *T. fluminalis* however taxonomic changes resulted in the transfer of these species to *Corbicula* (Mergele Von Mühlfeld, 1811). The *Corbicula* clams are distinguished from other bivalves by the triangular and chordate shell with concentric grooves rings without sinus pallial line (Morton, 1977). Species of *Corbicula* are found worldwide in fresh and saline waters, both as native and/or invasive species. These clams are benthic organisms and live buried in sediment, and can be found in large agglomerations of individuals. They are filter-deposit-feeders but pedal-suspension-feeding can also be performed - especially on invasive species (Hakenkamp & Palmer, 1999).

Corbicula clams usually present three reproductive seasons, one in early spring, a second in the middle of summer, and the third in the beginning of autumn (Doherty *et al.*, 1987). However, the reproductive seasons can change depending of the local temperature (Rosa *et al.,* 2011) and/or resources availablility in the environment. This genus is also distinguished by a wide spectrum of reproductive strategies, ranging from indirect development with free-swimming larvae (typical for estuarine *Corbicula* spp. tolerant to brackish water) to incubation of larvae in gills until the juveniles stage (restricted to freshwater taxa) (Siripattrawan *et al.,* 2000). Species of *Corbicula* can be dioecious-sexual species or hermaphroditic androgenetic (Konishi *et al.,* 1998; Korniushin & Glaubrecht, 2003). For instance, the lacustrine *Corbicula sandai* Reinchardt, 1878 and the estuarine species, *Corbicula japonica* Prime, 1864, are dioecious, free spawning and present unreduced monoflagellate sperm. Otherwise, the freshwater *Corbicula* clams are hermaphroditic androgenetic, present unreduced biflagellate sperm, range from diploid to tetraploid, and brood their larvae in their gills (Komaru *et al.,* 2000; Glaubrecht *et al.,* 2007; Pigneur *et al.,* 2012). In androgenetic *Corbicula* spp., cytological studies show that an unreduced sperm impregnates the

oocyte. The full maternal nuclear genome is then extruded from the oocyte as two polar bodies, while mitochondria and other organelles from the egg are reserved. Thus, the offspring receives only paternal nuclear chromosomes and are clones of their father (Kornishi *et al.,* 1998; Houki *et al.,* 2011; Pigneur *et al.,* 2012).

In androgenetic *Corbicula* clams, the unreduced sperm from one genetic lineage can also fertilize the egg of another lineage (Kornishi *et al.,* 1998). Thus, the nuclear genome of the first lineage has the mitochondrial genome of the second, an event called as 'egg parasitism' or mitochondrial capture which results in cytonuclear mismatches (Hedtke *et al.,* 2011). Further, egg parasitism allows an admixture of different nuclear genomes when the maternal nuclear genome is incompletely extruded (Komaru *et al.,* 2006; Hedtke *et al.,* 2011). As a consequence, in androgenetic *Corbicula*, outcrossing and recombination may occur. Therefore, the signature of such parasitism between lineages is an incongruence between the mitochondrial haplotype of that lineage and its phenotype or nuclear genome. Therefore, the androgenetic clonal reproduction obscure the *Corbicula* phylogenetic relationship (Hedtke *et al.,* 2011).

Despite advances in genetic studies with *Corbicula* clams (like Hedtke *et al.,* 2011; Pigneur *et al.,* 2012; Pigneur *et al.,* 2014b), little is known about the origin and/or development of morphological variability of these clams. Subsequently to the original proposal of *Corbicula* by Müller, 1774 (with the description of three species *C. fluminalis, C. fluminea,* and *C. fluviatilis*), many other extant species of the genus have been found in freshwater and estuarine habitats from Southeast Asia, the Pacific islands, and in parts of Europe and Africa (Araújo *et al.,* 1993; Rosa *et al.,* 2011; Franco *et al.,* 2012). Usually, morphological variations in the shell shape, growth rings, and umbo are the most common taxonomic characteristics used by early conchologists to determine species of *Corbicula* (Glaubrecht *et al.,* 2007; Graf, 2013). However, populations of these clams, in some geographic areas, have great morphological variability (Morton, 1977; Pfenninger *et al.,* 2002; Lee *et al.,* 2005). Therefore, most likely, the *Corbicula* taxonomy involves more species names than needed, thus, it is necessary to carefully assign a new specific name for different phenotypes of these clams. Indeed, it is necessary to join morphological, mitochondrial, and nuclear data to

properly attribute a specific species identification of *Corbicula*, especially when it comes to a complex of androgenetic lineages whose origin is not defined or its evolution (Hedtke *et al.*, 2011; Pigneur *et al.*, 2012).

Corbicula clams are efficient invasive species (Gatlin *et al.*, 2012). The geographical expansion of the invasive *Corbicula* lineages has been accelerated by human activities and globalization and, thus, these clams have been successfully introduced in freshwaters domains outside of their natural range, including Asia, Australia, Africa, and the Middle East (Araújo *et al.*, 1993; Park & Kim, 2003). The success as invasive species is likely associated with the fact that they are r-strategist with rapid population growth (which includes fast individual growth, early maturity, a short lifespan, multiple reproductive periods, high fecundity, a small larva), and extensive capacity of dispersion in freshwaters domains. Among the impacts, more associated to the presence of *Corbicula* spp. in an invaded range, are the reduction on phytoplankton density (Pigneur *et al.*, 2014b), spatial and trophic competition with native species (McMahon, 2002), induction of ecological changes such as those associated with local carbon dynamics (Kakenkamp & Palmer, 1999) and change in the compartmentalization of photosynthetic production with immediate consequences in the structure of the local aquatic community (Pigneur *et al.*, 2014b). Economic damages are usually associated to biofouling (Sousa *et al.*, 2008; Pigneur *et al.*, 2014b) and are especially important in industrial duct systems such as those of cooling systems (McMahon, 2002).

Here a synthesis of the bioinvasion by *Corbicula* spp. in South America is presented. This paper reviews the characteristics of the invasive lineages of *Corbicula* worldwide and updates the distribution range of *Corbicula* sp. form A/R lineage in South America. It further discusses possible traits that facilitate the invasion success of this lineage in new introduced environments and discusses the areas in which future studies are necessary to better understand the process and patterns of invasion of the species in the continent.

CORBICULA WORLDWIDE: *INVASIVE LINEAGES*

Among the androgenetic lineages of *Corbicula*, four are considered successful invaders. These forms are widely distributed in Europe, North and South America (Araújo *et al.*, 1993; Darrigran, 2002; Lee *et al.*, 2005; Pigneur *et al.*, 2011b). In North America, invasive *Corbicula* clams were apparently first introduced into the northwestern region of the United States in 1938, into the Columbia River (Britton & Morton, 1977; McMahon, 1982). In Europe, the first *Corbicula* report was in France and Portugal around 1980 (Araújo *et al.*, 1993) to *Corbicula fluminalis* (Müller, 1774). In South America, *Corbicula* clams were apparently introduced in the same year, 1980s at Argentina and Southern Brazil (Ituarte, 1981; Veitnheimer-Mendes, 1981).

The names for *Corbicula* lineages that we used follows Pigneur *et al.*, (2014a): *Corbicula* sp. form A/R (known in North America as form A and in Europe as form R); *Corbicula* sp. form B (known only from North America); *Corbicula* sp. form Rlc (reported only from Europe) and, *Corbicula* sp. form C/S (known from South America as form C and from Europe as form S).

These invasive clams are successful invaders despite the generalized lack of genetic diversity (Pigneur *et al.*, 2014a) and represent an example of the 'genetic paradox' (see Allendorf & Lundquist 2003). Each invasive *Corbicula* lineage has been correlated with a single mtDNA haplotype and nDNA genotype (Pigneur *et al.*, 2011). The *C.* sp. form A/R present solely the haplotype FW5 to mtDNA COI gene which is shared with *Corbicula leana* (Prime, 1864) and, present solely one nDNA genotype. The *C.* sp. form B present solely the haplotype FW1 to mtDNA COI gene and, present solely one nDNA genotype. The *C.* sp. form Rlc present solely the haplotype FW4 to mtDNA COI gene and, present solely one nDNA genotype. Despite the form B and Rlc lineages present distinct genotypes and morphotypes, the mtDNA COI haplotype mismatches in each other in only one nucleotide position (Pigneur *et al.*, 2011b). Finally, the *C.* sp. form C/S present solely the haplotype FW17 to mtDNA COI gene and, solely one nDNA genotype, however, form C (in South America) and form S (in Europe) diverge in their morphotypes (Pigneur et al. 2011). In addition, according to Pigneur *et al.*, (2012), all these invasive lineages are characterized by androgenetic

reproduction and are able to perform nuclear and mitochondrial captures between lineages. Based on mtDNA and nDNA, according to Pigneur et al. 2012, probably the nuclear and mitochondrial captures between *C.* sp. form A/R lineage and another unkown *Corbicula* lineage (from mainland Asia), could had originated the *C.* sp. form B lineage which present mixed genotype between both but with FW1 haplotype mtDNA COI gene from the second lineage. Thus, according to Pigneur *et al.*, (2014a), this second lineage probably also originated the *C.* sp. form Rlc, which present only one nucleotide difference at the COI gene when compared with *C.* sp. form B mtDNA COI haplotype.

From all invasive lineages, *Corbicula* sp. form A/R is the most widespread lineage, which has been detected in Australia, Africa, Eurasia and Americas (McMahon, 2002; Darrigran, 2002; Park & Kim, 2003; Karatayev *et al.*, 2005; Lee *et al.*, 2005; Pigneur *et al.*, 2011b; Pigneur *et al.*, 2014a). This lineage has been considered the most invasive alien species (IAS) of all freshwater bivalves (Ricciardi 2015). The putative potential of invasiveness of this lineage is related to its ability of colonizing a wide range of niches within the freshwater domain (Britton & Morton, 1979; Mansur *et al.*, 2004; Crespo *et al.*, 2015), which propitiate: (i) its large tolerance to salinity, (ii) an intermediate tolerance to pH levels as low as 5.6 and, and to (iii) its extraordinarily tolerance to high turbidity when compared to another invasive mollusk, like zebra mussel *Dreissena polymorpha* Pallas, 1771 (McMahon, 1999; Sousa *et al.*, 2008). However, these are not the unique traits that can be associated to their consistent success in the invasion of new environments (see further below).

CORBICULA SPP. IN SOUTH AMERICA

In South America, specimens of *Corbicula* spp. have been introduced accidentally probably by ballast water stored in merchant ships, in the River Río de La Plata estuary in Argentina, sometime between 1965 and 1975 (Ituarte, 1981). The first specimens, however, were collected from the sandy banks of Punta Lara in 1979 (Ituarte, 1981), East Argentina. Concurrently, other specimens determined as *Corbicula manilensis* (Philippi, 1844) - and subsequently recognized as *C. fluminea* by Mansur *et al.*, (2004) -

were found by Veitenheimer-Mendes (1981) around the city of Porto Alegre, Southern Brazil. Ituarte (1994) reported 1,974 specimens of *C. fluminea* collected in June of 1978, in the Jacuí and Guaíba Rivers (also in the surroundings of Porto Alegre). In addition, this author also reported *Corbicula largillierti* (Philippi, 1844) in Colonia, Uruguay. Populations of others *Corbicula* spp. were subsequently detected from the city of Magdalena (Río de la Plata River, East Argentina) to the upper course of the Paraná River (at the Province of Corrientes, Northeast Argentina), also in the Plata basin (Darrigran, 1992).

The South American nomenclature of lineages of *Corbicula* greatly differs with those recent literatures (like Hedtke *et al.*, 2011; Pigneur *et al.*, 2011, 2014a). Thus, in this section we use the names of lineages like Pigneur *et al.*, (2014a) to those specific *Corbicula* names attributed in South American papers so far, because of recent findings about the mitochondrial and nuclear capture between lineages. In South America, specimens of *Corbicula fluminea* (Müller, 1774) is most closely to *Corbicula* sp. form A/R lineage, specimens of *Corbicula largillierti* (Philippi, 1844) is most closely to *Corbicula* sp. form C/S, specimens of *Corbicula* cf. *fluminalis* (Müller, 1774) is most closely to *Corbicula* sp. form B. The *Corbicula* sp. form Rlc lineage is not correlated with any known South American species because it is not found in this region so far.

Historic publications reported the presence of *C.* sp. form A/R (Mansur & Garces, 1988; Darrigran & Pastorino, 1993; Beasley *et al.*, 2003; Vidigal *et al.*, 2005; Pimpão & Martins, 2008) (Fig. 2), *C.* sp. form C/S (Ituarte, 1984; Darrigran & Maroñas, 1989; Pereira *et al.*, 2000), *C.* sp. form B (Mansur *et al.*, 2004) and, recently, a new *Corbicula* sp. (Mansur *et al.*, 2012) was detected - the last two are exclusively found in Patos Lagoon, Porto Alegre city in Southern Brazil.

Since the introduction of *Corbicula* spp. in South America, it was observed that there are two picks with a great number of publications in South America (Fig. 1), including new reports of occurrences (between 2001-2005), description of new morphological variability (between 1981-1985) and ecological studies (between 1996-2000). On the other hand, few papers described the impacts of *C.* sp. form A/R as Boltovskoy *et al.*, (1997), Darrigran (2002) and Santos *et al.*, (2012). In addition, there

is only one study involving genetic data of *Corbicula* spp. in South America by Bagatini *et al.*, (2005).

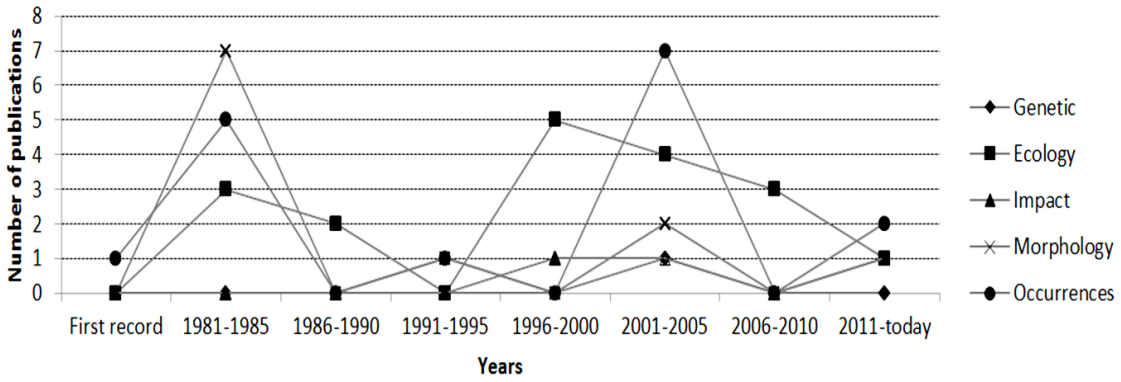
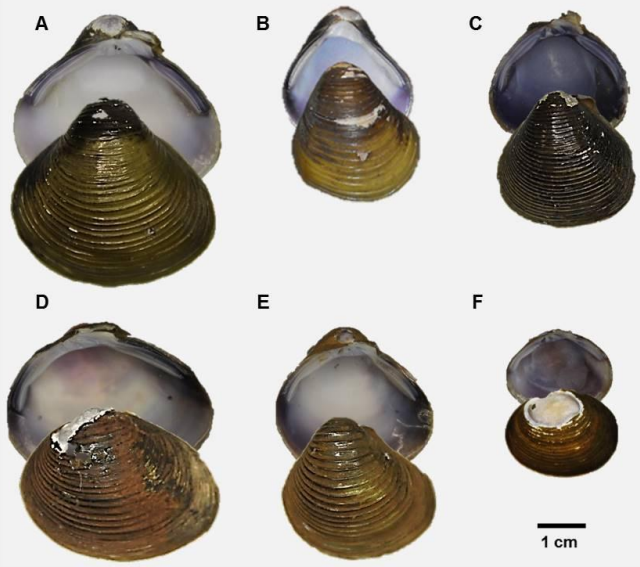


Figure 1. Temporal variation on the number of publications about the invasive *Corbicula* clams in South America, since Ituarte (1981). The published papers are separated by major topics (Supporting information 1).

Taxonomic problems with determination of species of *Corbicula* in South America are also common and previous publications have assigned specific names to *Corbicula* specimens based solely on morphological characteristics. According to Ituarte (1994), *C.* sp. form A/R presents a trigonal robust shell outline, inflated beaks and not eroded umbos, which extend into a rostrum posteriorly (like Box 1A). However, the variations in shell shape and outline are common in species of *Corbicula* (e.g. Pfenninger *et al.*, 2002) and are influenced by environmental factors such as substrate composition, hydrodynamic, and other local characteristics including climate (Sousa *et al.*, 2007). Furthermore, based on morphological characteristics Mansur et al. (2004), described the shell of *C.* sp. form A/R as being inequivalve and robust with high umbos, externally the shell is inflated and tapered at the beak, with spaced rings and bright yellowish brown/greenish periostracum (like Box 1A). The shell of *C.* sp. form B can be distinguished from *C.* sp. form A/R by the number of growth rings (9-10) and by the shell height (like Box 1B). Although the morphological variability supports the divergent taxonomic status of these two lineages of *Corbicula*, *C.* sp. form B seems to be very conservative while *C.* sp. form A/R presents high morphological variability (like Box 1D-E). Differing from the previous lineages, the shell of *C.* sp. form C/S is triangular-ovate with moderately flat umbo, thin valves with few spaced growth rings

(like Box 1C), and pallial line evident as described by Ituarte (1994). The divergent morphology of this form reported/described by Castillo *et al.*, (2007) from the Uruguay River, Southern Brazil, is interpreted as a consequence of plasticity associated to local environmental conditions, since; the specimens were collected from backwater area where there is no current with high concentrations of organic matter and clay.

However, according to Pfenninger *et al.*, (2002), intermediate morphotypes of *Corbicula* are found in European populationn, especially in those lineages originated by the cytonuclear mismatch between nuclear and mitochondrial captures, which, according to Hedtke *et al.*, 2008, are expected to occur in areas of sympatry. Surprisingly, recent findings (Ludwig, unpublished data) also reported intermediate morphotypes to *Corbicula* populations (Box 1F), which are associated with 'mixed' genotypes in South American rivers.



Box 1. Description of the shells of the lineages of *Corbicula* from South America (based on Ituarte 1994; Darrigran 2002; Mansur et al. 2012).

The shell of Corbiculidae is equivalve, approximately equilateral, ovate to trigonal in outline, typically robust and inflated at the beaks, and with a sculpture of concentric rings which may also be interrupted by concentric growth rings. (A,D,E) The shell of *Corbicula* Form A/R is robust with high umbos, internal surface white to slightly yellowish with the purple or brown marginal edge of the line pallial; pallial line not evident and rostrum may be present. (B) The shell of *Corbicula* Form B has a pronounced triangular shape, robust with high umbos. (C) The shell of *Corbicula* Form C/S is triangular-ovate with juxtaposed concentric rings and, low and round umbos, internally the color can be dark purple, the pallial line is evident. (F) Recently, an intermediate form was found with an ovoid shell and plain eroded umbo, closely juxtaposed concentric rings and, light purple internal surface and evident pallial line scar.

In Southern Brazil, the identity of three *Corbicula* morphotypes (like Box 1A, D, F) was verified by Bagatini *et al.*, (2005). These authors detected a single nDNA genotype (based on RAPD and ISSR markers) for different morphotypes. This morphological variability is probably stimulated by local environmental characteristics. Otherwise, in Northern Brazil, Pimpão & Martins (2008) reported the occurrence of *Corbicula* spp. in the Amazon region presenting thin valves and apparent concentric

growth rings. These specimens also exhibited a glossy, smooth periostracum from dark brown to black coloration. In large specimens, a prominent rostrum is present, which results in a more inequilateral shell while small specimens are more equilateral. Internally, the pallial line is whitish and the scars of the adductor muscles are very evident (like Box 1E).

Recently, the presence of a fourth species for South America, *Corbicula* sp., was reported in the Guaíba Lake by Mansur *et al.*, (2004), in the region of Porto Alegre city, Southern Brazil. These specimens have a strong and almost equilateral shell, low and round umbos, no rostrum, and shell slightly concave in the anterior margin of the umbos, delicate marginal lines and a shiny brown periostracum. *Corbicula* sp. is smaller than form C/S, robust and present little arcuate hinge; internally, the color is slightly pink. Furthermore, a new morphotype of *Corbicula* (Box 1F) was found recently in Porto União, Santa Catarina State in Southern Brazil (Ludwig, unpublished data) that is most similar to the morphotype from the Iguazu-Falls reported by Lee *et al.*, (2005).

CORBICULA SP. FORM A/R IN SOUTH AMERICA: *PRESENT DISTRIBUTION*

The first record of *Corbicula* sp. form A/R in South America was in Argentina, around 1979 by Ituarte (1981). Subsequently, this form was reported in sympatry with *Corbicula* sp. form C/S in the same area around 1982 (Darrigran & Pastorino, 1993). Later, Ituarte (1994) reported an extensive distribution of form A/R in Northern Argentina. This study showed that this lineage dispersed along the entire western shore - from the delta of the Paraná River to the city of Magdalena - and along the eastern shore of the River Río de La Plata estuary, in a discontinuous manner - from the upper course of the Paraná River to the lower course at San Nicolás. According to Ituarte (1994), form A/R was found along the Uruguayan coast up to the city of Santo Tomé, which is the northernmost record of the form in the Uruguay River Basin.

Other occurrences of form A/R from South American basins (Fig. 2) are from: the Orinoco basin, Venezuela, in 1987 (Martínez, 1987; Lasso *et al.*, 2009); rivers of the the Argentinian Patagonia, in 1997 (Cazzaniga, 1997; Cazzaniga & Pérez, 1999; Semenas & Flores, 2005); lower Paraná river in Argentina, in 1999 (Cataldo &

Boltovskoy, 1999); Patos Lagoon, Brazil, in 1981 (Veitnheimer-Mendes, 1981; Martins *et al.*, 2004); Paraguay river in Brazil, in 2002 (Callil & Mansur, 2002); Rosana River, Brazil, in 2005 (Bagatini *et al.*, 2005); in water bodies of the state of Minas Gerais, Brazil. in 2011 (Vidigal *et al.*, 2005; Maroneze *et al.*, 2011); Uruguay River, Brazil, 2007 (Castillo *et al.*, 2007); in the surroundings of the city of Brasília, in 2007 (Rodrigues *et al.*, 2007); in water bodies of the state of São Paulo, Brazil in 2007 (Suriani *et al.*, 2007); in São Francisco river, in 2009 (Borges *et al.*, 2009); in lower Amazon basin, in 2003 (Beasley *et al.*, 2003); in Negro river, Brazil, in 2008 (Pimpão & Martins, 2008); in Magdalena basin, in 2008, at the island of Salamanca, Colombia (Aristizábal, 2008) (for more details see Supporting information 1).

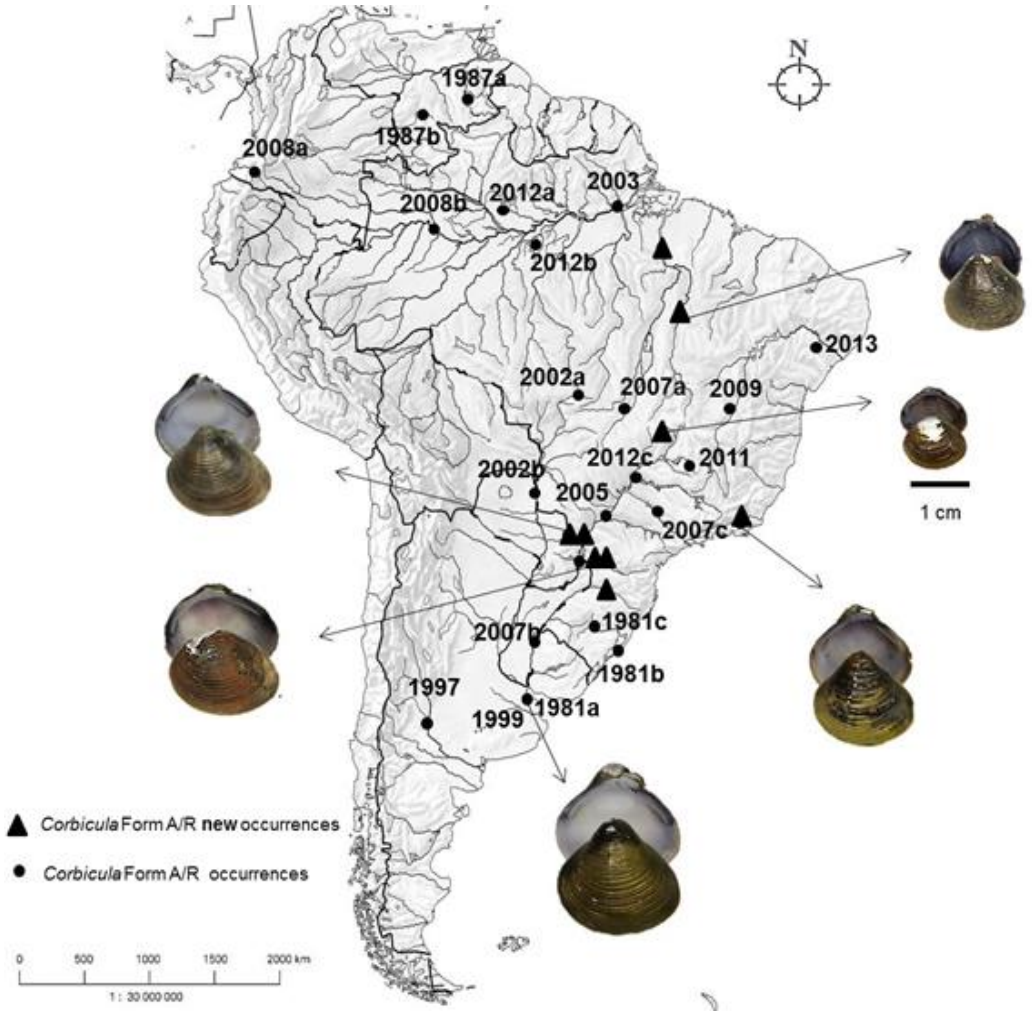


Figure 2. *Corbicula* sp. form A/R distribution in South America basing on (•) historic records (data information in text and Supporting information 1), and highlighting the shell morphology of (▲) new occurrences (this study, details in Supporting information 1).

The lack of studies involving the invasive *C.* sp. form A/R becomes evident when one realizes its present extensive distribution in South American basin. The occurrences presented above reveal that the Asian clam is already established in almost all hydrographic basins of South America.

FEATURES OF A SUCCESSFUL INVASIVE *CORBICULA* SPECIES

Potentially invasive organisms are usually those that do not have any known enemy into new introduced environment and, those that present exaptations which can maximize growth and reproduction in the introduced environment (e.g. Gould & Vrba, 1982; Wilson *et al.,* 2009). In the invasive *Corbicula* clams, some of those exaptations are: (i) strong and dark color shell that decreases corrosion in lotic waters (e.g. Ilarri *et al.,* 2014); (ii) efficient and inexpensive dispersion mechanisms, which may or may facilitate anthropogenic dispersion. Thus, human vectors can also promote faster, longer and/or 'jump' dispersions within or between rivers and hydrographic basins, as reported by Zhan *et al.,* (2013) for another species of invasive mollusk, the *Limnoperna fortunei* (the golden mussel) (Dunker, 1857). Belz *et al.,* (2012) suggested that small recreational boats and construction sand may represent important vectors of dispersion of the golden mussel. These are likely important dispersion vectors for *Corbicula* spp. as well. Fish may also represent important vectors for invasive mollusk species. Bivalves ingested by fishes survive the passage through the digestive tract closing their valves, as reported by Cantanhêde *et al.,* (2007) for *C. fluminea* in South American rivers and Belz *et al.,* (2012), for *L. fortunei*.

Furthermore, (iii) burying behavior to escape from predators and to allow additional pedal filter feeding. This filtration alternative can play a key role in oligotrophic habitats containing low phytoplankton concentrations and provides access to biomass and higher growth rates in habitats with high organic matter (Pigneur *et al.,* 2014). Other features that *Corbicula* clams share with other successful invasive

organisms is (iv) the capacity to rapidly adapt to the new environment (e.g. Colautti & Lau, 2015) - this capacity is called evolvability (sensu Kirschener & Gerhart, 1998). Further, invasive lineages of *Corbicula* present (v) androgenetic and clonal reproduction, which are observed in introduced regions (Pigneur *et al.*, 2012; Ludwig unpublished data). Androgenetic and clonal reproduction of *Corbicula* spp., Pigneur *et al.*, (2012), also suggest that the androgenetic reproduction may play an important role in favoring the establishment of these invasive clams in new areas. These clams are capable of self-fertilization and can quickly spread. Solely one individual it's capable of generate up to 90.000 descendants in a single reproductive season and rapidly originate a new population in the recently invaded range (see McMahon, 1999). Further, into introduced range, the androgenetic reproduction of individuals of a single lineage to 'parasitize' the maternal gametes of another lineage can improve their reproductive fitness (Pigneur *et al.*, 2014b).

Additionally, potentially invasive organisms present (vi) short life span with larval stages. For instance, Ludwig *et al.*, (2014) suggests that *Corbicula* clams do not incubate early larval stages in many South American rivers. This shift in the larval incubation process appears to be the result of local adaptation, since it was not reported previously. The premature release of larval stages into the plankton accelerates the dispersion of these organisms in the environment. Dispersion by larval stages of *Corbicula* is notable; Voelz *et al.*, (1998) suggested that these larvae can disperse at least 1.2 km/year upstream, even without human aid.

Finally, another fundamental aspect to understand the invasiveness of *Corbicula* clams is their (vii) ability to rapidly respond to environmental conditions by phenotypic plasticity (Agrawal 2001). In other words, the species genotype can express different phenotypes according to the immediate environmental pressure. Thus, plasticity can maximizes the individual's fitness in changing environments and may represent an important asset during colonization and persistence of invading lineages of *Corbicula* in new areas. As detected by Bagatini *et al.*, (2005), different phenotypes are expressed by a single genotype of *Corbicula* spp. in Southern Brazil. Phenotypic plasticity allows the colonization and establishment of invasive organisms in a new ecosystem by increasing

their fitness (i.e. providing better use of available resources like different substrates and occupying different trophic niches) to local conditions (e.g. Dybdahl & Kane, 2005; Keller &Taylor, 2008).

Recently, findings suggested that *Corbicula* sp. form A/R represents a widespread super-clone population which maintains the lack of genetic diversity (only one mtDNA COI haplotype and one genotype to each lineage in entire invasive area) through androgenetic reproduction (Pigneur *et al.*, 2014b; Ludwig unpublished data). Thus, the combination of those features listed above suggests that *Corbicla* spp. present (viii) robust genotype (see Agosta & Klemens, 2008) which facilitates the establishment of the introduced population.

THE IMPACT OF *CORBICULA* SPP. IN THE INVADED ENVIRONMENT

In North America, *C*. sp. form A/R has caused extensive economic damage to intake pipes, irrigation canals, and power-plants structures (Kramer-Wilt, 2008, in press; Ricciardi 2015). In Europe, invasive *Corbicula* spp. have decreased the biomass of annual primary production in River Meuse (Pigneur *et al.*, 2014a) and, according to Sampaio & Rodil (2014), these clams are considered ecosystem engineers in Portugal, which can alter the levels of sediment in the water, dissolved oxygen, density of the macrofauna, and biodiversity descriptors in general. Furthermore, these invasive clams can dominate in abundance, biomass, and secondary production natural ecosystems as was registered in Portugal by Sousa *et al.*, (2008). Also, they are benthic pedal- and filter-feeders and have one of the greatest filtration rates among bivalves (McMahon, 2002). Thus, as a consequence, according to Cohen *et al.,* (1984) and Pigneur *et al.*, (2014b), these clams can impact the concentration of dissolved oxygen by decreasing phytoplankton abundance and biomass. These clams can also directly affect the carbon cycling and organic matter dynamics (Hakenkamp & Palmer, 1999) and damage structures created by human (e.g. Zampatti & Darrigran, 2001).

In South America, there are few reports about the ecological and economic impact caused by species of *Corbicula* (e.g. Darrigran 2002; Santos *et al.,* 2012). These invasive clams have drastically reduced the populations of native bivalve from

Mycetopodidae and Hyriidae in Paraná River (Takeda *et al.*, 2000), in Pará state, Northern Brazil (Beasley *et al.*, 2003) and, competing with *Neocorbicula limosa* Maton, 1811 in Southern Brazil (Karatayev *et al.*, 2003; Silva & Stuff, 2011). In addition, they also have competed with another invasive mollusk, *Rapana venosa* (Valenciennes, 1846) and, in the structure of the food web in the River Río de la Plata estuary in Argentina (Lercari & Bergamino, 2011).

POLICY AND ECONOMIC IMPLICATIONS

The continuous introduction and spread of invasive *Corbicula* clams in Neotropical region seem inevitable and probably hard to completely eradicate, as public policies did not deal with the issue, despite significant ecological impacts (Darrigran, 2002, Silva & Stuff, 2011). Opposing to economic impacts in human activities as hydrographic power plants systems, usually ecological impacts do not alarm public agencies since they do not generate massive and conspicuous economical losses. Unfortunately, in Brazil, there is no public policy to control invasive *Corbicula* clams (neither many other invasive species, see Oliveira & Machado, 2009). However, there is a great concern of private agencies that these organisms may generate some kind of economic impact and affect human activities, especially in electrical system, such as *L. fortunei* (Darrigran, 2002). Overall, there is immediate need for more detailed field studies to evaluate the full impact of *Corbicula* clams in invaded environments; collaborative, international studies across boarder countries in Neotropical region and biogeographic regions will be necessary.

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REFERENCES

AGOSTA, S.J. & KLEMENS, J.A. 2008. Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. *Ecology Letters,* **11:** 1123-1134.

AGRAWAL, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science,* **294:** 321-326.

ALLENDORF, F.W. & LUNDQUIST, L.L. 2003. Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservation Biology* 17: 24 – 30, http://dx.doi.org/10.1046/j.1523-1739.2003.02365.x

ARAÚJO, R., MORENO, D. & RAMOS, M.A. 1993. The asiatic clam *Corbicula fluminea* (Muller 1774) (Bivalvia, Corbiculidae) in Europe**.** *American Malacological Bulletin,* **10**: 39-49.

ARISTIZÁBAL, M.V.D.L.H. 2008. Primer registro en Colombia de *Corbicula fluminea* (Mollusca: Bivalvia: Corbiculidae), una espécie invasora. *Boletín de Investtigaciones Marinas y Costeras,* **37**: 197-202.

BAGATINI, Y.M., PANARARI, R.S., HIGUTI, J., BENEDITO-CECILIO, E., PRIOLI, A.J. & PRIOLI, S.M.A.P. 2005. Morphological and molecular characterization of *Corbicula* (Mollusca: Bivalvia) at Rosana Resevoir, Brazil. *Acta Scientiarum Biological Science,* **27**: 397-404.

BEASLEY, C.R., TAGLIARO, C.H. & FIGUEIREDO, W.B. 2003. The occurrence of the Asian clam *Corbicula fluminea* in the lower, Amazon Base. *Acta Amazonica,* **33**: 317-324**.**

BELZ, C.E., DARRIGRAN, G., NETTO, O.S.M., BOEGER, W.A. & JUNIOR, P.J.R. 2012. Analysis of four dispersion vectors in Inland Waters: The case of the invading bivalves in South America. *Journal of Shellfish Research* 31: 777 – 784, http://dx.doi.org/10.2983/035.031.0322

BOLTOVSKOY, D., CORREA, N., CATALDO, D., STRIPEIKIS, J., & TUDINO, M. 1997. Environmental stress on *Corbicula fluminea* (Bivalvia) in the Paraná River Delta (Argentina): complex pollution-related disruption of population structures. *Archives für Hydrobiologie,* **138:** 483-507.

BORGES, H.L.F., CASTRO, R.P., ALVES, A.E., RAMALHO, D.F., LIMA, E.L.A., QUEIROZ, A.C.S., MELO, V.F. & SEVERI, W. 2009. Caracterização da comunidade de macroinvertebrados bentônicos no reservatório de sobradinho e submédio rio São Francisco. IX Jornada de Ensino, Pesquisa e Extensão, Universidade Federal Rural de Pernambuco, Recife.

BRITTON, J.C. & MORTON, B. 1979. *Corbicula* in North America: The evidence reviewed and evaluated. Proceeding, First International *Corbicula* Symposium, Texas Christian University: 249-287.

CALLIL, C.T. & MANSUR, M.C.D. 2002. Corbiculidae (Mollusca, Bivalvia) in Pantanal of Mato Grosso, high Paraguay Basin, Brazil: distribution and population density. *Amazoniana*, **17**: 1-15.

CANTANHÊDE, G., HAHN, N.S., GUBIANI, E.A. & FUGI, R. 2007. Invasive molluscs in the diet of Pterodoras granulosus (Valenciennes, 1821) (Pisces, Doradidae) in the Upper Paraná River floodplain, Brazil. *Ecology of Freshwater Fish*, **17***:* 47-53.

CASTILLO, A.R., BORTOLUZZI, L.R., OLIVEIRA, E.V. 2007. Distribuição e Densidade Populacional de *Corbicula fluminea* (Muller, 1774) do Arroio Imbaá, Rio Uruguai, Uruguaiana, Brasil. *Biodiversidade Pampeana*, **5**:25-29.

CATALDO, D. & BOLTOVSKOY, D. 1999. Population dynamics of *Corbicula fluminea* (Bivalvia) in the Paraná River Delta, Argentina. *Hydrobiologia.* **380**: 153-163.

CAZZANIGA, N.J. 1997. Asiatic clam, *Corbicula fluminea*, reaching Patagonia (Argentina). *Journal of Freshwater Ecology*, **12**: 629-630, http://dx.doi.org/10.1080/02705060.1997.9663577

CAZZANIGA, N.J. & PEREZ, C. 1999. Asiatic clam, *Corbicula fluminea*, in Northwestern Patagonia (Argentina). *Journal of Freshwater Ecology,* **14**: 551-52, http://dx.doi.org/10.1080/02705060.1999.9663713

COLAUTTI, R.I. & LAU, J.A. 2015. Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology,* **24: 1999-2007.** http://dx.doi.org/10.1111/mec.13162.

CRESPO, D., DOLBETH, M., LESTON, S., SOUSA, R. & PARDAL, M.A. 2015. Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. *Biological Invasions,* **17:** 2087-2101. http://dx.doi.org/10.1007/s10530-015-0862-y

COHEN, R.R., DRESLER, P.V., PHILLIPS, E.J. & CORY, R.L. 1984. The effect of the Asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography,* **29:** 170-180.

DARRIGRAN, G.A. & MAROÑAS, M.E. 1989. Crecimiento de las poblaciones de *Corbicula fluminea* (Müller, 1774) y *C. largillierti* (Philippi, 1844) (Bivalvia: Sphaeiacea) en el litoral de Punta Blanca, Estuario del Río de la Plata, República Argentina. *Comunicaciones de la Sociedad Malacológica del Uuruguay,* **7:** 56-57.

DARRIGRAN, G. 1992. Variacion temporal y espacial de la distribución de las especies de *Corbicula* Megerle, 1811 (Bivalvia, Corbiculidae), en el estuario del Rio de La Plata, Republica Argentina. *Neotropica,* **38**: 59-63.

DARRIGRAN, G. & PASTORINO, G. 1993. Bivalvos invasores en el Rio de Plata, Argentina. *Comunicaciones de la Sociedad Malacologica del Uruguay,* **64**: 64 – 65.

DARRIGRAN, G. 2002. Potential impact of filter-feeding invaders on temperate inland fresh water environments. *Biological Invasions,* **4**: 145-156.

DYBDAHL, M.F. & KANE, S.L. 2005. Adaptation vs. phenotypic plasticity in the success of a clonal invader. *Ecology,* **86:** 1592-1601.

DOHERTY, G.F., CHERRY, D.S. & JR, J.C. 1987. Spawning periodicity of the Asiatic clam *Corbicula fluminea* in the New River, Virginia. *American Midland Naturalist*, **117**: 71-82.

FOCHT, T. & VEITENHEIMER-MENDES. 2001. Distribuição sazonal e reprodução de *Neocorbicula limosa* (Maton) (Bivalvia, Corbiculidae) no lago Guaíba, Rio Grande do Sul, Brasil.

GATLIN, M.R., SHOUP, D.E. & LONG, J.M. 2012. Invasive zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) survive gut passage of

migratory fish species: implications for dispersal. *Biological Invasions,* **15**: 1195 – 1200, http://dx.doi.org/10.1007/s10530-012-0372-0

GLAUBRECHT, M., FÉHER, Z. & KOHLER, F. 2006. Inventorizing an Invader: Annotated Type Catalogue of Corbiculidae Gray, 1847 (Bivalvia, Heterodonta, Veneroidea), Including Old World Limnic *Corbicula* in The Natural History Museum Berlin. *Malacologia,* **49**: 243-272, http://dx.doi.org/10.4002/0076-2997-49.2.243

GOULD, S.J., & VRBA, E.S. 1982. Exaptation-a missing term in the Science of form. *Paleobiology,* 4-15.

GRAF, D.L. 2013. Patterns of freshwater bivalve global diversity and the state of phylogenetic studies on the Unionoida, Sphaeriidae, and Cyrenidae. *American Malacological Bulletin,* **31**: 135 – 153, http://dx.doi.org/10.4003/006.031.0106

HAKENKAMP, C.C. & PALMER, M.A. 1999. Introduced bivalves in freshwater ecosystems: the impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia,* **119**: 445-451, http://dx.doi.org/10.1007/s004420050806

HEDTKE, S.M., STANGER-HALL, K., BAKER, R.J., HILLIS, D.M. 2008. All-male asexuality: origin and maintenance of androgenesis in the Asian clam *Corbicula. Evolution,* **62**: 1119-1136, http://dx.doi.org/10.1111/j.1558-5646.2008.00344.x

HEDTKE, S.M., GLAUBRECHT, M. & HILLIS, D.M. 2011. Rare gene capture in predominantly androgenetic species. *PNAS,* **108**: 9520-9524, http://dx.doi.org/10.1073/pnas.1106742108

HOUKI, S., YAMADA, M., HONDA, T. & KOMARU, A. 2011. Origin and possible role of males in hermaphroditic androgenetic *Corbicula* clams. *Zoological Science,* **28**: 526 – 531.

ILARRI, M.I., SOUZA, A.T., ANTUNES, C., GUILHERMINO, L., & SOUSA, R. 2014. Influence of the invasive Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) on estuarine epibenthic assemblages. Estuarine, Coastal and Shelf Science, **143:** 12-19.

ITUARTE, C.F. 1981. Primera noticia acerca de la introduccion de pelecipodos asiaticos en el area rioplatense**.** *Neotropica,* **27**:79-82.

ITUARTE, C.F. 1984. Aspectos biológicos de las poblaciones de *Corbicula largillierti* Philippi (Mollusca Pelecypoda) en el río de la Plata. *Rev. Del Museo de La Plata. NS Zoologia*, **13.**

ITUARTE, C.F. 1994. *Corbicula* and *Neocorbicula* (Bivalvia: Corbiculidae) in the Parana, Uruguay, and Rio de La Plata Basins**.** *Nautilus,* **107**:129-135.

KARATAYEV, A.Y., BURLAKOVA, L.E., KESTERSON, T. & PADILLA, D.K. 2003. Dominance of the Asiatic clam, *Corbicula flumínea* (Müller), in the benthic community of a reservoir. *Journal of Shellfish Research,* **22:** 487-494.

KARATAYEV, A.Y., HOWELLS, R.G., BURLAKOVA, L.E. & SEWELL, B.D. 2005. History of spread and current distribution of *Corbicula fluminea* (Müller) in Texas. *Journal of Shellfish Research,* **24**: 553 – 559.

KELLER, S.R. & TAYLOR, D.R. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecology Letters,* **11:** 852-866. http://dx.doi.org/10.1111/j.1461-0248.2008.01188.x

KIRSCHNER, M. & GERHARDT, J. 1998. Evolvability. PNAS, **95:** 8420-8427.

KOMARU, A., OOKUBO, K., & KIUOMOTO, M. 2000. All meiotic chromosomes and both centrosomes at spindle pole in the zygotes discarded as two polar bodies in clam *Corbicula leana:* unusual polar body formation observed by antitubulin immunofluorescence. *Development genes and evolution,* **210:** 263-269.

KOMARU, A., KUMAMOTO, A., KATO, T., ISHIBASHI, R., OBATA, M., & NEMORO, T. 2006. A hypothesis of ploidy elevation by formation of a female pronucleus in the androgenetic clam *Corbicula fluminea* in the Tone River Estuary, Japan. *Zoological science,* **23:** 529-532.

KORNISHI, K., KAWAMURA, K.O.U.I.C.H.I., FURUITA, H., & KOMARU, A.K.I.R.A. 1998. Spermatogenesis of the freshwater clam *Corbicula* aff. *fluminea* Müller (Bivalvia: Corbiculidae). *Journal of Shellfish Research,* **17:** 185-190.

KORNIUSHIN, A.V., & GLAUBRECHT, M. 2003. Novel reproductive modes in freshwater clams: brooding and larval morphology in Southeast Asian taxa of *Corbicula* (Mollusca, Bivalvia, Corbiculidae). *Acta Zoologica,* **84:** 293-315.

KRAMMER-WILT, E. 2008. *Corbicula fluminea* (OF Müller, 1774) – Asian Clam.

LASSO, C.A., MARTÍNEZ-ESCARBASSIERE, R., CAPELO, J.C., MORALES-BETANCOURT, M.A., SÁNCHEZ-MAYA, A. 2009. Lista de los moluscos (Gastropoda-Bivalvia) dulceacuícolas y estuarinos de La cuenca del Orinoco (Venezuela). *Biota Colombiana,* **10**: 63-74.

LEE, T., SIRIPATTRAWAN, S., ITUARTE, C.F. & Ó FOIGHIL, D. 2005. Invasion of the clonal clams: *Corbicula* lineages in the New World. *American Malacological Bulletin,* **20**:113-122.

LERCARI, D. & BERGAMINO, L. 2011. Impacts of two invasive mollusks, *Rapana venosa* (Gastropoda) and *Corbicula fluminea* (Bivalvia), on the food web structure of the Río de la Plata estuary and nearshore oceanic ecosystem. *Biological Invasions,* **13:** 2053-2061. http://dx.doi.org/10.1007/s10530-011-0023-x

LUDWIG, S., TSCHÁ, M.K., PATELLA, R., OLIVEIRA, A.J. & BOEGER, W.A. 2014. Looking for a needle in a haystack: molecular detection of larvae on invasive *Corbicula* clams. *Management of Biological Invasions*, **5:** 143-149. http://dx.doi.org/10.3391/mbi.2014.5.2.07

MANSUR, M.C.D., & GARCES, L.M.M.P. 1988. Ocorrência e densidade de *Corbicula fuminea* (Müller, 1774) e *Neocorbicula limosa* (Maton, 1811) na Estação ecológica do Taim e áreas adjacentes, Rio Grande do Sul, Brasil. *Ilheringia, Série Zoologica,* **68:** 99-115.

MANSUR, M.C.D., CALLIL, C.T., CARDOSO, F.R., IBARRA, J.A.A., SILVA, J.S.V., SOUZA, R.C.C.L. 2004. Uma retrospectiva e mapeamento da invasão de espécies de *Corbicula* (Mollusca, Bivalvia, Veneroida, Corbiculidae) oriundas do sudeste asiático, na América do Sul. In Silva JSV and Souza RCCL, Água de lastro e bioinvasão. Interciência, Rio de Janeiro.

MANSUR, M.C.D., SANTOS, C.P., & NEHRKE, M.V. 2012 Corbiculidae na América do Sul: Espécies nativas e invasoras, dispersão e a situação das pesquisas no Brasil (Mollusca: Bivalvia). In: Ecos do XIX Encontro Brasileiro de Malacologia, Rio de Janeiro, Technical Books, pp 467.

MARONEZE, D.M., COSCARELLI, D., VIDIGAL, T.H.D.A., CALLISTO, M. 2011. First record of *Corbicula fluminea* (Muller, 1774) in the drainage basin of the Araguari River, Minas Gerais, Brazil. *Brazilian Journal of Biology*, **71**: 221-222, http://dx.doi.org/10.1590/S1519-69842011000100033

MARTÍNEZ, R. 1987. *Corbicula manilensis* molusco introducido en Venezuela. *Acta Cientifica Venezolana*, **38**: 384-385.

MARTINS, D.S., VEITENHEIMER-MENDES, I.L. & FACCIONI-HEUSER, M. 2004. *Corbicula* (Bivalvia, Corbiculidae) em simpatria no Lago Guaíba, Rio Grande do Sul, Brasil. *Biociências*, **12**: 129-138.

MCMAHON, R.F. 1982. The occurrence and spread of the introduced Asiatic freshwater clam, *Corbicula fluminea* (Müller) in North America**.** *Nautilus*, **96**: 134-141.

MCMAHON, R.F. 1999. Invasive Characteristics of the Freshwater Bivalve, *Corbicula fluminea***.** *Lewis Publisher*, 315-343.

MCMAHON, R.F. 2002. Evolutionary and physiological adaptations of aquatic invasive animals: *r* selection versus resistance. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**: 1235 – 1244.

MORTON, B. 1977. *Corbicula* in Asia. Proceeding, First International *Corbicula* Symposium, Texas Christian University, 15-38.

OLIVEIRA, A.E.S. & MACHADO, C.J.S. 2009. Quem é quem diante da presença de espécies exóticas no Brasil? Uma leitura do arcabouço institucional-legal voltada para a formulação de uma política pública nacional. Ambiente & Sociedade, **12**: 373-387.

PARK, J. & KIM, W. 2003. Two *Corbicula* (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment. *Molecular Phylogenetics and Evolution*, **29**: 529-539, http://dx.doi.org/10.1016/S1055-7903(03)00138-6

PEREIRA, D., VEITENHEIMER-MENDES, I.L., MANSUR, M.C.D., & SILVA, M.D. 2000. Malacofauna límnica do sistema de irrigação da microbacia do arroyo Capivara, Triunfo, RS, Brasil. *Biociências*, **8:** 137-157.

PFENNINGER, M., REINHARDT, F., STREIT, B. 2002. Evidence for cryptic hybridization between different evolutionary lineages of the invasive clam genus *Corbicula* (Veneroida, Bivalvia). *Journal of Evolutionary Biology*, **15**: 818-829, http://dx.doi.org/10.1046/j.1420-9101.2002.00440.x

PIGNEUR, L.M., MARESCAUX, J., ROLAND, K., ETOUNDI, E., DESCY, J.P. & VAN DONINCK, K. 2011b. Phylogeny and androgenesis in the invasive *Corbicula* clams (Bivalvia, Corbiculidae) in Western Europe. *BMC Evolutionary Biology*, **11**:147, http://dx.doi.org/10.1186/1471-2148-11-147

PIGNEUR, L.M., HEDTKE, S.M., ETOUNDI, E. & VAN DONINCK, K. 2012. Androgenesis: a review through the study of the shelfish shellfish *Corbicula* spp. *Heredity*, **108**: 581 – 591, http://dx.doi.org/10.1038/hdy.2012.3

PIGNEUR, L.M., ETOUNDI, E., ALDRIDGE, D.C., MARESCAUX, J., YASUDA, N. & VAN DONINCK, K. 2014a. Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. *Molecular Ecology*, **23:** 5102-5116.

PIGNEUR, L.M., FALISSE, E., ROLAND, K. EVERBECQ, E., DELIÉGE, J.F., SMITZ, J.S., VAN DONINCK, K., & DESCY, J.P. 2014b. Impact of invasive Asian clams, *Corbicula* spp., on a large river ecosystem. *Freshwater Biology*, **59:** 573-583.

PIMPÃO, D.M. & MARTINS, D.S. 2008. Occurrence of the Asian mollusk *Corbicula fluminea* (Müller 1774) (Bivalvia: Corbiculidae) in the lower Rio Negro, Central Amazoan Region, Brazil. *Acta Amazonica*, **38**: 589 – 592.

RICCIARDI, A. 2015. Ecology of invasive alien invertebrates. In: THORP, J., ROGERS, D.C. (Eds.), Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates, Academic Press, 83-91.

RODRIGUES, J.C.A., PIRES-JUNIOR, O.R., COUTINHO, M.F., MARTINS-SILVA, M.J. 2007. First occurrence of the Asian Clam *Corbicula fluminea* (Bivalvia: Corbiculidae) in the Paraná Lake, Brasília, Brazil**.** *Brazilian Journal of Biology*, **67**: 789-790.

ROSA, I.C., PEREIRA, J.L., GOMES, J., SARAIVA, P.M., GONÇALVES, F., COSTA, R. 2011. The Asian clam *Corbicula fluminea* in the European freshwater-dependent industry: A latent threat or a friendly enemy? *Ecological Econonimics,* **70**: 1805-1813, http://dx.doi.org/10.1016/j.ecolecon.2011.05.006

SAMPAIO, E. & RODIL, I.F. 2014. Effects of the invasive clam *Corbicula fluminea* (Müller, 1774) on a representative macrobenthic community from two estuaries at different stages of invasion. *Limnetica,* **33**: 249-262.

SANTOS, S.B., THIENGO, S.C., FERNANDEZ, M.A., MIYAHIRA, I.C., GONÇALVES, I.C.B., XIMENES, R.F., MANSUR, M.C.D., & PEREIRA, D. 2012. Espécies de moluscos límnicos invasores no Brasil, pp 25-49. In: Moluscos límnicos invasores no Brasil: biologia, prevenção e controle, Maria Cristina Dreher Mansur (Org.), pp 412. Redes Editora (ed), Porto Alegre. In: Fundación del Dr. Chang Yung Fa (ed) La construción de un puente cultural y de negocios entre Argentina y el Lejano Oriente, pp 93-128. Evergreen Group Press, Buenos Aires.

SEMENAS, L. & FLORES, V. 2005. Presence of *Corbicula fluminea* in the Upper Negro River Basin (Patagonia, Argentina). *Journal of Freshwater Ecology*, **20**: 615-616.

SILVA, L.F. & STUFF, T. 2011. Distribuição e abundância dos bivalves *Corbicula fluminea* (Müller, 1774) e *Neocorbicula limosa* (Maton, 1811) no lago Guaíba, Rio Grande do Sul, Brasil. *Fepam em Revista,* **5:** 13-18.

SIRIPATTRAWAN, S., PARK, J. & Ó FOIGHIL, D. 2000. Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. *Journal of Molluscan Studies,* **66**: 423-429, http://dx.doi.org/10.1093/mollusc/66.3.423

SOUSA, R., FREIRE, R., RUFINO, M., MÉNDEZ, J., GASPAR, M., ANTUNES, C., GUILHERMINO, L. 2007. Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Muller, 17774) in two Portuguese estuarines. *Estuarine, Coastal Shelf Science,* **74:** 166-174.

SOUSA, R., NOGUEIRA, A.J.A., GASPAR, M.B., ANTUNES, C., GUILHERMINO, L. 2008. Growth and extremely high production of the non-indigenous invasive

species *Corbicula fluminea* (Muller 1774): Possible implication for ecosystem functioning. *Estuarine, Coastal Shelf Science*, **80**: 289-295, http://dx.doi.org/10.1016/j.ecss.2008.08.006

SURIANI, A.L., FRANÇA, A.L.R.S., ROCHA, O. 2007. A malacofauna bentônica das represas do médio rio Tietê (São Paulo, Brasil) e uma avaliação ecológica das espécies exóticas invasoras, *Melanoides tuberculata* (Muller) e *Corbicula fluminea* (Muller). *Revista Brasileira de Zoologia* 24: 21-32, http://dx.doi.org/10.1590/S0101-81752007000100003

TAKEDA, A.M., HIGUTI, J., FUJITA, D.S. & BUBENA, M.R. 2000. Proliferação de uma espécie de bivalve invasora, *Corbicula flumínea*, na área alagável do Alto Rio Paraná (Brasil). In: Seminário Brasileiro sobre Água de Lastro 1, Arraial do Cabo, p.11.

VEITENHEIMER-MENDES, I.L. 1981. *Corbicula manilenssis,* (Philippi, 1844) molusco asiático, na bacia do Jacuí e do Guaíba, Rio Grande do Sul, Brasil (Bivalvia, Corbiculidae). *Ilheringia Serie Zoologica*, **60**: 63-74.

VIDIGAL, T.H.D.A., MARQUES, M.M.G.S.M., LIMA, H.P. & BARBOSA, F.A.R. 2005. Gastrópodes e bivalves límnicos do trecho médio da bacia do Rio Doce, Minas Gerais, Brasil. *Lundiana*, **6**: 67-76.

VOELZ, N.J., MCARTHUR, J.V. & RADER, R.B. 1998. Upstream Mobility of the Asiatic Clam *Corbicula fluminea*: Identifying Potential Dispersal Agents. *Journal of Freshwater Ecology*, 13: 39-45.

WILSON, J.R.U., DORMONTT, E.E., PRENTIS, P.J., LOWE, A.J. & RICHARDSON, D.M. 2009. Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology and Evolution*, **24**: 136-144, http://dx.doi.org/10.1016/j.tree.2008.10.007

ZAMPATTI, L. & DARRIGRAN, G. 2001. Generación y Transferencia del Conocimiento. El inicio de un intercambio eficiente. In: Fundación del Dr. Chang Yung Fa (ed) La construción de un puente cultural y de negocios entre Argentina y el Lejano Oriente, pp 93-128. Evergreen Group Press, Buenos Aires.

ZHAN, A., PAREPELIZIN, P.V., GHABOOLI, S., PAOLUCCI, E., SYLVESTER, F., SARDIÑA, P., CRISTESCU, M.E. & MACISSAC, H.J. 2012. Scale-dependent post-establishment spread and genetic diversity in an invading mollusk in South America. *Diversity and Distribution,* **18:** 1042-1055. http://dx.doi.org/10.1111/j.1472-4642.2012.00894.x

Supporting information 1. Historic records for the presence of *Corbicula* Form A/R (*Corbicula fluminea*) in South American hydrographic basins.

River	Sample Station	Record coordinates		Record date	Reference
		Latitude	Longitude		
Amazonas	Alenquer Cametá Caixuanã Melgaço	01º57'S 02°15'S 01°56'40"S 01°46'S	54º43'W 49°30'W 51°18'57"W 50°38'W	October 1998 August 1999 November 1999 June 2000	Beasley et al. 2003
Negro	Lago do Tupé	03º01'25,2"S	60º15'58,6"W	23 January 2005	Pimpão and Martins 2008
San Juan Caripe		10º17'N 10º10'26"N	62º57'W 63º30'09"W	1987	Martinez 1987
Magdalena	Caño los Almendros	11º01'N	74º46'W	2004-2005	Aristizábal 2008
Colorado	Argentine Patagonia	39º41'S	62º26'W	1997	Cazzaniga 1997
Guaíba Lake	Itapuã	30º20'S	50º50'W	June of 2002	Martins et al. 2004
Uruguay	Arroio Imbaá	29º46'33,2"S	56º57'08,7"W	May 2005	Castillo et al. 2007
Río de la Plata	Paraná de las Palmas	34°17'S	58°31'W	October 1995	Cataldo and Boltovskoy (1999)
Cuiabá	Rosário do Oeste Várzea Grande Santo Antônio do Leverger	14°49'S 15°37'S 15°52'S	56°24'W 56°08'W 56°04'W	August 1999	Callil & Mansur (2002)
		Not available	Not available	Not available	Mansur et al. (2012)
São Paulo	Barra Bonita Bariri Ibitinga	22°31'8"S 22°9'13"S 21°45'38"S	48°32'7"W 48°45'9"W 48°59'27"W	November 2002	Suriani et al. (2007)
Rosana	Rosana	22°35'24"S	52°49'51"W	7 November 2003	Bagatini et al. (2005)
Araguari	Amador Aguiar Hydroeletric Plant	18°20'S	46°00'W	July 2008	Maroneze et al. (2011)
Paranoá	Brasília	15°43'49"S	47°53'28"W	October 2004	Rodrigues et al. (2007)
São Francisco	Sobradinho Reservoir	09°25'S	40°51'W	July 2008	Borges et al. (2009)
São Francisco	Paulo Afonso Reservoir	9°41’60”S	37°39’48”W	September 2010	Santana et al. (2013)

CAPÍTULO II

Invasive *Corbicula* spp. (Bivalvia, Corbiculidae) in South America: androgenetic lineages, hybrids and morphological variability[§]

Abstract

Androgenetic *Corbicula* sp. form A/R, C/S, B and Rlc lineages are successful invaders in Americas and Europe. They are considered major alien invasive species because of their impacts on aquatic ecosystems and industrial cooling systems. Hermaphroditic androgenetic lineages of *Corbicula* are found over large geographic distances and exclusively in invasive area, while sexual species are restricted to native range. Thus, the androgenetic reproduction likely played an important role during the establishment process of the invasive *Corbicula* clams. Due to their morphological diversity, specific determination of *Corbicula* using morphological data alone often results in erroneous taxonomic assignment. Thus, the present study identified the South-American *Corbicula* lineages based on morphometric data, the COI fragment of the mtDNA, and ten microsatellite loci in order to clarify their taxonomic identification and phylogenetic relationships with the invasive androgenetic lineages present in Europe and North America and, their native Asian sexual and androgenetic congeners. We detected two *Corbicula* lineages based on mtDNA COI in South America: *C.* sp. form A/R and *C.* sp. form C/S. "Mixed" specimens presenting intermediate morphotypes and 'hybrid' genotypes were also detected in some sampled sites. In addition, extensive morphological variation was detected in *C.* sp. form A/R populations but all associated to a single genotype/mt haplotype FW5. Based on microsatellite data, 3D-FCA and DAPC analysis, it was possible to identify the presence of established *C.* sp. form A/R and C/S populations in South American rivers. Thus, our results support the existence of two possibly androgenetic *Corbicula* lineages, different phenotypes in *C.* sp. form A/R, and "mixed" populations between invasive lineages in South America.

Keywords: *Corbicula*, South America, lineages, androgenetic, phylogeny, hybrids

Running title: The invasive *Corbicula* spp. in South America

[§] Artigo formatado conforme à revista Biological Invasions

Introduction

Native to Asia, Australia, Africa, and the Middle East (Araújo et al. 1993; Park and Kim 2003), species of the clam genus *Corbicula* are present in estuarine and freshwater environments. The genus includes dioecious sexual (restricted to native range) and hermaphroditic androgenetic lineages (including invasive species) across the globe (Pigneur et al. 2014b). Compared to native *Corbicula* lineages, the invasive *Corbicula* lineages have low level of genetic diversity in their introduced range (the American and European continents; Siripattrawan et al. 2000; Lee et al. 2005; Hedtke et al. 2008; Pigneur et al. 2011b), which is correlated with androgenetic and clonal reproduction (Pigneur et al. 2014b). In Androgenetic lineages of freshwater *Corbicula*, four successful invaders (named here as Pigneur et al. 2014b) are presently known: *C.* sp. A/R (known in North America as form A and in Europe as form R), C/S (known in South America as form C and in Europe as form S), B (known in North American as form B) and Rlc (detected only in Europe) forms which are found in Europe, North and South America (Araújo et al. 1993; Darrigran 2002; Lee et al. 2005; Pigneur et al. 2011b).

Biflagellate unreduced sperm characterizes hermaphroditic androgenetic *Corbicula* lineages (Konishi et al. 1998, Komaru et al. 1997). In this mode of reproduction, the offspring are paternal clones as they only inherit the male nuclear chromosomes (see details in Houki et al. 2011; Pigneur et al. 2012). Also, the sperm from one androgenetic lineage can also fertilize the egg of another lineage. Thus, the nuclear genome of the first lineage presents the mtDNA of the second; this is known as "egg parasitism" or mitochondrial-capture event (Komaru et al. 2006). This peculiarity allows an admixture of different nuclear genomes when the maternal nuclear genome is incompletely extruded (Komaru et al. 2006; Pigneur et al. 2012). Therefore, the signature of such parasitism is incongruence between mitochondrial haplotype of that lineage and its phenotype or nuclear genome (Hedtke et al. 2008).

In freshwater ecosystems, *Corbicula* clams are considered one of the most invasive mollusks, owing to their large geographic distribution, the high densities they

can reach (e.g. 90.000 descendants in one reproductive period, McMahon 1999), can impact the phytoplankton abundance (Pigneur et al. 2014a) and invasive behavior (Sousa et al. 2008). The morphological diversity and androgenetic and clonal reproduction can also propitiate local adaptation of these clams. Thus, all these factors can maximize their invasion success in new environments.

The first report for the presence of specimens of *Corbicula* in South America was in Argentina, around 1980s (Ituarte 1981). Since then, the number of publications has increased (Ludwig, unpublished data) and their current distribution encompasses the Argentine Patagonia (Semenas and Flores 2005), the Negro River in Amazon basin, Northern Brazil (Pimpão and Martins 2008), the Orinoco basin in Venezuela (Lasso et al. 2009) and island of Salamanca in Colombia (Aristizábal 2008). Four *Corbicula* spp., defined based solely on morphological characters, are presently reported from South America: (i) *Corbicula fluminea* (Müller, 1774), (ii) *Corbicula largillierti* (Philippi, 1844), (iii) *Corbicula* cf. *fluminalis* (Muller, 1774), and (iv) *Corbicula* sp. (detected by Mansur et al. 2004). The first two species are found widely distributed in South American rivers while the last two are found in Patos Lagoon and Guaíba Lake, Porto Alegre city, Southern Brazil (Mansur et al. 2012). Genetically, according to Lee et al. (2005), there are two lineages in South America: *Corbicula* A/R and C/S forms. Both present low genetic variability at the mtDNA and are correlated with a single genotype. These authors also detected hybrid specimens in Iguazu Falls, presenting "mixed" genotypes between B and C/S forms and intermediate morphotype.

Recently researches (e.g. Pigneur et al. 2014) have sampled Corbicula specimens across the introduced range, however, the number of populations and the extent of sampling in South America was greatly limited. Thus, in this study, geographically wider sampling effort was made to identify *Corbicula* lineages and evaluate population structure. To achieve this goal, morphometric geometrics, and analysis of partial mtDNA COI gene and microsatellite nuclear markers were combined to characterize the several *Corbicula* populations distributed along South American hydrographic basins.

Materials and Methods

Sample collection and mtDNA amplification

In Brazilian and Argentine rivers, 236 *Corbicula* specimens were collected (Table 1, Appendix 1). Specimens were preserved in 96% ethanol. In the laboratory, the shells were separated from the soft tissue. The right shell of each specimen was photographed with a Canon Rebel EOS T3 digital camera and used in morphological analyses. Tissue samples were preserved and used in genetic analyses.

Approximately 700 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified from 236 specimens 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, 1x 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 30s at 94°C, 30s at 44°C and 40s at 72°C, and then a final extension of 5 min at 72°C. Amplified fragments (both strands) were sequenced in an Applied Biosystems 3130 automatic sequencer using the same amplification primers. Sequences were assembled, edited and a consensus was generated using Geneious® 6.1.2 (Biomatters; Available at http://www.geneious.com/). The consensus sequences of the individuals were compared to reference sequences in GenBank to identify similarities and cluster sequences into haplotypes. Subsequently, mtDNA COI gene haplotypes of *Corbicula* spp. available in GenBank (Supporting information 2) were added into the alignment for phylogenetic analyses.

Table 1. Sampling details and genetic diversity indices for mitochondrial cytochrome c oxidase subunit I (COI) gene and ten microsatellite markers for the *Corbicula* clams in South America. *N*, sample size for different molecular markers in different populations; *n*, number of COI haplotypes; *h*, haplotypic diversity; π, nucleotide diversity; *A* allelic richness; H_o observed heterozygosity and H_E expected heterozygosity computed at 10 microsatellite loci.

ID	Location/State/Country	Coordinates	Morpho type Form[1]	*COI N*	Haplotype	*n*	*h*	π	Lineage [2]	*N microsat*	Genotype	*A*	He	H_o
Argentina														
ARG1	Río de la Plata, La Plata, Bs.	34°55′0″S 57°57′0″W	C/S	11	FW17	1	0	0	C/S	11	C/S	1,58	0,41	0,64
ARG2	Río de la Plata, La Plata, Bs.	34°55′0″S 57°57′0″W	A/R	13	FW5	1	0	0	A/R	12	A/R	1,35	0,33	0,55
Brazil														
PP	Praia da Prata, TO	10°13’29”S 48°22’3”W	C/S	13	FW17	1	0	0	C/S	10	C/S	1,51	0,34	0,64
CLM	Capitao leonidas Marques, PR	25°32’36”S 53°29’33”W	A/R	10	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,31
IGU	Prainha 3 lagoas, Foz do Iguaçu, PR	25°26’48”S 54°30’16”W	A/R	14	FW5	1	0	0	A/R	3	A/R	1,6	0,28	0,55
GUA	Rio Paraná, Guaíra, PR	24°04'S 54°15'W	A/R	17	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,50
PU	Rio Iguaçu, Porto União, SC	26°14’16”S 51°04’40”W	IF	13	FWBra1	1	0	0	FWBra1	5	?[3]	1,42	0,19	0,32
JAC	Rio Jacuí, Agudo, RS	29°37’58’’S 53°14’33"W	IF	12	FW5	1	0	0	A/R	3	?[3]	1,31	0,25	0,5
BAR	Lago Guaíba, Barra do Ribeiro, RS	30°25'S 51°12'W	A/R	15	FW5	1	0	0	A/R	3	?[3]	1.42	0,17	0,33
GO	Rio Claro, Jataí, GO	17°57'0"S 51°43'21"W	A/R	9	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,5
RJ	Cabiunas, Silva Jardim, RJ Rio Guandu, Nova iguaçu, RJ	22°39'7"S 42°24'19"W 22°50'27"S 43°36'32"W	A/R	11	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,5
MAT	Arroio Tovoraipi, Mata, RS	29°34'46"S 54°25'16"W	A/R	23	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,50
IMI	Rio Iguatemi, Iguatemi, MS	23°44'S 54°33'W	A/R	20	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,50
ITU	Pedral do Tauri, Itupiranga, PA	05°08'S 49°10'W	A/R	28	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,50
ITA	UHE Itá, Itá, SC	27°16'S 52°22'W	A/R	12	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,50
ROS	Rio do Corvo, Rosana, SP	22°21'S 52°42'W	A/R	15	FW5	1	0	0	A/R	3	A/R	1,31	0,25	0,50
Invasive lineages /code[4]														
C. sp. form A/R (AA1)		North American form A and European Form R			FW5		0	0	A/R		A/R	1,6	0,25	0,5
C. sp. form B (AB1)		North American Form B			FW1		0	0	B		B	1,9	0,31	0,56
C. sp. form C/S (S1)		South American form C and European form S			FW17		0	0	C/S		C/S	2,5	0,38	0,58
C. sp. form Rlc (Rlc4)		European form Rlc			FW4		0	0	Rlc		Rlc	1,38	0,19	0,38

[1] Grouping resulted from Morphometric Geometric analyze (Fig.6) and the names were assigned following Pigneur et al. (2014b). [2]Lineage resulted from Bayesian Inference analyze (Fig. 3). [3] Populations with mixed genotype, detected in this study. [4]Genetic diversity of invasive *Corbicula* lineages from Pigneur et al. (2014b). (?) Indicate no proper identification.

Phylogenetic analyses

The evolutionary relationships among the COI haplotypes were examined using Bayesian Inference (BI) phylogeny reconstructions in order to identify the COI lineages of *Corbicula* in South America. The evolutionary model for the phylogenetic analysis of COI sequences was selected using jModelTest software (Posada, 2008), and based on Akaike information criteria (AIC), the best-fit model for dataset was TrN+I+Γ for all codon positions. The BI analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) implemented by the CIPRES Science Gateway (available at http://www.phylo.org/news/mrbayes). We ran four independent analyses with four Markov Chain Monte Carlo (MCMC) and 10 million generations each. We evaluated burn-in by plotting the log likelihood scores for each sampling point using TRACER v1.5 (Rambaut and Drummond 2007). Therefore, the first 25% were discarded as burn-in for each run. The residual trees were used to estimate a consensus tree using LogCombiner v.1.5.4 (part of the BEAST package, Drummond & Rambaut 2007). For all analyses, a published COI sequence of *Neocorbicula limosa* (Maton, 1811) was used as out-group. Phylogenetic trees were visualized and edited using FigTree v1.3.1 (Rambaut 2009).

Initially, the genetic diversity (number of haplotypes *n*, haplotype diversity *h* and nucleotide diversity π) was estimated using DnaSP 5.0 (Librado and Rozas 2009). It was not possible to identify any variability in the COI of the *Corbicula* clams sampled (Table 1). The low genetic variability found in the COI led us to select only few specimens for amplification of the microsatellites molecular markers as previous studies (Pigneur et al. 2014).

Microsatellite analysis

South American specimens of *Corbicula* were genotyped using ten loci of microsatellite nuclear markers developed by Pigneur et al. (2011a): ClA01, ClA02, ClA03, ClB03, ClB11, ClC01, ClC12, ClD06, ClE01 and ClD12. For each locus, the amplification was performed following the protocol of Pigneur et al. (2011a; 2014). The fragments were analyzed on ABI 3130XL Genetic Analyzer with GeneScan-500 (LIZ) size standard (Applied Biosystems). Subsequently, the raw data was visualized and scored using GENEMAPPER (Applied Biosystems). Then, the results were checked in MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to estimate stutter and scoring errors, and the proportion of null alleles at each locus.

The genetic diversity indices were also measured, using *adegenet* R package (Jombart 2008) in R software version 2.15.2 (R development Core Team, 2008), including the allelic richness (*A*) mean per locus and, per population, observed (H_o) and expected (H_E) heterozygosity with each *Corbicula* population sampled and including those diversity from invasive *Corbicula* lineages from Pigneur et al. (2014b). In addition, genetic differentiation between all *Corbicula* lineages detected in this study was computed through mean pairwise F_{ST} (Weir & Cockerman 1984) and, the Hill & Robertson (1984) indirect gene flow estimative was also calculated, once it is possible to detect gene flow between populations/lineages; these analysis were performed in GENETIX v.4.05 (Belkhir et al. 2004). Subsequently, a three-dimensional factorial correspondence analysis (3D-FCA) was also performed in GENETIX, to identify similarities between all analyzed genotypes and to cluster individuals into genetic populations**.** Additionally, a Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010) was performed to describe the diversity between pre-defined groups of observations, corresponding to all sampled populations in this study. This analysis was conducted to investigate individual genetic data and to identify the genetic structure among *Corbicula* populations, as proposed by Pigneur et al. (2014b). This method, implemented by the *adegenet* package in R, does not require the Hardy-Weinberg expectations or linkage equilibrium to be met. Both methods (3D-FCA and DAPC) were used because they do not assume Hardy-Weinberg Equilibrium, especially for asexual *Corbicula* lineages (see details in Pigneur et al. 2014b), which violate these rules and consequently may lead to fake clustering through this Bayesian method. The actual DAPC procedure consists of two steps. First, original data (*Corbicula* genotypes) are transformed (centered, in our case) and subjected to PCA. Second, the retained PCs are analyzed using Linear Discriminant Analysis based on the pre-identified populations. Preliminarily, data are grouped using k-means, a clustering algorithm that finds a given number of clusters by maximizing the variation between populations, to avoid over fitting during discrimination using DAPC. The optimal number of principal components was estimated using the *optim.a.score* function in *adegenet*. K-means clustering was conducted with all 30 principal components to sort samples into prior groups. Clustering solutions for different k values are compared calculating Bayesian Information Criterion (BIC) using the *find.clusters* between 1 to 20 clusters, if any of these populations were different from each

other. Thus, this procedure recognizes closely related genotypes which are clustered based on similarity; those specimens with mixed genotypes can also be detected by this method. In all analyses, our results were compared with those published by Pigneur et al. (2014b); thus, the following genotypes of the invasive *Corbicula* lineages were included in the analyses: *C. sp.* form A/R, *C. sp*. form B, *C. sp*. form C/S and *C. sp*. form Rlc (Table 1).

Morphological analysis

Qualitative and quantitative data were acquired from the right valve of each collected specimen, totalizing 236 individuals. From these data, geometric morphometric analyzes were used to evaluate morphological consistency of each *Corbicula* COI haplotype and genotype found. Quantitative characteristics from the shells were collected to determine if the specimens present morphological variability as previously detected by Bagatini et al. (2005) and, if the morphology of hybrid specimens present intermediate characteristics, as reported by Lee et al. (2005).

Geometric morphometric analyses based on two-dimensional (2D) anatomical landmarks were performed using 11 internal homologous points, following Sousa et al. (2007) (Figure 1). The coordinates of each landmark were obtained, three times to each individual to decrease the measurement errors, using tpsDig2 software (Rohlf 2006). The first landmark was the point of junction of the anterior adductor scar with the mantle and the shell. Landmarks 2-3 represent the length of the anterior lateral tooth. Landmarks 4-7 represent the cardinal tooth. Landmarks 8-9 represent the length of the hinge. Landmarks 9-10 represent the posterior lateral tooth and landmark 11 was located at the junction point between the posterior adductor scar with the mantle and shell. Thus, landmarks 11-1 represent the distance between the adductor scars.

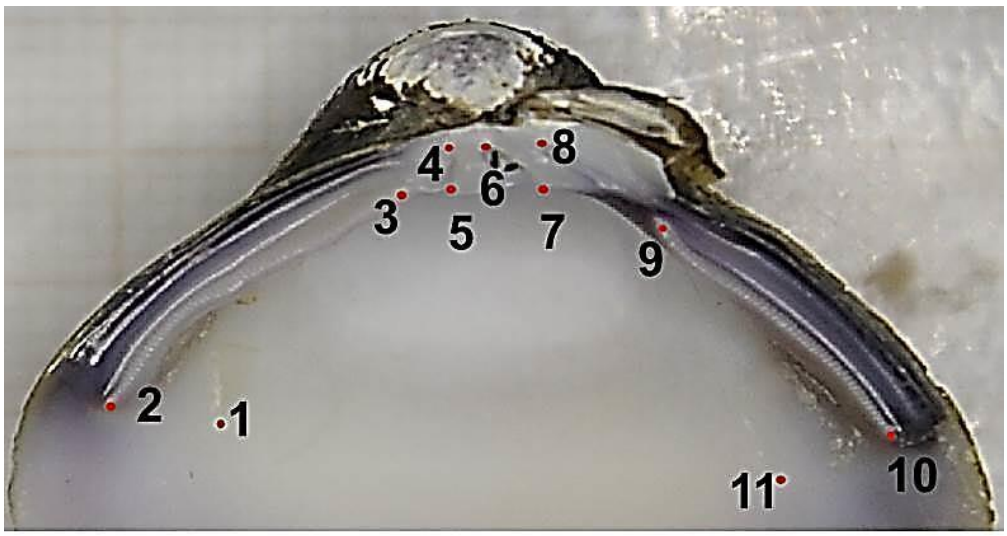

Figure 1. *Corbicula* specimen with the 11 landmarks pointed that were used to geometric morphometric analysis.

Furthermore, shape variables generated from the x, y coordinates with the effects of any differences in translation, rotation, and scale mathematically held constant were considered. These variables were used to construct a matrix for subsequent statistical analysis and to generate a graphical representation. The morphological variation among samples was examined through principal component analysis (PCA). In order to assess the variation among *Corbicula* COI haplotypes and genotypes within populations, a Canonical Variate Analysis (CVA) was used. For both analysis, *Corbicula* lineages were assigned by color according to their respective mtDNA COI haplotypes detected through Bayesian phylogeny (Fig. 2), and according to *Corbicula* genotypes assigned according to the results of the 3D-FCA and DAPC (Fig. 3; Fig.4). Thus, it was possible to check if there is significant morphological variation between the mtDNA COI haplotypes, and to check if the intermediate morphotypes found could indicate hybrids specimens from the original invasive *Corbicula* lineages. This was done because, according to Pigneur et al. (2011), when individual exhibit a mitochondrial/morphotype mismatch, the nuclear genotype is congruent with their morphotype. Thus, as Lee et al. (2005), we expected to find specimens with different mtDNA COI haplotypes, intermediate morphotype and "mixed" genotypes. Furthermore, the generalized Procrustes ANOVA analysis (GPA) algorithm (Dryden & Mardia 1998) was

performed to test for differences among mtDNA COI lineages and nDNA genotypes. In addition, a Regression analyses was performed to estimate the correctly attribution of each specimen to it's respectively lineage. All geometric morphometric analyses were performed in the MorphoJ software (Klingenberg, 2011) and the statistical analysis were perfomed using PAST 2.16 (Hammer et al. 2001).

Results

Phylogenetic relationship and Genetic diversity

Our alignment consisted of 283 sequences COI sequences, each of 500 bp long. Of these, 47 sequences were obtained from GenBank (Supporting Information 2) and 236 were sequenced herein. Phylogenetic analyses revealed the presence of two lineages of *Corbicula* and were identified based on their haplotypes, which are not unique to the South American continent: FW5 and FW17. Based on nomenclature used by Pigneur et al. (2014b), the lineages are *C.* sp. form A/R and *C.* sp. form C/S. The haplotype FW5 is diagnostic for *C.* sp. form A/R lineage and was found in ARG2, CLM, IGU, JAC, GO, RJ, BAR, MAT, IMI, ITU, ITA, ROS and GUA populations (see Table 1 for abbreviations). Haplotype FW17 is diagnostic for *C.* sp. form C/S lineage and was found in ARG1 and PP populations. A third haplotype was found in PU specimens, called in this study as FWBra1 (GenBank accession number: xxxx). Based on the interspecific Bayesian relationships of COI haplotypes, FWBra1, is closely related to C2 haplotype (Fig. 2). However, FWBra1 is not a representative of any known *Corbicula* lineage, thus, it is called *C. sp*. FWBra1 in this study.

South American samples of *Corbicula* clams show somewhat reduced levels of genetic variability (mean H_o=0.31-0.64) compared to those reported to invasive lineages for North American (0.5-0.56) and European (0.38-0.58) populations (Pigneur et al. 2014b). Mean allelic richness (A) of *C.* sp. form A/R (1.31-1.6) was lower than *C.* sp. form C/S (1.51-1.58). Mean H_E in *C.* sp. form A/R ranged from 0.25 to 0.33 and from 0.34 to 0.41 in Form C/S. In nine sites, where *C.* sp. form A/R was identified, the mean A (A=1.31), mean H_e (H_e =0.25), and mean H_o (H_o=0.50) were the same/equal. However, the genetic diversity was slightly higher in ARG2 (A=1.35, H_E=0.33, H_o=0.55) and IGU (A=1.6, H_E=0.28, H_o=0.55), that were also

identified as *C.* sp. form A/R lineage. In ARG1 and PP *C.* sp. form C/S specimens, the genetic diversity was slightly higher than Form A/R (Table 1).

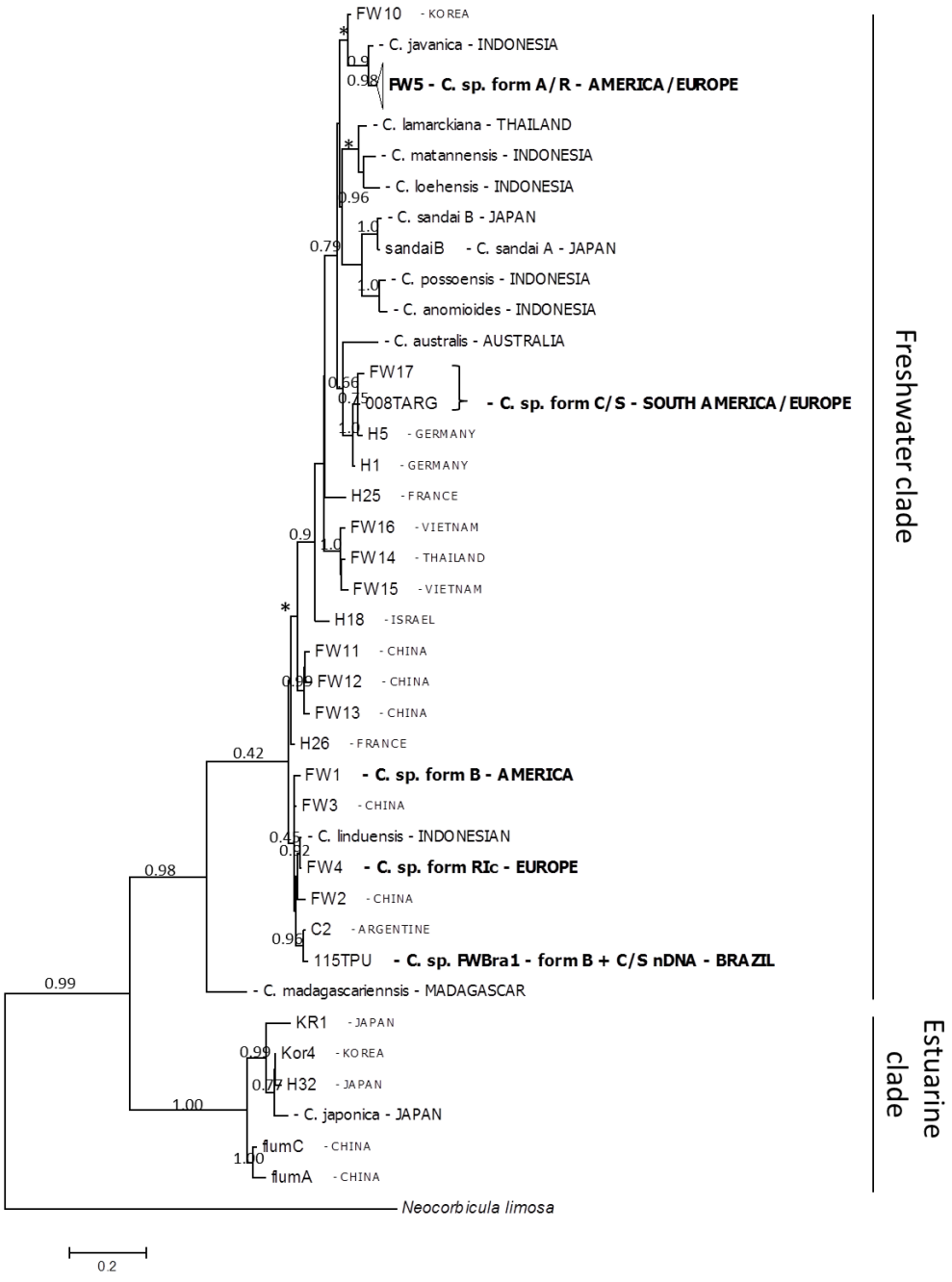

Figure 2. Bayesian inference trees based on a 500 bp fragment of mtDNA COI haplotypes from estuarine and freshwater *Corbicula* spp. with their respective occurrence region. Bayesian posterior probabilities are indicated

at branch length, and the asteristic symbol indicates the lowest posterior probabilities (≤0.40). The invasive *Corbicula* lineages clade are indicated in bold as *C.* sp. form A/R, *C.* sp. form C/S, *C.* sp. form B and *C.* sp. form Rlc and their COI haplotypes FW5, FW17, FW1 and FW4 respectively. Origin and GenBank accession numbers of sequences are presented in Supporting Information 2. The FWBra1 lineage indicated in bold indicates the cytonuclear mismatche (mtDNA vs nDNA) detected in PU specimens. Sequence of *Neocorbicula limosa* was used as outgroup.

Genetic structure

For all 10 polymorphic microsatellite loci, we had 11.64% of missing data in all 33 alleles detected: five alleles in ClD12 locus, four alleles in ClA01, ClA02, ClA03 and ClD06 loci, three alleles in ClC01 and ClC12 loci and two alleles in ClB03, ClB11 and ClE01 loci. As Pigneur et al. (2011a), the ClA03 locus was not possible to amplify in Form C/S specimens detected in ARG1 and PP sites.

Genetic differentiation between all *Corbicula* populations (Table 2), using polymorphic microsatellite loci, including the invasive lineages (gray lines), was strong. The highest F_{ST} values were found in PU populations when compared with others South American populations, ranging from 0.3812 to 0.6795. ARG1 and PP populations presented negative F_{ST} (-0.0242 and -0.0162 respectively) when compared with *C.* sp. form C/S (S1, Table 2) indicating that they are more genetic closely related than with other lineages. ARG1, ARG2, PU, PP and BAR and JAC populations presented high HR, when compared with other populations, indicating that in these populations there are some genetic recombination, what could indicate that in this sites there are receiving *Corbicula* propagules, especially in ARG and BAR sites which are close to port regions. However, the low HR in IGU, ITU, GUA, CLM, GO, RJ, MAT, IMI, ROS and ITA between each other, indicate that there is few or no genetic recombination; thus, this evidence reinforces the findings of Pigneur et al. (2011b; 2014b) that *C.* sp. form A/R can clonally reproduce into introduced regions.

Plotting the results of 3D-FCA with GENETIX confirmed the grouping of the 20 populations in six clusters (Fig.3). All factors combinations, 1x2, 1x3 and 2x3, well-distinguished the *C.* sp. form C/S (including S1, ARG1 and PP, blue circle), *C.* sp. form B, *C.* sp. form A/R (formed by AA1, ARG2, BAR, IGU, ITU, GUA, CLM, GO, RJ, MAT, IMI, ROS and ITA, green circle), *C.* sp. form Rlc, and JAC (pink square) and PU (yellow square) clusters (Fig.3).

Table 2. Pairwise F_{ST} per *Corbicula* populations from South America based on Weir & Cockerham (diagonal below) and Robertson & Hill effect (diagonal above) after Bonferroni corrections (p≥0.01)

	ARG2	ARG1	PU	PP	BAR	IGU	JAC	ITU	GUA	CLM	GO	RJ	MAT	IMI	ROS	ITA	AA1	AB1	S1	Rlc
ARG2		0,3928	0,4748	0,4267	0,2261	0,0085	0,3298	0,0222	0,1358	0,0222	0,0222	0,0222	0,0222	0,0222	0,2222	0,0222	0,207	0,401	0,496	0,505
ARG1	0,3928		0,3680	0,0793	0,4046	0,3556	0,5113	0,5410	0,5181	0,4244	0,4244	0,4281	0,427	0,4208	0,4244	0,4281	0,529	0,399	**-0,025**	0,218
PU	0,6156	0,3680		0,2264	0,4072	0,4191	0,4347	0,5067	0,4879	0,4992	0,4992	0,5017	0,4968	0,496	0,4991	0,5017	0,513	0,542	0,167	0,378
PP	0,5203	0,0250	0,3812		0,4743	0,3803	0,4265	0,4618	0,4321	0,4567	0,4567	0,4609	0,4591	0,4525	0,4567	0,4609	0,468	0,304	**-0,015**	0,263
BAR	0,2385	0,5327	0,6707	0,5660		0,2187	0,4500	0,2580	0,2580	0,2520	0,2520	0,2580	0,2580	0,2500	0,2518	0,2580	0,271	0,306	0,507	0,407
IGU	0,0112	0,5110	0,6084	0,5340	0,2727		0,2500	0,0714	0,0714	0,0714	0,0714	0,0714	0,0714	0,0714	0,0714	0,0714	0,202	0,311	0,350	0,396
JAC	0,4176	0,4724	0,5735	0,4982	0,6190	0,4375		0,3437	0,3437	0,3437	0,3437	0,3453	0,3438	0,3437	0,3437	0,3453	0,425	0,394	0,387	0,474
ITU	0,0175	0,4960	0,6795	0,5231	0,2778	0,1000	0,4737		0,1250	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,202	0,374	0,457	0,499
GUA	0,0960	0,4747	0,6518	0,5021	0,2778	0,1000	0,4737	0,0909		0,1250	0,1500	0,1217	0,1250	0,1250	0,1250	0,1271	0,281	0,374	0,430	0,474
CLM	0,0175	0,4971	0,6699	0,5192	0,2727	0,1000	0,4737	0,0000	0,0909		0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,191	0,374	0,458	0,494
GO	0,0175	0,4971	0,6699	0,5192	0,2727	0,1000	0,4737	0,0000	0,0909	0,0000		0,0000	0,0000	0,0000	0,0000	0,0000	0,202	0,374	0,458	0,496
RJ	0,0175	0,4971	0,6747	0,5192	0,2778	0,1000	0,4737	0,0000	0,0909	0,0000	0,0000		0,0000	0,0000	0,0000	0,0000	0,202	0,374	0,455	0,491
MAT	0,0175	0,4946	0,6699	0,5158	0,2778	0,1000	0,4737	0,0000	0,0909	0,0000	0,0000	0,0000		0,0000	0,0000	0,0000	0,202	0,368	0,450	0,491
IMI	0,0175	0,4971	0,6699	0,5192	0,2727	0,1000	0,4737	0,0000	0,0909	0,0000	0,0000	0,0000	0,0000		0,0000	0,0000	0,191	0,379	0,461	0,499
ROS	0,0175	0,4971	0,6699	0,5192	0,2778	0,1000	0,4737	0,0000	0,0909	0,0000	0,0000	0,0000	0,0000	0,0000		0,0000	0,202	0,374	0,456	0,494
ITA	0,0175	0,4971	0,6747	0,5192	0,2778	0,1000	0,4737	0,0000	0,0909	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000		0,202	0,374	0,455	0,491
AA1	0,2031	0,4779	0,6254	0,5089	0,2500	0,2857	0,5333	0,2222	0,2758	0,2195	0,2222	0,2222	0,2222	0,2222	0,2195	0,2222				
AB1	0,4058	0,2677	0,6287	0,3028	0,3448	0,4444	0,5116	0,4324	0,4324	0,4324	0,4324	0,4324	0,4286	0,4324	0,4324	0,4324				
S1	0,5611	**-0,0242**	0,1948	**-0,0162**	0,5957	0,5641	0,4324	0,5571	0,5373	0,5532	0,5532	0,5532	0,5493	0,5532	0,5532	0,5532				
Rlc	0,6418	0,4089	0,5555	0,4375	0,6666	0,6818	0,6986	0,7105	0,6986	0,7059	0,7105	0,7059	0,7059	0,7105	0,7059	0,7059				

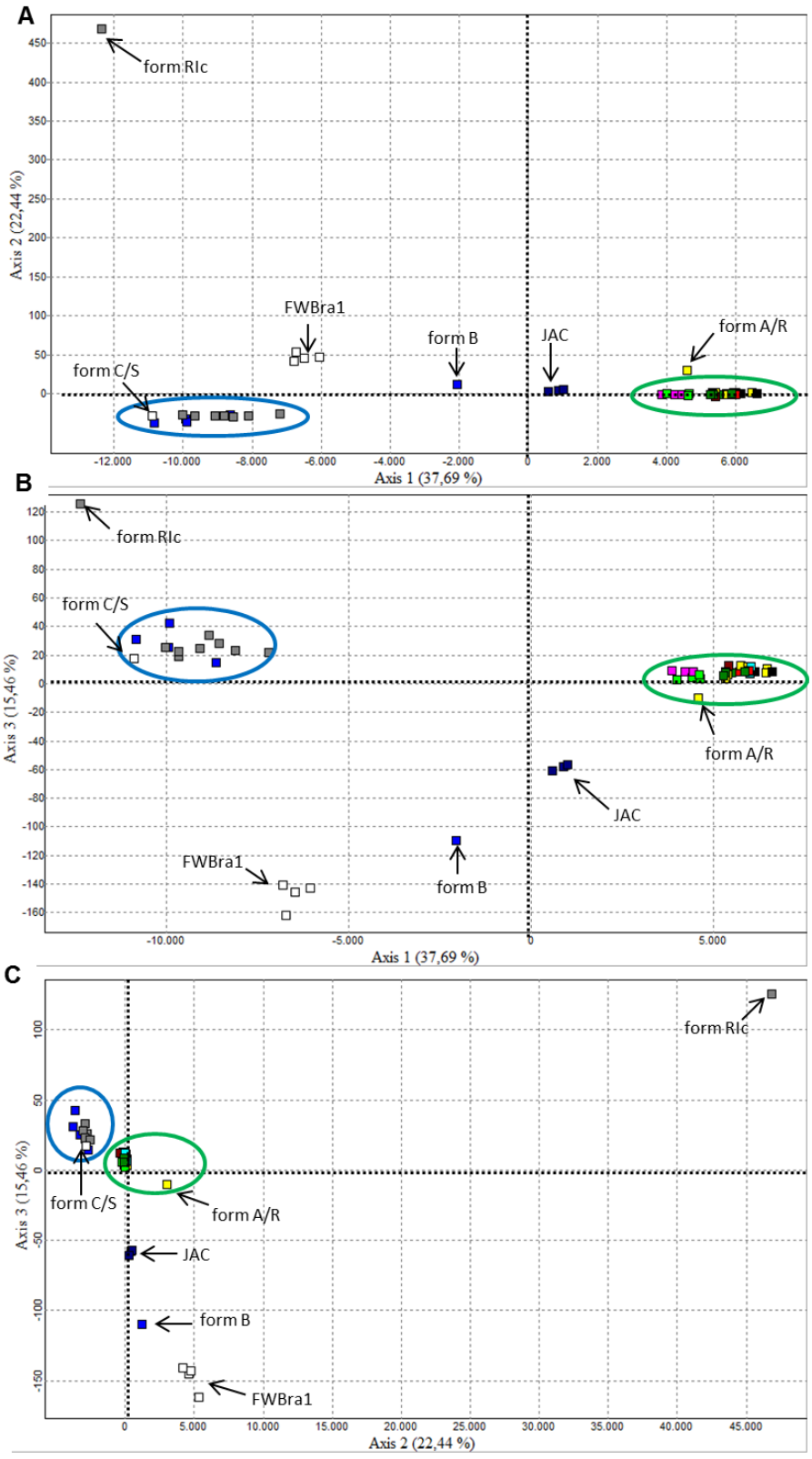
A
form RIc
FWBra1
form B
JAC
form A/R
form C/S
Axis 2 (22,44 %)
Axis 1 (37,69 %)
B
form RIc
form C/S
form A/R
JAC
FWBra1
form B
Axis 3 (15,46 %)
Axis 1 (37,69 %)
C
form RIc
form C/S
form A/R
JAC
form B
FWBra1
Axis 3 (15,46 %)
Axis 2 (22,44 %)

Figure 3. A three-dimensional factorial correspondence analyze (3D-FCA) of *Corbicula* populations from South America, showing different Axis combinations: (A) Axis 1 (36.63%) and Axis 2 (21.02%); (B) Axis 1 and Axis 3 (13.86%); and (C) Axis 2 and Axis 3. The detected invasive *Corbicula* lineages are indicated by circles: green and blue circles refer to *C.* sp. form A/R and C/S genotypes, respectively. The arrows indicate the *Corbicula* invasive lineages genotypes: *C.* sp. form A/R, *C.* sp. form B, *C.* sp. form C/S and *C.* sp. Rlc from Pigneur et al. (2014b). The others genotypes detected in this study are also indicated, FWBra1 to PU specimens and JAC specimens (see details at Supporting Information 3).

The DAPC analysis covered a range of possible clusters from 1 to 20. The lowest BIC value corresponded to $K = 6$. In the DAPC analysis, five principal components and four discriminant functions retained 97.5% of variance. The first cluster included ARG2, ITU, CLM, GO, RJ, MAT, IMI, GUA, IGU, ROS, ITA and AA1 genotypes, thus, these populations presented high genotype similarity with *C.* sp. form A/R. The second cluster included the BAR genotype and AB1, indicating high genotype similarity with *C.* sp. form B. The third cluster included ARG1, PP and S1, and presented high genotype similarity with *C.* sp. form C/S genotype. The fourth, fifth and sixth clusters included only PU, JAC and Rlc, respectively (Fig. 3), indicating different genotypes comparing with all others detected in this study. The consistency between the prior and posterior assignments was 98.2%.

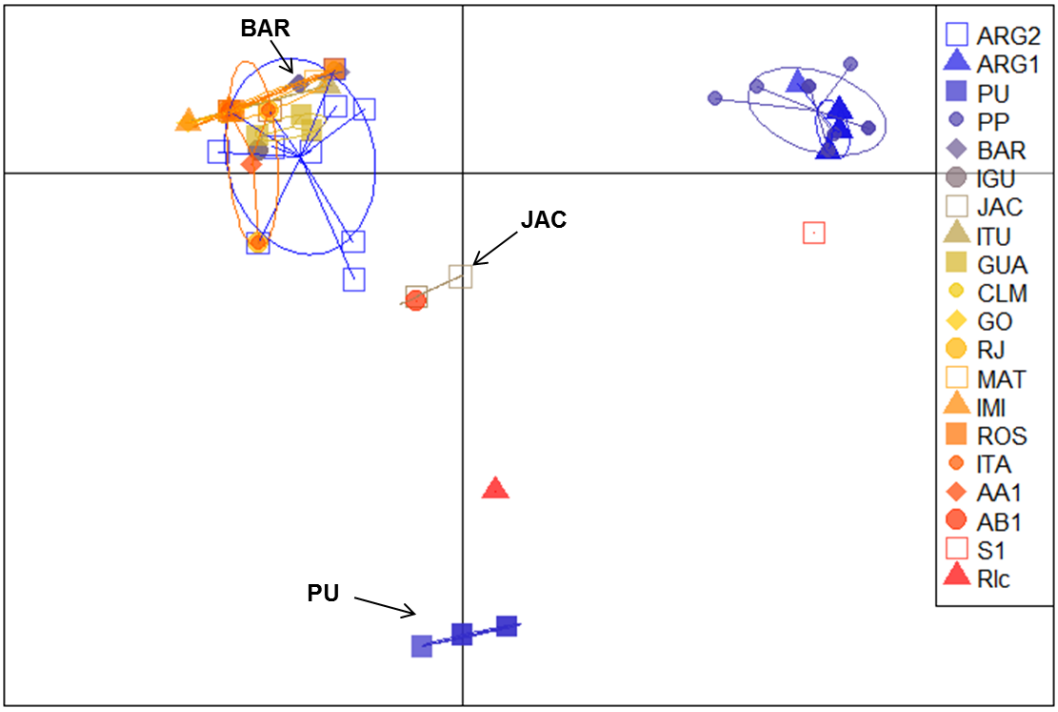

Figure 4. Population structure in *Corbicula* clams from South America based on 10 microsatellite loci**.** Scatter plot from the Discriminant Analysis of Principal Components (DAPC) based on five PCs and four

discriminant functions based on *k*-means clustering (*k*=6), with populations assigned by different symbols and colors (see legend). The arrows point to BAR, JAC and PU specimens that presented cytonuclear mismatches in this study.

The 3D-FCA and DAPC results show similarity between the *C.* sp. form C/S and A/R genotypes of the South American specimens with previous studies (Pigneur et al. 2011b and Pigneur et al. 2014b). However, both analysis diverged from BAR and PU results. The 3D-FCA indicated that BAR specimens are clustered with form A/R group (Fig. 2) while *table.value* function from DAPC clustered BAR specimens with *C.* sp. form B genotype, however, the output result (Fig. 4) indicated BAR specimens between form A/R group and *C.* sp. form B. Based on that, in this study it is attributed that BAR specimens are "mixed" populations between *C.* sp. form A/R and *C.* sp. form B. On the other hand, PU specimens clearly presented "mixed" genotype probably between form C/S and form B (Fig. 2), while *table.value* function from DAPC completely separated PU genotype from others, however, the output result indicated PU specimens very close to *C.* sp. form Rlc and very far from *C.* sp. form C/S group and *C.* sp. form B (Fig.3). Thus, when the data was analyzed once more, the allele's distribution between PU and form C/S-form B and between PU and Form Rlc, was concluded that PU specimens share more alleles with form C/S and form B than Rlc, despite the DAPC output display a divergent result.

Morphology and geometric morphometric

A total of 236 individuals were analyzed and, based on the morphotypes described by Pfenninger et al. (2002) and Pigneur et al. (2011b), we identified 200 individuals of *C.* sp. form A/R morphotype and 24 individuals of *C.* sp. form C/S morphotype. Twelve specimens of *Corbicula* presented a divergent morphotype – called herein as Intermediate Form (IF) (Table 1). The IF specimens presents ovoid shell and plan eroded umbo, closely juxtaposed concentric rings, light-purple internal color, and pallial line scar evident (Fig. 5B). *Corbicula* sp. form A/R morphotype (Fig.5A, 5D and 5E) is distinguished from the remaining by presenting a robust shell with high umbos, internally the color can be white to slightly yellowish with the marginal edge of the line pallial purple or brown, the pallial line is not evident, and rostrum may be present.

While *C.* sp. form C\S morphotype presents triangular ovate shell with juxtaposed concentric rings and, low and rounded umbos, internally the color can be dark purple; the pallial line is evident (Fig. 5C). However, IF of *Corbicula* (Fig. 5B) found in PU and JAC populations is similar to the *Corbicula* morphotype from Iguazu Falls found by Lee et al. (2005) – the shell resembles form C/S but visibly less triangular and lightly coarse and, with spaced external co-marginal rings.

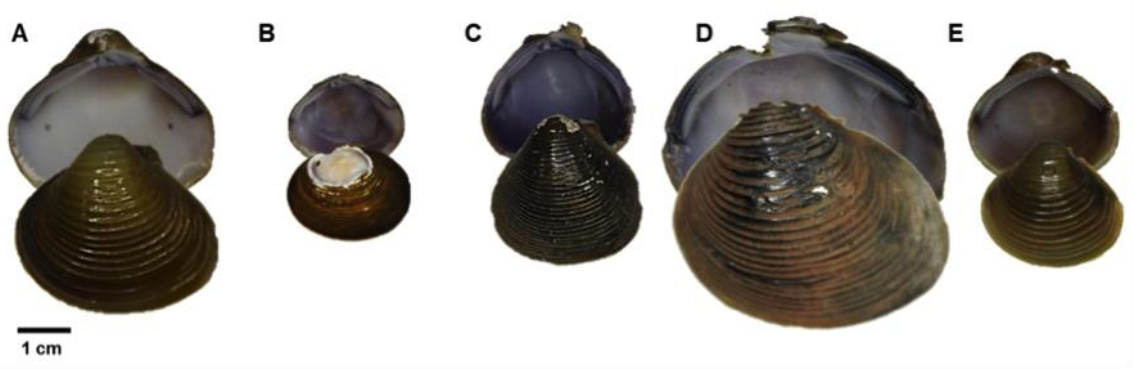

Figure 5. *Corbicula* morphotypes found in South American rivers. (A) Morphotype found in ARG2, ITU, GUA, MAT, IMI and ROS populations. (B) Morphotype found in PU and JAC populations; (C) Morphotype found in PP and ARG1 populations. (D) Morphotype found in BAR and CLM populations. (E) Morphotype found in IGU, GUA, RJ and ITA populations.

The PCA from morphometric geometric - with each mtDNA COI lineage assigned by color – (Fig. 6) resulted in two distinct vectors and described 99.7% of the total variability between specimens. The PC1 axis described 67.5% and the PC2 axis described 32.4% of the total variation. The CV1 axis account for 35.23% and the CV2 account for 31.5% of the total variation. The PCA with mtDNA COI lineages assigned showed a wide variation within *Corbicula* sp. form A/R (green dots) and was observed that the PC1 completely sepated the *C.* sp. form C/S (blue dots) morphotype from IF morphotype of PU specimens (Fig. 6A). In the other hand, the PC1 with nDNA genotypes assigned exhibited a complemented result which completely separated the *C.* sp. form C/S morphotype from IF morphotype of JAC and PU specimens and, the PC2 also exhibited the same pattern (Fig. 6C).

CVA clusters, with mtDNA COI haplotypes assigned, showed that even with a wide morphological variation within *C.* sp. form A/R group, the CV1 could distinguished between *C.* sp. form C/S morphotype, on the other hand, the CV2 completely separated the *C.* sp. FWBra1 from others mtDNA COI *Corbicula* lineages. Thus, CVA with mtDNA COI lineages assigned showed that morphotype are strictly correlated with their haplotypes (Wilk's lambda=0.082, F=33.93, P≤0.001), except for few specimens; but those did not present any mismatch between mtDNA COI/morphotype/nDNA (Fig. 6B). Furthermore, CVA with nDNA genotypes assigned yielded two distinct axes and described 92.64% of the total variability among lineages (Fig.6D). The CV1 completely separated the *C.* sp. form A/R and *C.* sp. form C/S morphotypes from JAC and PU morphotypes and, the CV2 completely separated the *C.* sp. form A/R and JAC morphotypes from *C.* sp. form C/S morphotype (Wilk's lambda=0.067, F=37.91, P≤0.001).

In addition, pairwise comparisons based on the generalized Procrustes ANOVA analyses (p-values = <0.0001) showed that based on centroid size was not significative difference between mtDNA COI lineages (df=4, F=3.24, P=0.0168), oh the other hand, based on shell shape was significative different between mtDNA COI lineages (df=72, F=6.05, P≤0.0001). In addition, the Regression analyses based on PC1 and PC2 was possible to predict correctly each specimen in 69.87% to it's respectively lineage.

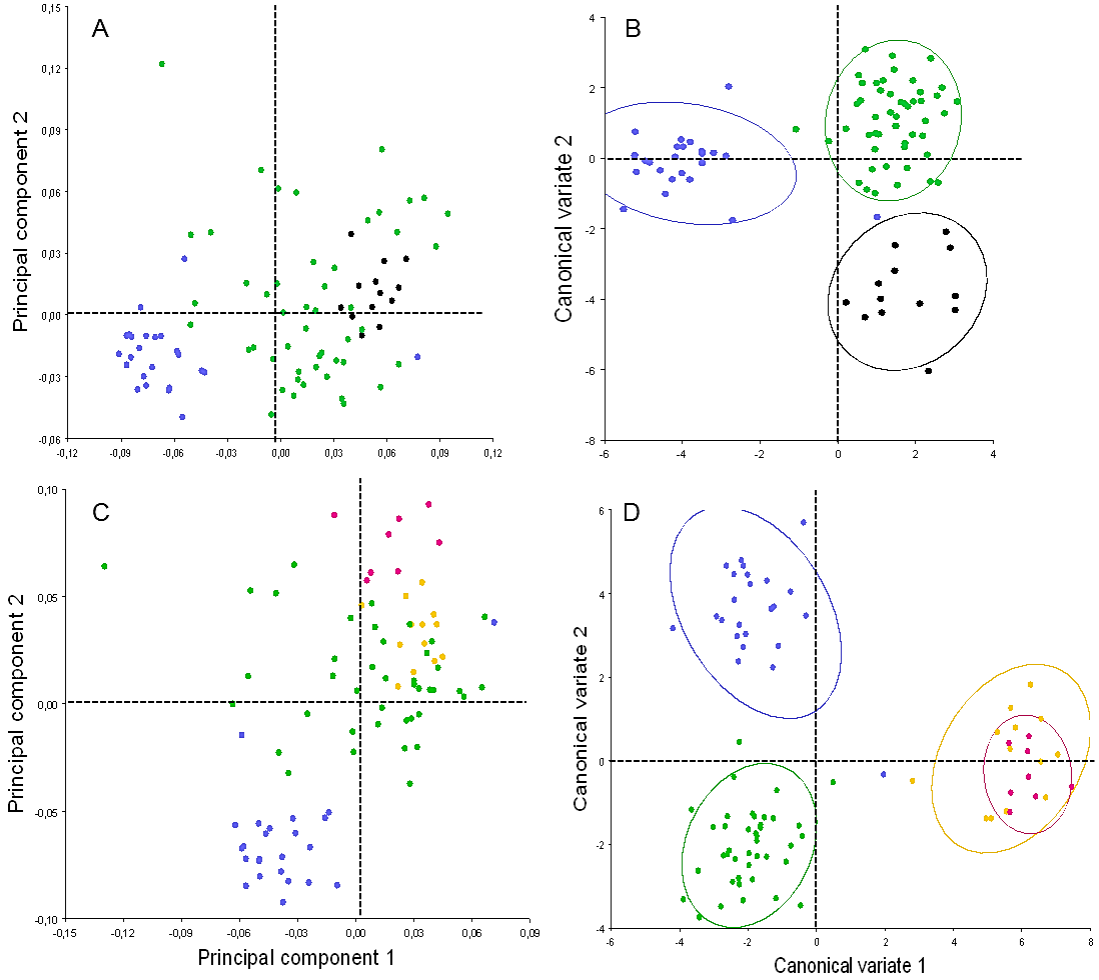

Figure 6. Geometric morphometric results: from PCA (A) and CVA (B) with mtDNA COI *Corbicula* lineages assigned through phylogenetic COI relationships: Blue dots and ellipses correspond to form C/S lineage, green dots and ellipses correspond to form A/R lineage, black dots and ellipses correspond to PU (present the FWBra1 haplotype in mtDNA COI) and JAC specimens. From PCA (C) and CVA (D) with nDNA *Corbicula* genotypes assigned through 3D-FCA: yellow dots and ellipses correspond to PU specimens and, pink dots and ellipses correspond to JAC specimens. To form C/S and A/R the colors are the same as A and B.

Discussion

Five important observations emerge from this study. First, our findings show that there are two androgenetic invasive *Corbicula* lineages, *C.* sp. form C/S and *C.* sp. form A/R, and the last one is clonally expanding its distribution in South America. Second, results presented here indicated 'mixed' populations in three distinct sites. Third, in one

of those ‘mixed’ populations, a new mtDNA COI haplotype was detected, namely FWBra1. Fourth, *Corbicula* lineages co-occur at the same site where probably “egg parasitism” happens. Fifth, in our analysis, there are positive indications of multiple introductions of *Corbicula* spp. in South America.

Corbicula lineages in South America

The present study reveals the existence of two invasive androgenetic *Corbicula* lineages established in South America: *Corbicula* sp. form A/R and *Corbicula* sp. form C/S. The *C.* sp. form C/S lineage was detected in ARG and PP sites, presenting the COI FW17 haplotype, Form C morphotype and, similar multilocus genotype (MLG) with *C.* sp. form C/S from Pigneur et al. (2014a). The South American Form C and European Form S - here this lineage is namely as *C.* sp. form C/S - was introduced almost at the same time in Argentina/Brazil (Ituarte 1994) and in Europe (Haeslopp 1992), in surroundings of 1980s. According to Pigneur et al. (2014a), most likely that propagules of *C.* sp. form C were accidently introduced in Europe from South America, since this lineage is only found in both continents and it is not found in native area.

The *Corbicula* sp. form A/R lineage detected in this study presented similar MLG to the *C.* sp. form A/R from Pigneur et al. (2014a), a dominant COI FW5 haplotype and various morphotypes. This lineage was detected in ITU, IGU, MAT, RJ, ITA, CLM, ARG2, GO, IMI, GUA and ROS sites. Besides that, within these populations, a wide varieties of morphotypes were detected. Usually, in freshwater mussels, gradual changes in shell morphology can be observed, such as *Melanoides tuberculatus* (Müller, 1774) (see Peso et al. 2011). Thus, the various morphotypes detected are likely influenced by spatial and local abiotic characteristics, as suggested for European *Corbicula* populations (e.g. Pfenninger et al. 2002; Sousa et al. 2008; Pigneur et al. 2011b). However, little is known about the influence of the local characteristics of the new invaded environment in shaping the shells of invasive *Corbicula* spp.

The low levels of variability found in both invasive lineages in South America is consistent with previous studies (Lee et al. 2005; Pigneur et al. 2014a). However, it is still possible to genetically identify different individuals (Fig. 3-4). Based on our data,

the introduced populations of *C.* sp. form A/R and form C/S from South America are less diverse genetically, relative to others invasive lineages from North America and Europe (Pigneur et al. 2014b); indeed, there is more variability between lineages than within populations. Therefore, the low genetic variability could be a consequence of successive bottlenecks during the introduction process, as was also detected for others invasive freshwater bivalves, such as the zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Gosling et al. 2008) and, the golden mussel *Limnoperna fortunei* (Dunker, 1874) in South America (Zhan et al. 2012).

Based on our data, we postulate that specimens of *Corbicula* sp. form A/R (ITU, RJ, GO, ITA, IMI, ROS, CLM and MAT sites) and form C/S (ARG1 and PP) from South America may be composed of clones (e.g. Pigneur et al. 2011b). We sustain this hypothesis based on low/absent differentiation between/within populations and, based on the fact that, each lineage are strictly correlated with solely one mtDNA haplotype and one generalist genotype (at least into the nuclear markers used in this study) (e.g. Pigneur et al. 2014b; this study). Furthermore, *C.* sp. form A/R populations presented various phenotypes with a broad environmental tolerance capacity (as was detected to Portuguese specimens by Sousa et al. 2008) and, become more plastic and generalized, reaching a wider geographical and ecological distribution (Pigneur et al. 2014b; Crespo et al. 2015). In addition, recently findings of Ludwig et al. (2014), demonstrated that *Corbicula* spp. do not always incubate early larval stages in their gills (at least in South American rivers) propitiating new pool of clones rapidly and resulting in high dispersion rates during the reproductive seasons. All these novel discoveries strongly indicate that clonal *Corbicula* spp. invasiveness are not affected by loss of genetic variability neither by natural barriers into new environment. Nevertheless, further studies should attempt to correlate the clonal fitness and invasiveness, in South American *Corbicula* sp. form A/R, and evaluate if they are potentiated by phenotypic plasticity to local environment conditions.

Connectivity and multiple introductions

The introduction process of an exotic population into new environment can give us a unique opportunity to evaluate invasion dynamics in expanding populations (e.g. Betancur-R et al. 2011). However, while our ability to identify the dispersion mechanisms during invasion process remains limited, they can orient efforts to control invasive species. According to Zhan et al. (2012), human activities have aided the propagation of invasive aquatic species in South America, even indirectly via sand transportation (which could contain benthic organisms that are dredged together with sand) and/or incrusted organisms in hulls of small boats, both methods reported for *L. fortunei* (Belz et al. 2013). However, according to Voelz et al. (1998), we cannot ignore the fact that free-swimming *Corbicula* larvae can also disperse naturally at least 1.2 km/year upstream without human aid. Thereby, dispersal mechanisms and later range expansion of *C.* sp. form A/R in South America was assessed by the recombination RH rates, which were higher between sites next to port regions, ARG-JAC and ARG-ITU. Based on historic publications, ARG site has been considered as one putative introduction point of *Corbicula* spp. in South American rivers (Ituarte 1994).

ARG site is close to international commercial ports in Argentina, which is located at mouth of Río de La Plata River estuary –one of the largest rivers in the world, into which flows Brazilian and Uruguayans rivers. The specific geographic position of this river in South America and the connectivity with tributaries of neighborhood countries grant the dissemination of new propagules into South American continental waters (e.g. Ghabooli et al. 2013). The highest RH were also found in PU-ITA and PP-ITU, what could indicate genetic recombination between them. However, this result should be carefully interpreted because specimens from PU-ITA and PP-ITU are not representatives of the same genetic lineage. The PU site is located on the Iguaçu River and the ITA site is located on Uruguay River, and are far from each other by solely 171.03 km. Further, both sites are located in different states (Paraná and Santa Catarina state, respectively), without any connection between each other since both rivers flow independently into Río de La Plata River estuary. Based on our data, ARG could represent the genetic pool source to IGU, GUA, CLM, RJ, GO, MAT, IMI, ROS and ITA populations.

In addition, the Patos Lagoon, in Southern Brazil, is also considered as another putative introduction point of *Corbicula* spp. in Brazil (Mansur et al. 2004). Based on historic publications, there are four *Corbicula* spp. living sympatrically in Patos Lagoon, *Corbicula* Form A/R, C/S, B and *C. sp*. (Mansur et al. 2012; Santos et al. 2012). In this study, in JAC and BAR sites were detected 'mixed' populations, most probably between Form A/R and Form B. JAC site is far from Patos Lagoon by 240.1 km and it is allocated at the Agudo city on Jacuí River, which flows into Patos Lagoon. In addition, BAR site is located at Barra do Ribeiro city on Guaíba Lake, while is only 87 km far; both water bodies flow directly into Patos Lagoon. Based on such proximity of those sites with the Patos Lagoon, the high RH rates in both sites indicate that there is genetic recombination between them and, most likely, the propagules are indeed coming from the Patos Lagoon.

In the far north of Brazil, PP site is located at Palmas city and ITU site is located at Itupiranga city, which are 572. 6 km far from each other but both are located at the banks of Tocantins River, which flows directly into Amazoan Delta, next to Belém port region. The Tocantins River is very important river to fluvial navigation at Northern Brazil, it cross four Brazilian states (Goiás, Tocantins, Pará and Maranhão) and, there are six hydroelectric power plants installed in its waters. Exchange of propagules of *Corbicula* spp. between/within rivers, since it was detected high recombination RH rates between those populations.

Herein, we postulate that based on our data and historic publications, multiple introductions can explain the distribution of *Corbicula* spp. in South America, once in ARG (Río de La Plata river, Argentina), second in BAR (through Patos Lagoon, Porto Alegre state, Southern Brazil) and third in ITU (through Amazoan Golf, Pará state, Northern Brazil). We sustain that a third independent introduction in Belém, which is also port region and is connected with ITU site by the Tocantins River. Based on, the fact that the Tocantins River (located in the Tocantins river basin) has no direct connection with the other basins to the South of Brazil and, most likely, PP and ITU are receiving genetic pools from another source; in this case from Amazon Delta. Nevertheless, also, we cannot rule out the hypothesis that dispersal mediated by humans

may be facilitating the propagules dispersion for the basins to the north of Brazil from south region.

Cytonuclear mismatches in 'mixed' populations

"Mixed" genotypes resulted from cytonuclear mismatches, admixture and/or hybridization invasion could spark the origin of new variants and even more successful invaders and become invasive pest species as was detected to frogs (Arano et al. 1995) and plants (Thompson 1991). In the present study, cytonuclear mismatches were detected in PU, JAC and BAR specimens. Based on our results, PU specimens - called in this study as *C.* sp. FWBra1 - presented mixed genotypes probably between form B and form C/S (likely the spermatozoon of B lineage parasitized the egg of C/S lineage), IF morphotype and unique COI haplotype, FWBra1 (Fig. 2). Interesting, JAC specimens present the same COI FW5 haplotype than *C.* sp. form A/R, but showed mixed genotypes, probably between form B and form A/R and IF morphotypes. In addition, BAR specimens also have the same COI FW5 haplotype than *C.* sp. form A/R, but with mixed genotype between form B and A/R and their shells present well-projected rostrum contrasting to the traditional description as presented by Ituarte (1981) for *Corbicula* sp. According to Hedtke et al. (2008), cytonuclear mismatches are resulted of "egg parasitism" between *Corbicula* lineages, which their offspring present paternal nuclear DNA while it keeps the maternal mitochondrial DNA. This cytonuclear mismatch has been observed in other invasive *Corbicula* populations from Europe (Pfenninger et al. 2002; Pigneur et al. 2011b), Asia (Park et al. 2002) and America (Lee et al. 2005; Hedtke et al. 2011; this study).

The detection of new COI haplotype (FWBra1) in PU specimens in this study, which is maternally inherited and for now is restricted to this population, could suggest 'hybrid' specimens. However, we aware that the cytonuclear mismatch is a distinct process of hybridization, so this finding should be carefully interpreted as representing 'hybrid' specimens.

The Hybridization-Invasion hypothesis (Ellstrand & Schierenbeck 2000) suggest that interspecific hybridization may promote invasiveness based on several

mechanisms, such as hybridization, can create novel/intermediate phenotypes relative to the parental taxa, increasing the likelihood of survival and the success of establishment in novel habitats (e.g. Rius and Darling (2014), as detected in the IF morphotype in all mixed populations of this study. Thus, the expression of new phenotypes in PU, JAC and BAR populations can also have an important paper in post colonization adaptation and niche divergence, which might allow opportunities for local adaptation that were previously inaccessible. Furthermore, cytonuclear mismatch can lead to increased genetic variation, especially in heterosis due to hybridization accompanied by mechanisms that stabilize heterotic lineage as polyploidy and/or clonal growth. Thus, the resulting hybrids may experience increased invasiveness. However, in this study the opposite was observed. Low heterozygosity was observed in all hybrid specimens probably because of their androgenetic clonal reproduction, as suggested by Facon et al. (2005) for hybrids in *M. tuberculatus*. Based on our data, none of the detected 'mixed' populations presented unique alleles, even in PU specimens which presented distinct COI haplotype, suggesting that the 'mixed' populations have recently colonized these regions. However, it is presently not possible to infer where their origins nor where the egg parasitism between both lineages occurred.

Sympatric areas

Numerous cases of cytonuclear mismatches have been documented for invasive *Corbicula* populations in which different lineages live sympatrically (Pfenninger et al. 2002; Pigneur et al. 2011b). In this study, two lineages of *Corbicula* were reported living sympatrically at the sites ARG - *Corbicula* form C/S and A/R - but no specimen presented any evidence (cytonuclear mismatches between mtDNA and nDNA) that "egg parasitism" occurred between them. However, at the BAR site, the specimens presented cytonuclear mismatches between the mtDNA (FW5 haplotype) and nDNA (probably between form B and form A/R genotypes) and their shells exhibited a well-projected rostrum. Since there are reports in literature for the presence of four *Corbicula* species (A/R, C/S, B and *C.* sp. forms) living in sympatry at this site (i.e. Guaíba Lake, Porto Alegre state, Southern Brazil) (Mansur et al. 2004), "egg parasitism" most likely exists

between these lineages, BAR specimens supports this proposal. However, more specimens should be sampled to better investigate if the "egg parasitism" also occurs between other *Corbicula* lineages in the BAR site.

The high HR recombination effect in BAR population could also result from admixture. According to Rius and Darling (2014), intraspecific genetic admixture occurs when multiple divergent genetic lineages come intogene-flow contact and interbreed, usually in sympatric zones. This can generate novel allelic combinations that can be beneficial i.e. short-term increased population fitness and increased adaptive potential. Thus, the BAR population, which is close to port regions, are most probably receiving novel genetic material of propagules from different *Corbicula* lineages and from other parts of the world. However, we recognize that the sample size in our study is limited and any interpretation requires caution.

Corbicula clams are among the best-studied and most widely introduced mollusks worldwide and, yet there is much more to learn about factors that potentiate the invasion success in South America and, somewhere else. It is vital to consider invasive *Corbicula* lineages as case-by-case due to the androgenetic clonal reproduction mode during the introduction process that may result in distinct patterns of invasion. Our study indicates that populations of invasive *Corbicula* clams were multiple introduced and are established in South America. Also, *Corbicula* sp. form A/R is clonally expanding its range in South American rivers and can present various phenotypes, thus, its identification should be done with precaution and we suggest include molecular data to properly do it.

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References

Arano B, Llorent G, Gardia-Paris M & Herrero P (1995) Species translocation menaces iberian waterfrogs. Conservation Biology, 9: 196-198.

Araújo R, Moreno D, Ramos MA (1993) The asiatic clam *Corbicula fluminea* (Muller 1774) (Bivalvia, Corbiculidae) in Europe**.** *American Malacological Bulletin* 10: 39-49.

Aristizábal MVDLH (2008) Primer registro en Colombia de *Corbicula fluminea* (Mollusca: Bivalvia: Corbiculidae), una espécie invasora. *Boletín de Investtigaciones Marinas y Costeras* 37: 197-202.

Bagatini YM, Panarari RS, Higuti J, Benedito-Cecilio E, Prioli AJ, Prioli SMAP (2005) Morphological and molecular characterization of *Corbicula* (Mollusca: Bivalvia) at Rosana Resevoir, Brazil. *Acta Scientiarum Biological Science* 27: 397-404.

Belkhir K, Borsa P, Chikhi L, Raufast N & Bonhomm F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, Université de Montpellier, Montpellier, France.

Belz CE, Darrigran G, Netto OSM, Boeger WA & Junior, PJR (2012) Analysis of four dispersion vectors in Inland Waters: The case of the invading bivalves in South America. Journal of Shellfish Research, 31: 777-784, http://dx.doi.org/10.2983/035.031.0322

Crespo D, Dolbeth M, Leston S, Sousa R and Pardal MA (2015) Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. Biol Invasions, http:dx.doi.org/10.1007/s10530-015-0862.y

Darrigran G (2002) Potential impact of filter-feeding invaders on temperate inland fresh water environments. *Biological Invasions* 4: 145-156.

Drummond AJ & Rambaut A (2007) BEAST: Bayesian eolutionary analysis by sampling trees. BMC Evol Biol, 7: 214. http://dx.doi.org/10.1186/1471-2148-7-214

Dryden IL and Mardia KV (1998) Statistical shape analysis (Vol. 4). New York: John Wiley & Sons.

Ellstrand NC & Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences, 97: 7043-7050.

Facon B, Jame P, Pointier JP & David P (2005) Hybridization and invasiveness in the freshwater snail Melanoides tuberculata: hybrid vigour is more important than increase in genetic variance. Journal of Evolutionary Biology, 18: 524-535.

Folmer O, Black M, Hoeh W et al (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294-299.

Ghabooli S, Zhan A, Sardiña P, Paolucci E, Sylvester F, Perepelizin PV et al (2013) Genetic diversity in introduced golden mussel populations corresponds to vector activity. Plos One, 8: e59328. http://dx.doi.org/10.1371/journal.pone.0059328

Gosling E, Astanei I and Was A (2008) Genetic variability in Irish populations of the invasive zebra mussel, *Dreissena polymorpha*: discordant estimates of population differentiation from allozymes and microsatellites. Fresh Biol, 53: 1303-1315. http://dx.doi.org/10.1111/j.1365-2427.2008.01965.x

Hedtke SM, Stanger-Hall K, Baker RJ and Hillis DM (2008) All-male asexuality: origin and maintenance of androgenesis in the Asian clam *Corbicula*. Evolution, 62: 1119-1136. http://dx.doi.org/10.1111/j.1558-5646.2008.00344.x

Hedtke SM, Glaubrecht M and Hillis DM (2011) Rare gene capture in predominantly androgenetic species. PNAS, 108: 9520-9524. http://dx.doi.org/10.1073/pnas.1106742108

Houki S, Yamada M, Honda T and Komaru A (2011) Origin and Possible role of males in hermaphroditic androgenetic *Corbicula* clams. Zool Scie, 28: 526-531. http://dx.doi.org/10.2108/zsj.28.526

Ituarte CF (1981) Primera noticia acerca de la introduccion de pelecipodos asiaticos en el area rioplatense**.** *Neotropica* 27:79-82.

Ituarte CF (1994) Corbicula and Neocorbicula (Bivalvia: Corbiculidae) in the Paraná, Uruguay, and Río de La Plata Basins. Nautilus, 107: 129-135.

Jombart T (2008) adegenet: a R package for the multivare analysis for genetic markers. Bioinformatics, 24: 1403-1405.

Jombart T, Devillard S and Balloux F (2010) Discriminat analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics, 11: 1-15.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resources, 11: 353-357. http://dx.doi.org/10.1111/j.1755-0998.2010.02924.x

Komaru A, Konishi K, Nakayama I, Kobayashi T, Sakai H and Kawamura K (1997) Hermaphroditic freshwater clams in the genus *Corbicula* produce non-reductional spermatozoa with somatic DNA content. Biol Bull, 193: 320-323.

Komaru A, Kumamoto A, Kato T, Ishibashi R, Obata M and Nemoto T (2006) A hypothesis of ploidy elevation by formation of a female pronucleus in the androgenetic clam *Corbicula fluminea* in the Tone River estuary, Japan. Zool Sci, 23: 529-532. http://dx.doi.org/10.2108/zsj.23.529

Konishi K, Kawamura K, Furuita H and Komaru A (1998) Spermatogenesis of the freshwater Clam *Corbicula* aff. *fluminea* Müller (Bivalvia: Corbiculidae). J Shellf Res, 17: 185-189.

Lasso CA, Martínez-Escarbassiere R, Capelo JC, Morales-Betancourt MA, Sánchez-Maya A (2009). Lista de los moluscos (Gastropoda-Bivalvia) dulceacuícolas y estuarinos de La cuenca del Orinoco (Venezuela). Biota Colombiana, 10: 63-74.

Lee CE (2002). Evolutionary genetics of invasive species. Trends Ecol Evol 17: 386–391.

Lee T, Siripattrawan S, Ituarte C and Ó Foighil D (2005) Invasion of the clonal clams: *Corbicula* lineages in the New World. Amer Malac Bull, 20: 113-122.

Librado P and Rozas (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451-1452. http://dx.doi.org/10.1093/bioinformatics/btp187

Mansur MCD, Callil CT, Cardoso FR, Ibarra JAA, Silva JSV, Souza RCCL (2004) Uma retrospectiva e mapeamento da invasão de espécies de *Corbicula* (Mollusca, Bivalvia, Veneroida, Corbiculidae) oriundas do sudeste asiático, na América do Sul. In Silva JSV and Souza RCCL, Água de lastro e bioinvasão. Interciência, Rio de Janeiro.

Mansur MCD, Santos CP, Nehrke MV (2012) Corbiculidae na América do Sul: Espécies nativas e invasoras, dispersão e a situação das pesquisas no Brasil (Mollusca: Bivalvia). In: Ecos do XIX Encontro Brasileiro de Malacologia, Rio de Janeiro, Technical Books, pp 467.

McMahon RF (1999) Invasive Characteristics of the Freshwater Bivalve, *Corbicula fluminea***.** *Lewis Publisher*: 315-343.

Park JK, Lee JS and Kim W (2002) A single mitochondrial lineage is shared by morphologically and allozymatically distinct freshwater *Corbicula* clams. Mol Cells, 14: 318-322.

Park J and Kim W (2003) Two *Corbicula* (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment. *Molecular Phylogenetics and Evolution* 29: 529-539, http://dx.doi.org/10.1016/S1055-7903(03)00138-6.

Peso JG, Pérez DC, Vogler RE (2011) The invasive snail *Melanoides tuberculata* in Argentina and Paraguay. Limnologica, Ecol and Management of Inland Waters, 41: 281-284. http://dx.doi.org/10.1016/j.limno.2010.12.001

Pfenninger M, Reinhardt F & Streit B (2002) Evidence for cryptic hybridization between different evolutionary lineages of the invasive clam genus *Corbicula* (Veneroida, Bivalvia). J Evol Biol, 15: 818-829.

Pigneur LM, Risterucci AM, Dauchot N, Li X & Van Doninck K (2011a) Development of novel microsatellite markers to identify the different invasive lineages in the Corbicula complex and to assess androgenesis. Molecular ecology resources 11: 573-577.

Pigneur LM, Marescaux J, Roland K, Etoundi E, Descy JP and VanDoninck K (2011b) Phylogeny and androgenesis in the invasive *Corbicula* clams (Bivalvia, Corbiculidae) in Western Europe. BMC Evol Biol 11: 147. http://dx.doi.org/10.1186/1471-2148-11-147

Pigneur LM, Hedtke SM, Etoundi E and Van Doninck K (2012) Androgenesis: a review through the study of the shelfish shellfish *Corbicula* spp. *Heredity* 108: 581 – 591, http://dx.doi.org/10.1038/hdy.2012.3

Pigneur LM, Etoundi E, Aldridge DC, Marescaux J, Yasuda N and Van Doninck K (2014b). Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. Mol Ecol, 23: 5102-5116. http://dx.doi.org/10.1111/mec.12912.

Pimpão DM and Martins DS (2008) Occurrence of the Asian mollusk *Corbicula fluminea* (Müller 1774) (Bivalvia: Corbiculidae) in the lower Rio Negro, Central Amazoan Region, Brazil. *Acta Amazonica* 38: 589 – 592.

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol, 25: 1253-1256. http://dx.doi.org/10.1093/mollbev/msn083

Rambaut A and Drummond AJ (2007) Tracer v1.4: MCMC tracer analyses tool. See http://tree.bio.ed.ac.uk/software/tracer/[PMCfreearticle]. PubMed.

Rambaut A (2009) FigTree v1.3.1: Tree figure drawing tool. Website: http://tree.bio.ed.ac.uk/software/figtree.

Rius M and Darling JA (2014) How important is intraspecific genetic admixture to the success of colonizing populations? Trends in Ecol & Evol, 29: 233-242. http://dx.doi.org/10.1016/j.tree.2014.02.003

Robertson A & Hill WG (1984) Deviation from Hardy-Weinberg proportions: sampling variances and use in estimation of inbreeding coefficient. Genetics, 107: 703-718.

Rohlf FJ (2006) TpsDig2 software, ver.2.10. State Univ. New York, Stony Brook.

Ronquist F & Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19: 1572-1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Santos SB, Thiengo SC, Fernandez MA et al (2012) Espécies de moluscos límnicos invasores no Brasil. In: Moluscos límnicos invasores do Brasil: biologia, prevenção e controle, Porto Alegre, Redes Editora, pp 412.

Semenas L and Flores V (2005) Presence of *Corbicula fluminea* in the Upper Negro River Basin (Patagonia, Argentina). *Journal of Freshwater Ecology* 20: 615-616.

Siripattrawan S, Park JK and Ó Foighil (2000) Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. J Mollu Stud, 66: 423-429.

Sousa R, Freire R, Rufino M, Méndez J, Gaspar M et al. (2007) Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries. Estuarine, Coastal and Shelf Science, 74: 166-174.

Sousa R, Nogueira AJA, Gaspar MB, Antunes C and Guilhermino L (2008) Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): Possible implications for ecosystem functioning. Estuarine, Coastal and Shelf Scie 80: 289-295.

Thompson JD (1991) The biology of an invasive plant. BioScience, 41: 393-401.

Van Oosterhout C, Hutchinson WF, Wills DPM and Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping erros in microsatellite data. Mol Ecol, 4: 535-538. http://dx.doi.org/10.1111/j.1471-8286.2004.00684.x

Voelz NJ, McArthur JV & Rader RB (1998) Upstream mobility of the Asiatic clam *Corbicula fluminea:* Identifying potential dispersal agents. Journal of Freshwater Ecology, 13: 39-45.

Weir BS & Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution, 1358-1370.

Zhan A, Perepelizin PV, Ghabooli S, Paolucci E, Sylvester F, Sardina P et al. (2012) Scale-dependent post-establishment spread and genetic diversity in na invading

mollusk in South America. Diversity and distributions, 18: 1042-1055. http://dx.doi.org/10.1111/j.1472-4642.2012.00894.x

Supporting Information 1. Sampling sites of *Corbicula* spp. distributed in South American hydrographic basins. The populations' names and coordinates are specified in Table 1.

Supporting information 2. mtDNA COI haplotypes of *Corbicula* spp. from GenBank utilized in this study to reconstruct the haplotype relationships phylogeny.

Haplotype	Taxon	Location	GenBank
austr	*C. australis*	Australia	AF196274
sandaiA	*C. sandai* A	Japan	AF196272
sandaiB	*C. sandai* B	Japan	AF196273
C2	*C.* sp. form C	Argentina	AF519512
FW2	*C.* sp.	China	AF457989
FW3	*C.* sp.	China	AF457990
FW7	*C. fluminea*	France	AF269094
FW8	*C.* sp.	Taiwan	AF457991
FW1	*C. fluminea*	Korea	AF196269
H26	*Corbicula* sp.	France	AY097303
H25	*Corbicula* sp.	France	AY097302
FW4	*Corbicula* sp. Form RIc	France	GU721084
FW17	*Corbicula* sp. Form C	Argentina	AF519508
H5	*Corbicula* sp.	Germany	AY097282
H1	*Corbicula* sp.	Germany	AY097262
Kor4	*C.* sp.	Korea	EU090399
japonica	*C. japonica*	Japan	AF196271
KR1	*C. japonica*	Japan	AF367440
flumA	*C. fluminalis* A	China	AF457996
FW9	*C. javanica*	Indonesia	AF457993
FW11	*C.* sp.	China	AF457994
FW14	*C. fluminea*	Thailand	AF196270
flumC	*C. fluminalis* C	China	AF457998
FW5	*Corbicula* sp. Form A	USA	AF519497
H8	*Corbicula* sp.	Germany	AY097285
FW13	*Corbicula* sp.	China	AF457999
FW12	*Corbicula* sp.	China	AF457995
FW16	*C.* sp.	Vietnam	AF468018
FW15	*C.* sp.	Vietnam	AF468017
H32	*C.* sp.	Japan	AY097312
H18	*C.* sp.	Israel	AY097295
FW10	*Corbicula* sp.	Korea	AF457992
lindu	*C. linduensis*	Indonesia	DQ285579
anomio	*C. anomioides*	Indonesia	DQ285605
posso	*C. possoensis*	Indonesia	DQ285598
mada	*C. madagascariensis*	Madagascar	AF196275
lamar	*C. lamarckiana*	Thailand	DQ285578
loeh81	*C. loehensis*	Indonesia	DQ285581

mata87	*C. matannensis*	Indonesia	DQ285587
Neocorbicula limosa	*N. limosa*	Argentina	AF196277

Supporting information 3. Microsatelite data of *Corbicula* spp. from South America. Invasive *Corbicula* MLG is indicated in gray lines.

Pop code	Spp. code	Microsatellite loci									
		A01	A02	A03	B03	B11	C01	C12	D06	E01	D12
ARG2	1	184198	000000	000000	233239	311311	175179	226226	199207	213213	274278
ARG2	2	198198	000000	192192	233239	311311	175179	226226	199207	213213	274278
ARG2	14	184198	000000	192192	233239	000000	175179	226226	199207	213213	274278
ARG2	16	184198	110114	192192	233239	311311	175179	226226	199207	213213	274278
ARG2	17	198198	110114	192192	233239	000000	175179	226226	000000	213213	274278
ARG2	20	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
ARG2	47	184198	110114	192192	233239	311311	175179	226226	000000	213213	274278
ARG2	48	198198	110114	000000	233239	311311	175179	226226	199207	213213	274278
ARG2	49	184198	110114	192192	233239	000000	175179	226226	000000	000000	274278
ARG2	50	184198	110114	000000	233239	311311	175179	226226	199207	000000	274278
ARG2	51	198198	110114	192192	233239	311311	175179	226226	199207	000000	274278
ARG1	3	198198	112116	000000	233239	311311	173175	000000	237237	209209	264274
ARG1	4	198198	112116	000000	233239	311311	173175	000000	237237	209209	264274
ARG1	6	198198	112116	000000	233239	311311	173175	000000	237237	209209	264274
ARG1	8	198198	112116	000000	233239	311313	173175	226228	237237	209209	262272
ARG1	9	198198	112116	000000	233239	311313	173175	226228	237237	209209	264274
ARG1	10	198198	112116	000000	233239	311313	173175	226228	000000	209209	262272
ARG1	11	198198	112116	000000	233239	311313	173175	226228	237237	209209	262272
ARG1	12	198198	112116	000000	233239	311313	173175	226228	237237	209209	262272
ARG1	13	198198	112116	000000	233239	311313	173175	226228	237237	209209	262272
ARG1	15	198198	112116	000000	233239	311313	173175	226228	237237	209209	264274
ARG1	18	198198	112116	000000	233239	311313	173175	226228	237237	209209	262272
ARG1	19	198198	112116	000000	233239	311313	173175	226228	237237	209209	264274
ARG1	21	198198	112116	000000	233239	311313	173175	226228	237237	209209	264274
PU	109	206206	112112	190190	233239	311313	173175	226228	237237	000000	274274
PU	111	206206	112112	190190	233239	311313	173175	226228	237237	000000	274274
PU	112	206206	112112	190190	233239	311313	173175	226226	237237	000000	274274
PU	115	206206	112112	190190	233239	311313	173175	226226	237237	000000	274274
PU	116	206206	112112	190190	000000	311313	173175	226226	237237	000000	274274
PP	156	198198	112116	000000	233239	311313	173175	226228	237237	209209	264274
PP	157	198198	112116	000000	233239	311313	173175	226228	237237	209209	000000
PP	158	198198	112116	000000	233239	311313	173175	226228	237237	209209	264274

PP	159	198198	112116	000000	233239	311313	173175	000000	237237	209209	264274
PP	160	198198	112116	000000	233239	311313	173175	000000	237237	209209	264274
PP	161	198198	112116	000000	233239	000000	173175	000000	000000	209209	264274
PP	162	198198	112116	000000	233239	000000	173175	226228	000000	209209	264274
PP	163	198198	112116	000000	233239	000000	173175	226228	000000	209209	264274
PP	164	198198	112116	000000	000000	311311	173175	226228	000000	209209	264274
PP	165	198198	112116	000000	233239	311311	173175	226228	000000	209209	264274
BAR	306	198198	110110	000000	000000	311311	173175	226226	199199	213213	274278
BAR	308	198198	110110	000000	000000	311311	173175	226226	199199	213213	274278
BAR	309	198198	110110	000000	233239	311311	173175	226226	199199	213213	274278
IGU	202	184198	000000	192192	233239	311311	175179	226226	199207	213213	274278
IGU	203	184198	000000	192192	233239	311311	175179	226226	199207	213213	274278
IGU	205	184198	000000	192192	233239	311311	175179	226226	199207	213213	274278
JAC	220	206206	112114	192192	233239	311311	175179	226226	199207	209209	274278
JAC	221	206206	112114	192192	233239	311311	175179	226226	199207	209209	274278
JAC	222	206206	112114	192192	233239	311311	175179	226226	199207	209209	000000
ITU	374	198198	110114	192192	233239	311311	175179	226226	000000	213213	000000
ITU	375	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
ITU	377	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
GUA	529	198198	110112	192192	233239	311311	175179	226226	199207	213213	274278
GUA	533	198198	110112	192192	233239	311311	175179	226226	199207	213213	000000
GUA	535	198198	110112	192192	233239	311311	175179	226226	000000	213213	274278
CLM	184	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
CLM	185	198198	110114	192192	233239	311311	000000	226226	199207	213213	274278
CLM	186	198198	110114	000000	233239	311311	175179	226226	199207	213213	274278
GO	141	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
GO	142	198198	110114	192192	000000	311311	175179	226226	199207	213213	274278
GO	143	198198	110114	192192	233239	311311	000000	226226	199207	213213	274278
RJ	281	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
RJ	285	198198	110114	192192	233239	311311	175179	226226	199207	000000	274278
RJ	286	198198	000000	192192	233239	311311	175179	226226	199207	213213	274278
MAT	349	198198	110114	192192	233239	311311	175179	000000	199207	213213	274278
MAT	350	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
MAT	351	198198	000000	192192	233239	311311	175179	226226	199207	213213	274278
IMI	440	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
IMI	441	198198	110114	192192	000000	311311	175179	226226	199207	213213	274278
IMI	442	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
ROS	523	198198	110114	192192	233239	311311	000000	226226	199207	213213	274278
ROS	524	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
ROS	525	198198	110114	000000	233239	311311	175179	226226	199207	213213	274278
ITA	488	198198	000000	192192	233239	311311	175179	226226	199207	213213	274278

ITA	489	198198	110114	192192	233239	311311	175179	226226	199207	213213	274278
ITA	490	198198	110114	192192	233239	311311	175179	226226	199207	000000	274278
AA1	AA1	198198	110114	190194	233239	311311	175179	226226	199207	213213	274278
AB1	AB1	198198	112114	188188	233239	311311	173175	226228	199211	000000	274278
S1	S1	000000	112116	000000	233239	311313	173175	226228	237237	209209	264274
Rlc4	Rlc4	196196	112116	190190	239239	311313	173175	230230	000000	000000	274274

CAPÍTULO III

Morphological and genetical insights on invasion of *Corbicula* sp. form A/R (Bivalvia, Corbiculidae) in South America

Aim Our goal was to examine the invasion genetics of *Corbicula* sp. form A/R; its population structure within continental waters, its genetic diversity compared with others invasive and native populations. This study also evaluated if its genetic pattern suggests single and/or multiple introductions, and to reconstruct, within the limitations of the available data, the invasion history of this clam in South America.
Location South America
Methods Specimens of *Corbicula* sp. form A/R from 11 locations were collected from South America rivers and analyzed in conjunction with previously published data from invasive and native populations. Genetic variation within and among groups was quantified, and the genetic structure was inferred via spatial analyses of molecular variance, Mantel and DAPC. Furthermore, dispersal patterns, gene flow, and first migrants were analyzed to improve our knowledge about dispersion during invasion process.
Results Microsatellite data of *Corbicula* sp. form A/R indicate that all South American populations reproduce clonally and present low level of genetic diversity relative to their native relatives. The low genetic variability detected in this study is most probably results from of accentuated bottleneck, genetic drift, and clonal reproduction. Besides that, first migrants were detected between native and South American populations in distinct points, indicating that there is gene flow between native and invasive range.
Main conclusions Wide distributed morphotypes were detected and probably are influenced by temperature and latitudinal scale, thus, suggesting that *Corbicula* sp. form A/R presents phenotypic plasticity. The clonal reproduction in androgenetic invasive *Corbicula* isolated them from native range, however, there is still some genetic exchange between invasive and native areas. Multiple introductions and admixture of new propagules from multiple geographic areas may propitiate the maintenance of clonal diversity of this clam in South America. Its low genetic diversity was found in all invasive *Corbicula* populations and, *C.* sp. form A/R across invasive range present similar multilocus genotypes, indicating that it may belong to metapopulation of superclones. Nevertheless, the low genetic diversity in invasive range do not affect the invasiveness of *Corbicula* sp. form A/R, at least in South American rivers.

Keywords *Corbicula*, plasticity, admixture, multiple introductions, South America, superclones.

Running title Invasion genetic of *Corbicula* sp. form A/R

[§]Artigo formatado segundo à revista Diversity and Distribution.

INTRODUCTION

From an evolutionary perspective, biological invasions are exceptional natural experiments for studying the genetic and ecological factors that shape colonization and expansion of alien invasive species (AIS) into new habitats (Betacun-R *et al*., 2011; Frish *et al.,* 2012). *Corbicula* clams are bivalve mollusks originated from Asia, Middle East, Africa and Australia and are found in estuarine and freshwater environments (Glaubrecht *et al.,* 2007; Park and Kim, 2000). Recent findings demonstrated that estuarine species, such as *Corbicula japonica* Prime, 1864, present unreduced biflagellate sperm and sexual reproduction in natural area. On the other hand, freshwater species, as *Corbicula leana* Prime, 1864, present unreduced mono-flagellate sperm and asexual reproduction (Komaru *et al.,* 1997). Usually, asexual reproduction happens when the descendants inherit a replica of one the genome from of the parents that is unchanged by recombination, and the best-known forms are parthenogenesis and androgenesis. Asexual forms are rare to find but Komaru *et al.,* (1998) demonstrated that obligate androgenesis may occur in species of *Corbicula*. Further, new insights about androgenesis in *Corbicula* were demonstrated over the years (Komaru *et al.,* 2001; Ishibashi *et al.,* 2003; Komaru *et al.,* 2006); Houki et al. 2011; Hedtke *et al.,* 2011; Pigneur *et al.,* 2012; Pigneur *et al.,* 2014a, Ludwig unpublished data).

Besides the native *Corbicula* species, there are, at least until today, four known invasive *Corbicula* lineages, which quickly spread throughout the American and European continents (Counts, 1981; Ituarte, 1981; Lee *et al.,* 2005; Araújo *et al.,* 1993). These invasive lineages most probably were accidently introduced in those continents by propagules stored in the ballast water of merchant ships, originating from the native range of the species. The discharge of ballast water favors the introduction of new propagules into the new environment. The invasive lineages are: (i) *Corbicula* sp. form B is found in American continent, presenting a well-pronounced triangle shell shape and, FW1 mtDNA haplotype; (ii) *Corbicula* sp. form C/S is found only in South America and Europe, presenting thin shell, juxtaposed growth rings and internally the palial line is evident and, FW17 mtDNA haplotype; (iii) *Corbicula* sp. form Rlc is found only in Europe, presents robust light shells whose interior is white-yellow. The last invasive clam is the most widespread across the globe, (iv) *Corbicula* sp. form A/R (the Asian clam). The Asian clam is found in North America, South America and Europe (Renard *et al.,* 2000; Siripattrawan *et al.,* 2000; Park *et al.,* 2002; Lee *et al.,* 2005; Hedtke *et al.,* 2008; Pigneur *et al.,* 2011a; Pigneur *et al.,* 2014a), which present robust shell, high umbos, spaced growth rings yellowish, internal surface, and palial line not evident. This lineage is genetically recognized by the presence of a unique FW5 mtDNA haplotype and specific multilocus genotype (MLG) (Pigneur *et al.,* 2014a).

Androgenetic lineages of *Corbicula*, in invaded areas, present clonal reproduction and low genetic variability (Pigneur *et al.,* 2014a). This peculiar reproduction mode is

thought to facilitate their establishment in recent introduced environments, especially because they can self-fertilize and a single individual can start a new population, rapidly reaching high densities (McMahon, 1999). Although androgenetic *Corbicula* clams often present low genetic variability based on mtDNA, the capacity to quickly spread into new ranges may be the result of: (i) phenotypic plasticity (e.g. Glaubrecht *et al.*, 2007), (ii) ability to occupy a wide range of niches within the freshwater domain (e.g. Sousa *et al.*, 2008), (iii) tolerance to waters with low salinity (e.g. McMahon, 1999) (iv) rapid population growth and short life span (e.g. McMahon, 2002) and, (v) multiple reproductive periods (e.g. Gatlin *et al.*, 2012). Once established and in expansion, several studies have documented that *Corbicula* clams can impact the introduced environment increasing the oxygen uptake and affect the fluxes of nutrient across the water interface (Zhang *et al.*, 2011). *Corbicula* spp. can also impact the organic matter dynamics in sandy streams (Hakenkamp and Palmer, 1999) and directly impact the phytoplankton abundance in rivers (Strayer *et al.*, 2008; Pigneur *et al.*, 2014b). Despite of the fact that the invasive *Corbicula* sp. form A/R can cause ecological and/or economic impacts, it also provides an ideal model to investigate evolutionary questions, as a natural experiment in evolution by the scientific community (Sax and Brown, 2001; Keller and Taylor, 2010; Cristescu 2015).

In South America, *Corbicula* clams were probably introduced around 1975 in the Río de La Plata River in Argentina (Ituarte, 1981), the Patos Lagoon, southern of Brazil (Veitenheimer-Mendes, 1981), and Venezuela around 1985 (Martinez, 1987). After that, the number of occurrences increased and recently findings demonstrated that these clams are already established and expanding their range into additional South American rivers (Ludwig unpublished data). Previous studies demonstrated the presence of two androgenetic invasive lineages in South America (Lee *et al.*, 2005; Ludwig unpublished data), *C.* sp. form A/R and *C.* sp. form C/S. Further, Ludwig (unpublished data) identified hybrids specimens in three Brazilian populations, with each population associated with solely one mtDNA haplotype, various morphotypes, and genotypes "mixed" between invasive lineages. In that study, it was postulated that multiple introductions might explain the actual distribution of *Corbicula* clams in South America; however, the invasion history of these clams were not assessed.

To improve our knowledge about the *Corbicula* invasion and colonization processes into South America, it is essential to study its evolutionary history (e.g. Cristescu 2015). Patterns of genetic variation have proven indispensable for determining the likely source of founder population (e.g. Keller and Taylor, 2010). However, studying evolutionary history of *Corbicula* clams can be a challenge. According to Hedtke *et al.*, (2011), androgenetic *Corbicula* clams present mitochondrial and nuclear capture or "egg parasitism". "Egg parasitism" happens when the unreduced sperm from

one genetic lineage fertilize the egg of another lineage. During the fertilization process, the maternal nDNA of the egg is extruded, resulting a descendant with maternal mtDNA of one lineage although presents paternal nDNA of the other lineage (Hedtke *et al.*, 2008). As a consequence, "egg parasitism" allows admixture of distinct nuclear genomes when the maternal nDNA is partly extruded (Komaru *et al.*, 2006). This cytonuclear mismatch has been detected in North American, European and South American specimens (Lee *et al.*, 2005; Pigneur *et al.*, 2011; Ludwig unpublished data). This peculiarity makes it more difficult to understand the phylogenetic relationships between mtDNA haplotypes, since it only show the maternal inheritance. Thus, with genotype data it is also possible to understand the evolutionary history of these clams; otherwise, it makes them more complex and intriguing. Although, identifying source populations is challenging especially to *Corbicula* clams, genetic characteristics of these populations can provide helpful insights into whether or not invasive populations representatively capture the diversity of native populations (e.g. Darling *et al.*, 2008). Thus, understanding the ecological parameters of source propagule can elucidate the invasive organism's capacity for range expansion (e.g. Zhan et al. 2012). Thus, this study aimed to improve the knowledge on the invasion genetics of *C.* sp. form A/R using highly variable molecular markers (i.e. microsatellites) in South America, testing hypotheses about the number of successful invasion events, dispersion, and morphological plasticity.

METHODS

Sampling preservation, mtDNA COI amplification and genotyping

In Brazilian and Argentine rivers, 200 *Corbicula* specimens were collected (Fig. 1) (Table 1). *Corbicula* specimens were preserved in 96% ethanol and the shell of each one was preserved dry. In laboratory, the shells were separated from the soft tissue to genetic analysis. Each specimen had the right shell photographed with Canon EOS Rebel T3 digital camera and, the image was used for morphological analyses. Total genomic DNA was isolated from the mantle tissue using EZ-DNA kit (Biosystems, Brazil). Gonadal tissues were specifically avoided to prevent comparisons of no orthologous sequences due to the presence of androgenetic *Corbicula* lineage (Ludwig unpublished data).

Mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified from all 500 specimens 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, 1x Reaction buffer, 200 µM of dNTPs, 0.5 µM of both primers and 1 µL of Taq DNA polymerase (Life Technologies). PCR conditions

were: 5 min at 95°C followed by 35 cycles of 30s at 94°C, 30s at 44°C and 40s at 72°C, and then a final extension of 5 min at 72°C. Amplified fragments (both strands) were sequenced in an Applied Biosystems 3130 automatic sequencer using the same amplification primers. Sequences were assembled, edited and a consensus was generated using Geneious® 6.1.2 (Biomatters; Available at http://www.geneious.com/). The consensus sequences of the individuals were compared to reference sequences in GenBank to identify its haplotypes. Thus, all COI sequences of *Corbicula* specimens of this study matched with FW5 haplotype (access number: AF519497). According to Pigneur et al. (2011; 2014), mtDNA COI FW5 haplotype is strictly correlated to clones of *Corbicula* sp. form A/R in invaded area.

Since, the *Corbicula* sp. form A/R specimens of this study were recognized as clones, based on mtDNA and nDNA, only few individuals of each population were chosen to be genotyped (Table 1), assuming that low genetic variability would be detected among sampled populations. To genotype the specimens, 10 polymorphic microsatellite markers were used from Pigneur et al. (2011): C1A01, C1A02, C1A03, C1B03, C1C01, C1C12, C1D06, C1E01 and C1D12. PCR reactions were performed separately for each microsatellite locus and the amplification was performed in 25µL total volume including 1 µl of gDNA, 1x GoTaq reaction buffer (Promega), 2mM of dNTPs (Promega), 20 of µM of both primers and 0.1 U of GoTaq DNA polymerase (Promega). The PCR cycling conditions was performed following Pigneur et al. (2014b). PCR products were eletrophoretically separated on ABI 3130XL Genetic Analyzer with GeneScan-500 (LIZ) size standard (Applied Biosystem), and allele sizes were visualized and scored using the PeackScanner Software v1.0 (Applied Biosystems) and GENEMAPPER (Applied Biosystems). Subsequently, the results were check at MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.,* 2004) to estimate stutter errors, and the proportion of null alleles at each locus. Based on previous studies (Pigneur *et al.*, 2014a; Ludwig unpublished data, Chapter II), is expected low genetic variability across *Corbicula* clams.

Table 1. Sampling details and genetic diversity indices for ten microsatellite markers for the Corbicula Form A/R clams in South America. N microsat =sample size for ten microssatelite loci in different populations. NA = information not available.

Region/ ID	Location/City/State	Coordinates (decimal)	Morphotype[1]	COI lineage[2]	N microsat	Genotype
(a) South American (AS)						
Argentina						
ARG2	Río de la Plata, La Plata, Bs.	34,916 -57,950	A/R	A/R	11	A/R
Brasil						
CLM	Capitao leonidas Marques, PR	25,543 -53,49	A/R	A/R	3	A/R
IGU	Prainha 3 lagoas, Foz do Iguaçu, PR	25,446 -54,504	A/R	A/R	3	A/R
GO	Rio Claro, Jataí, GO	17,95 -51,72	A/R	A/R	3	A/R
RJ	Cabiunas, Silva Jardim, RJ Rio Guandu, Nova iguaçu, RJ	22,84 -43,6	A/R	A/R	3	A/R
MAT	Arroio Tovoraipi, Mata, RS	29,579 -54,42	A/R	A/R	3	A/R
IMI	Rio Iguatemi, Iguatemi, MS	23,73 -54,55	A/R	A/R	3	A/R
ITU	Pedral do Tauri, Itupiranga, PA	5,133 -49,166	A/R	A/R	3	A/R
ITA	UHE Itá, Itá, SC	27,26 -52,36	A/R	A/R	3	A/R
ROS	Rio do Corvo, Rosana, SP	22,35 -52,7	A/R	A/R	3	A/R
GUA	Rio Paraná, Guaíra, PR	24,066 -54,25	A/R	A/R	3	A/R
	Mean Alellic richness in AS populations A= 1.34					
	Mean expected heterozygosity He= 0.26					
	Mean observed heterozygosity Ho= 0.49					
	Mean inbreeding index *F*is= -0.5109					
(b) Invasive linheages (IN)						
Code[3]						
C. sp. Form A/R (AA1)	North American form A and European Form R	30,633 -97,684	A/R	A/R	3	A/R
C. sp. Form B (AB1)	North American Form B	30,633 -97,684	B	B	3	B
C. sp. Form C/S (S1)	South American form C and European form S	47,561 -7,632	C/S	C/S	3	C/S
C. sp. (Hw)	North America Hawaii	19,068 -155,765	I	I	3	Hw
C. sp. Form Rlc (Rlc4)	European form Rlc	43,956 -4,2719	Rlc	Rlc	3	Rlc
	Mean Alellic richness in IN populations A= 1.73					
	Mean expected heterozygosity He= 0.275					
	Mean observed heterozygosity Ho= 0.42					

		Mean inbreeding index *F*is= -0.5305				
(c) NAT						
Native lineages/code[3]						
C. sp. (Fu)	China	22,285 -114,157	NA	Fu	3	Fu
C. sp. (BA)	China	22,285 -114,157	NA	BA	3	BA
C. sp. (Vt)	Vietnam	21,024 -105,841	NA	Vt	3	Vt
C. sp. (CR')	Vietnam	21,024 -139,69	NA	CR'	3	CR'
C.leana (KMT)	Japan	35,689 -139,69	NA	KMT	3	KMT
C.sp.(EHM)	Japan	33,784 -32,861	NA	EHM	3	EHM
C. japonica (Jp)	Asia	35,689 -139,69	NA	Jp	3	Jp
C. sandai (CS)	Asia	35,156 -135,943	NA	CS	3	CS
C. fluminalis africana (ZA)	Africa	20,688 -27,098	NA	ZA	3	ZA
		Mean Alellic richness in NAT populations A= 2.84				
		Mean expected heterozygosity He= 0.411				
		Mean observed heterozygosity Ho= 0.432				
		Mean inbreeding index *F*is= -0.5573				

[1]Grouping resulted from Geometric Morphometric analysis assigned following Ludwig (unpublished data, Chapter II). [2] Lineage resulted from Bayesian phylogeny (Ludwig unpublished data, Chapter II). [3] Genetic information of *Corbicula* lineages (invasive and native areas) from Pigneur et al. (2014b).

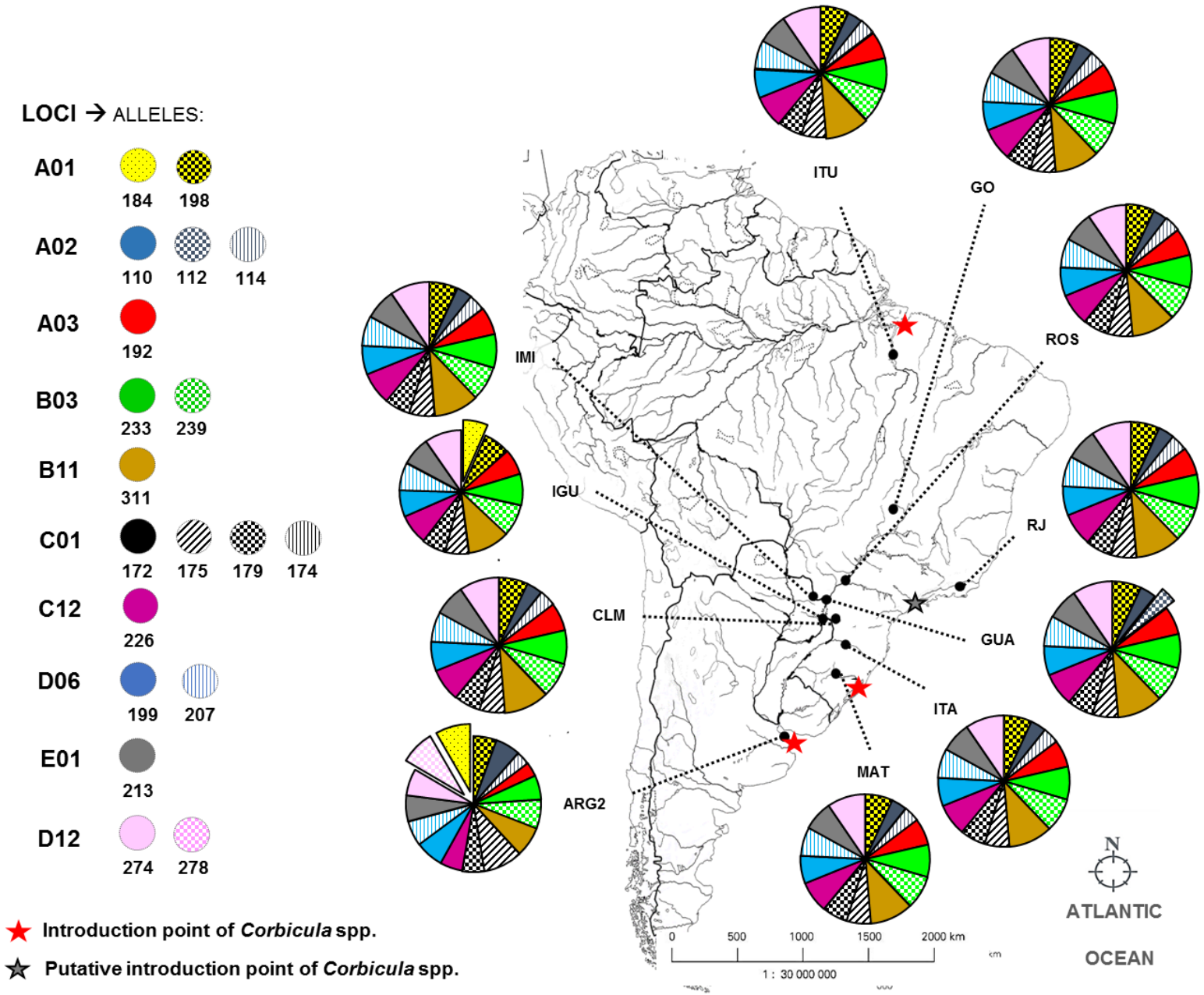

Figure 1. Distribution of *Corbicula* populations in South American rivers and their respective alleles. Rare alleles are highlighted. Introduction points of *Corbicula* spp. in South America are assigned with red star symbol and, putative introduction point is assigned with gray star symbol. (Details in Supporting Information 1).

Morphological analyses

A dataset comprising 500 pictures, from each specimen previously identified as *C.* sp. form A/R, was analyzed based on 3 linear measures, following instructions of Sousa *et al.*, (2007). Linear distances from each individual were measured (three times for each individual and media was done after that to decrease the possible errors) per site for its maximum length (L), height (H), and width (W) using a digital caliper (resolution of 0.01 mm). To standardize the variables for size, we calculated the height/length, (L/H) length/width (L/W), and width/height (H/W) ratios for all specimens and populations. Subsequently, mean annual temperature of each site was compiled through INMET (Instituto Nacional de Meteorologia, www.inmet.gov.br/portal/) and literature (Rodrigues et al. 2004; Vilanova et al. 2005; Fisch et al. 2006; Blain 2010). The abiotic variable was used to infer if the morphological variation is correlated with changing temperatures along latitudinal scale.

Subsequently, a Principal Components Analysis (PCA) was performed to simplify descriptions of morphological variation between *C.* sp. form A/R South American specimens through var-covar matrix. The collected linear data were corrected for size and rotational distances between the marks of each individual whilst keeping the relation of the marks for each individual constant. Subsequently, Canonical Variate Analyses (CVA) and MANOVA were assessed to estimate morphological variation among populations, through Hotelling's p-values with sequential Bonferroni significance. In addition, a Regression Analysis was also performed to estimate the correlation of principal components of morphological variation found in *C.* sp. form A/R with temperature variations and latitude distribution. In addition, a pairwised matrix with Procrustes distances was calculated for subsequent Mantel correlation test. All statistical analyses were performed using the PAST software package (Hammer *et al.*, 2001).

Genetic diversity, population differentiation, and structure

Mean genetic diversity of South American *C.* sp. form A/R populations was estimated using *adegenet* package (Jombart, 2008) in R software version 2.15.2 (R Development Core Team, 2011), which included mean of allelic richness (A) per locus and per population, and observed (H_o) and expected (H_E) heterozygosis in all populations.

To estimate population differentiation and structure of *C.* sp. form A/R from South America region, two sets of analysis were performed comparing within South American populations (SA) and comparing with their counterparts from invasive (IN) and native (NAT) populations (Table 1) (that were identified by Pigneur *et al.*, 2014). First, a membership probability assignment was performed between SA, IN and NAT populations to identify spatial structure and possible admixture event between them. To identify spatial structure in *Corbicula* spp. populations an exploratory Discriminant Analysis of Principal Components

(DAPC) was assessed using the *adegenet* package (Jombart, 2008) for software R (R Development Core Team, 2011). This analyze was performed without prior information on individual populations. Whenever group priors were unknown, the number of clusters was assessed using the *find.clusters* function, which runs successive *K*-means clustering with increasing number of clusters (*k*). For selecting the optimal number of clusters, it was applied the Bayesian Information Criterion (BIC) for assessing the best-supported model, and therefore the number and nature of clusters, as recommend by Jombart *et al.*, (2010). Thus, the membership probability assignment was computed through *compoplot* function based on previous DAPC priors, without prior information of sampling location. Second, a Principal Coordinates Analysis (PCoA) was conducted comparing the alleles' distribution across SA populations with IN and NAT at individual and population level, and were clustered based on DAPC analyses, using the GenAlex 6.5 (Peakall & Smouse 2012). PCoA is a multivariate statistical analysis that uses summarized genetic distances between individual multi-locus genotypes to cluster individuals relative to each other in a multidimensional space, without the assumptions of Hardy Weinberg neither Linkage disequilibrium (since androgenetic clonal *Corbicula* spp. do not fit to those priors).

Correlations test

Mantel test (Mantel, 1967) was applied correlating pairwise population comparisons at geographic and morphological distances to examine the effect of landscape features on morphological structure within SA populations. For geographic distance, the closest linear distance between pairs of populations was calculated (for those sites for which coordinates were not available in Pigneur *et al.*, 2014a, the capital of the country was used as reference as coordinates reference, see Table 1), estimated using Google Earth (http://earth.google.com) and, the data was transformed in log using GenAlex. For morphological distances, the Procrustes distance between populations from PCA using the 3 linear measures was calculated, as described previously. A multivariate analysis of variance (MANOVA) was performed through estimation of coefficient of correlation (R^2) and *p*-values (for 10.000 permutations) to estimate the correlation between all 3 morphological rations and mean temperature for each sampled site with latitudinal scale, using PAST.

First-generation of migrants

The number of first-generation migrants into each location was assessed using the Bayesian assignment method by Rannala & Mountain (1997) implemented in GENECLASS2 (Piry *et al.*, 2004). This method computes the probability that multilocus genotype (MLG) of each individual will be encountered in a given population and is a more appropriate test when population differentiation is low and loci deviate from Hardy-Weinberg equilibrium (Rannala

& Mountain, 1997). For the analysis, the database included only 4 loci (ClB03, ClB11, ClC01, ClC12) that amplified in more than 90% of the total individuals (total: 77 individuals). The statistical criterion computed for likelihood estimation was $L_{HOME}/L_{MAX\_NOT\_HOME}$, as it was considered that the ratio L_{HOME} to the highest likelihood value among all population samples excluding the population where the individual was sampled $L_{MAX\_NOT\_HOME}$ (Piry *et al.*, 2004). In other words, we wanted to identify the individual whose genotype is excluded from the site population in which it was captured with the most likely source population /region of each migrant. For the probability of computation, it was combined the Monte Carlo resampling procedure of Paetkau *et al.,* (2004) with the likelihood criteria of Rannala & Mountain (1997) with 10.000 simulated individuals and α=0.05 as error estimative.

RESULTS

Morphological variation and Mantel test

We analyzed a total of 500 individuals identified as *C.* sp. form A/R (see Ludwig unpublished data). Based on 3 linear measurements, principal components 1 and 2 (85.67% of variance, Fig. 2A) and 1 and 3 (68.49% of variance, Fig. 2B) showed high intraspecific morphological variability between populations. Thus, PC1 discriminated the shape variations (Wilk's lambda=0.9831, p=0.1204) and, the PC2 discriminated the size variations (Wilk's lambda=0.8274, p=5.692E-11) between *C.* sp. form A/R populations from South America. Based on Procrustes distances (range 0.021 – 0.1243), only six pair of populations did not exhibit a significant difference, these are ARG2-GO, ARG2-MAT, ARG2-RJ, GO-IGU, GO-RJ and IMI-MAT (Table 2). Populations from ARG2 presented high values for L/W ratios (mean = 4.394 cm) while individuals from ROS and ITA presented high values for L/H (mean =1.200 cm). The results from overall MANOVA through Hotelling's p-values pointed significative difference between *C.* sp. form A/R populations (Wilk's lambda=0.815, F=13.4, P=2.207E-10). The CV1 completely separated ITA and ROS populations from others with 84.67% of morphological variation and, the CV2 completely separated IMI, GO, ITU and GUA populations from others with 12.89% of morphological variation (Fig. 2C). The test on dependent variables through Regression analysis resulted that shape variations (Principal Component 1) are moderate correlated and significative (R^2=0.0359, F=4.66, p=0.0103) with Latitudinal distribution than size variations (Principal Component 2), which are moderate correlated and significative (R^2=0.0967, F=13.39, p=2.997E-06) with Temperature variations.

Furthermore, the Mantel test revealed weak correlation (R^2=0.0457) but significant ($p \leq 0.001$) for morphological variation across geographical distance between SA populations.

Table 2. Distance matrix of pairwise Procrustes distance based on linear measures and their respective significance *p*-values ($p \leq 0.0001$ in bold) (above diagonal) and pairwise geographic distance (km log transformed) in *C.* sp. form A/R population from South America. Data was used to perform Mantel test.

	ARG2	IGU	ITU	GUA	CLM	GO	RJ	MAT	IMI	ROS	ITA
ARG2		3.043	3.536	3.1	3.052	3.298	3.287	2.833	3.11	3.173	3.002
IGU	**0.0768**		3.367	2.195	2.014	2.946	3.059	2.663	2.283	2.592	2.47
ITU	**0.0855**	**0.0978**		3.338	3.365	3.162	3.314	3.443	3.332	3.291	3.395
GUA	**0.0725**	**0.1093**	**0.0528**		2.261	2.863	3.04	2.788	1.692	2.396	2.606
CLM	**0.0931**	**0.0937**	**0.0291**	**0.0568**		2.937	3.02	2.662	2.36	2.562	2.348
GO	0.0465	0.0542	**0.0768**	**0.071**	**0.0756**		3.003	3.121	2.85	2.7	3.016
RJ	0.0668	**0.0708**	**0.046**	**0.0609**	**0.0455**	0.050		3.119	3.051	2.972	3.005
MAT	0.0667	**0.1243**	**0.0713**	**0.0416**	**0.0845**	**0.082**	**0.0736**		2.814	2.915	2.516
IMI	**0.0385**	**0.0536**	**0.0745**	**0.072**	**0.0788**	**0.021**	**0.049**	0.0773		2.389	2.654
ROS	**0.0539**	**0.0775**	**0.0413**	**0.056**	**0.053**	**0.0534**	**0.038**	**0.0635**	**0.0478**		2.739
ITA	**0.0703**	**0.0561**	**0.0667**	**0.0714**	**0.0604**	**0.0362**	**0.0322**	**0.0884**	**0.0425**	**0.0544**	

Populational genetic structure

From all 10 microsatellite loci, 60% of them were polymorphic in SA populations, with 1-4 alleles per locus overall and a range of 15-16 alleles per population (Fig. 1). Mean allelic richness differed between SA populations and, all invasive populations (including South America) and native populations, 1.19, 1.47 and 2.84 respectively. The expected heterozygosity was higher in NAT than SA and IN regions, but not significative different between SA and IN regions. Based on alleles' distribution within SA populations (Fig. 1), was observed that IGU and ARG2 populations share the 184 allele at ClA01 locus, and is not found in any other population/lineage of this study. In ARG2, it was detected the presence of allele 278 at D12 locus which is also found in Jp (*C. japonica*) population (Fig. 1). In addition, GUA population also presented a distinct allele from others SA population, however it is not unique to this region. Overall, all SA populations presented that 192 allele at locus A03 but this allele is not found in others *C.* sp. form A/R populations (see AA1 genotype in Supporting information 1), indeed it is shared with Hw (Hawaii) and CS (*C. sandai*) populations.

Principal Coordinate Analysis (PCoA) of alleles' distribution (Fig. 3) among SA populations showed IGU population separated from others but closest to ARG2 (Fig. 3A). Overall, the PCoA exhibited a large cluster including SA and AA1 populations, which are closest to Jp (estuarine *C. japonica* from native area), and Hw populations than the other invasive (S1, AB and Rlc) and native populations (Fig. 3B-D). Based on PCoA pattern between SA, IN and NAT populations, there is no evidence that ARG2 and IGU present private alleles.

In the DAPC analysis, performed without any a priori group assignment (Fig. 3E), an optimal number of clusters was estimated with the *find.clusters* function; 40 axes that represented more than 84% of the total variance were retained. The program covered a range of possible clusters from 1 to 10. The lowest BIC value corresponded to k=5 (see Suporting Information 3). For DAPC analysis, 25 PCA axes and 4 discriminant functions were retained. One of the clusters included individuals of SA, AA1, Jp and two individuals of Hw. A second cluster joined AB1, Rlc, EHM, BA, two individuals of Fu, two individuals of KMT, and one individual of Vt. The third cluster included S1, CR', one individual of Hw, one individual of KMT, one individual of Fu and two individuals of Vt. The fourth cluster included only CS individuals and the fifth included only ZA individuals. Overall, the membership probability showed that from 25 analyzed populations, five clusters were formed across *Corbicula* populations of the world (Fig. 3B).

First-generation of migrants

The 83 *Corbicula* specimens included in the first-generation migrants assignment analysis, 5 individuals (from 4 loci, α=0.05) were assigned as first-generation migrants to a new location. The 4 loci detected first migrants between (the following sites are donor and receiver, respectively) ARG2 and GO (*L*-log 1.991), KMT and MAT (*L*-log 1.279), ARG2 and Hw (*L*-log 3.051), Vt and ARG2 (*L*-log 1.831) and, Jp and ITU (*L*-log 1.864) (details in Supporting Information 3).

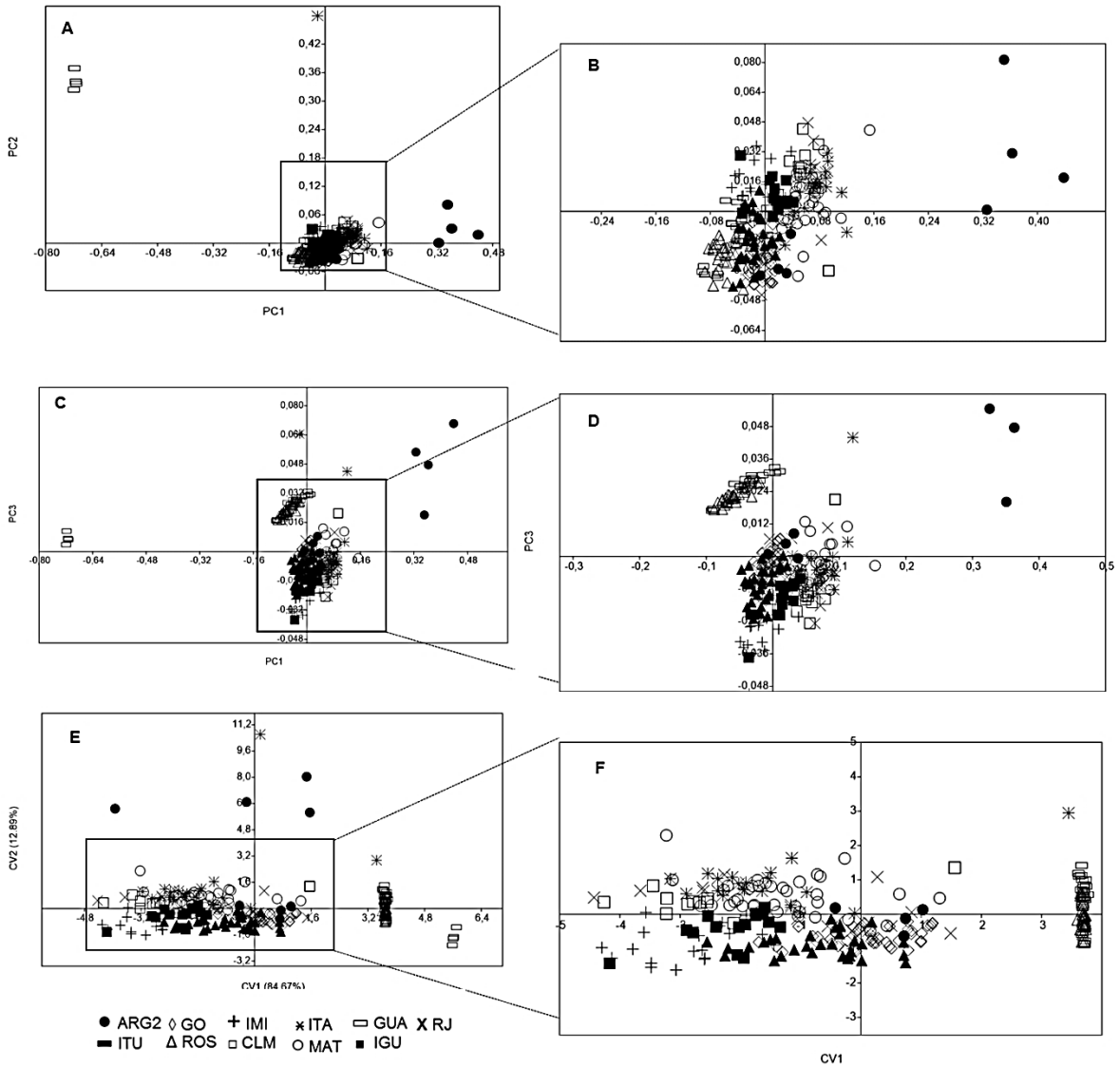

Figure 2. Morphological variations in South American *C.* sp. form A/R populations evaluated by Principal Components Analysis (A-D) and Canonical Variate Analysis (E-F). Each symbol corresponds to distinct populations (see legend).

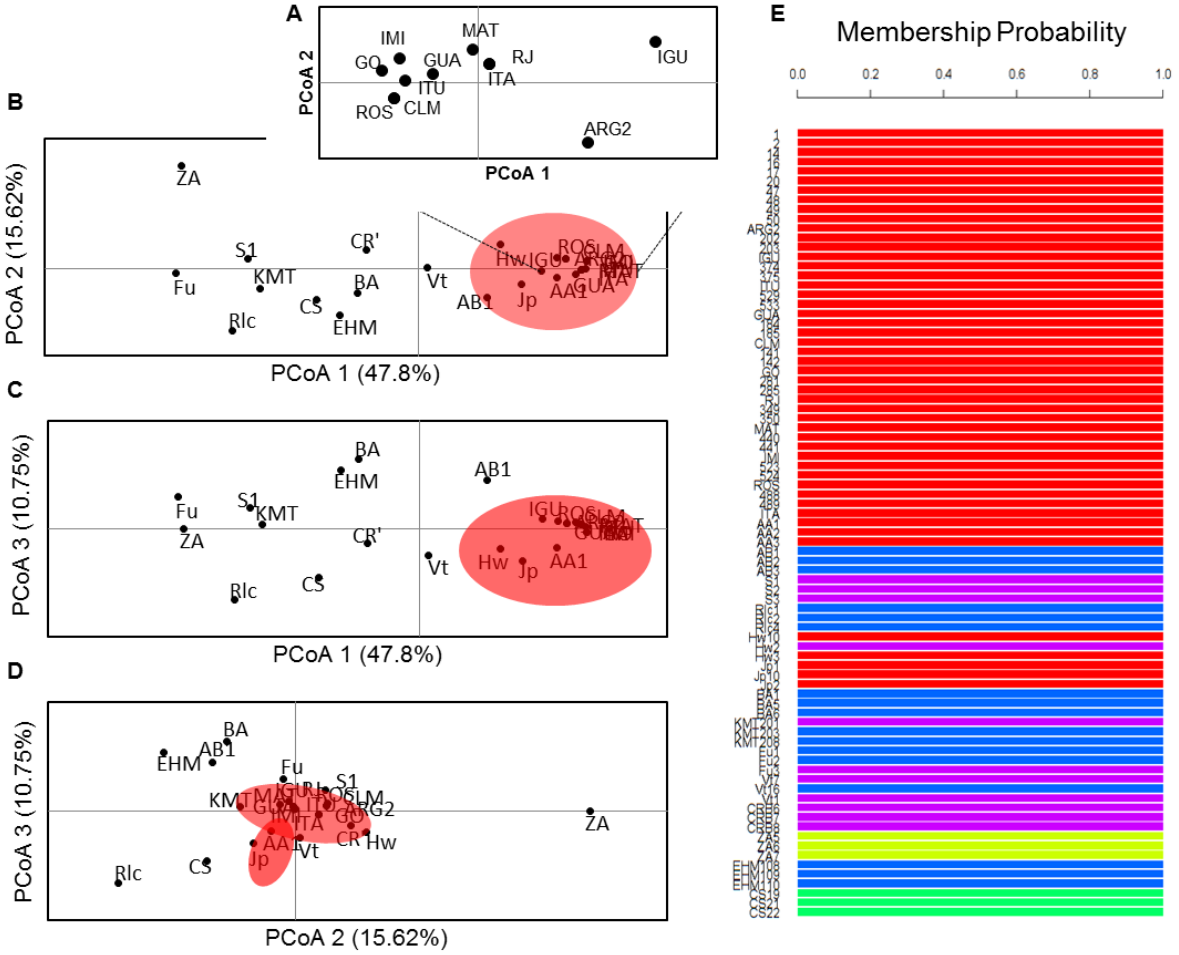

Figure 3. Principal coordinates analysis (PCoA) using allele's distribution genetic distance from *Corbicula* populations, comparing: (A) within South American populations, (B-D) South American populations and Invasive and Native populations plotted in distinct axis combinations. The red circle indicates Cluster 1 (which grouped SA, AA1, Hw and Jp individuals) from DAPC plot (E). Discriminant analysis of principal components (DAPC) results between South American *Corbicula* sp. form A/R populations and their counterparts from invasive and native area (E). Assignment probabilities of individuals with no local sampling *prior* with *k*=5. Each individual is represented by a horizontal bar partitioned into *K*-colored segments that represent its estimated membership cluster in each of the inferred groups. The populations' code is indicated in Table 1.

DISCUSSION

Corbicula sp. *form A/R superclones*

South American populations combined, presented lowerst mean allelic richness values than NAT populations 1.34 and 2.89, respectively. The PCoA results (Fig. 4A-D) were congruent

with DAPC (Fig. 4E), which clustered SA, AA1, Hw and Jp together. This finding diverges from previously studies with mtDNA haplotype phylogenetic relationship (Pigneur *et al.*, 2014a; Ludwig unpublished data), which completely separated Jp from others *Corbicula* spp. This divergence is associated with the fact that Jp (*C. japonica*) is an estuarine sexual species in its native area and present unreduced mono-flagellate sperm while invasive androgenetic/clonal lineage are freshwater species presenting biflagellate sperm. Thus, as previously studies suggested (Hedtke *et al.*, 2008), there is an incongruence between the mtDNA and nDNA within *Corbicula*, which is most probably is associated with "egg parasitism" (Hedtke *et al.*, 2008, 2011). All SA populations analyzed in this study were previously identified as *C.* sp. form A/R based on the presence of mtDNA FW5 haplotype, which is closely related to the native freshwater androgenetic *C. leana*, and high genotypic similarity with AA1 (clonal invader from South America, North America and Europe) (Pigneur *et al.*, 2014a).

In this study, high genotypic similarity was detected between nDNA of SA populations (based on allele's distribution, PCoA and DAPC analysis) with nDNA of Jp. According to Pigneur *et al.*, (2014a), when cytonuclear mismatches are identified within *Corbicula*, the mitochondrial inheritance comes from maternal lineage and the nuclear inheritance comes from paternal lineage. This finding indicates that *C. sp.* form A/R populations of South America could result from "egg parasitism" between maternal mtDNA from the androgenetic *C. leana* and paternal nDNA of the sexual *C. japonica*, keeping the presence of 192 allele and unreduced biflagellate sperm as maternal inheritance. Additionally, according to Bengtsson (2003), subpopulations of asexual/clonal organisms can exhibit some degree of genetic variability between them when the asexual/clonal lineage (*C.* sp. form A/R) recently derived from their parents (in this case *C. leana* and *C. japonica*). This statement proved to be true when was compared SA, IN and NAT populations and, based on alelle's distribution, was observed great similarity between Jp and KMT genotypes with SA and AA1 genotypes. Herein we postulate, based on our data, that the androgenetic clonal *C.* sp. form A/R populations across globe are part of metapopulation of superclones and that it is most probably the result of cytonuclear mismatch or 'hybrids' between *C. leana* mtDNA and *C. japonica* nDNA lineages.

Population' structure of South American C. sp. *form A/R*

The lack of genetic variability in South American *Corbicula* spp. in both mtDNA (Ludwig unpublished data) and in nDNA (this study) can be the result of successive bottlenecks during the introduction process associated with clonal propagation within continental waters. Further, was observed in IGU a private 184 allele at the locus A01, shared with specimens at ARG2; thus, until today, is unique to South America region. This finding indicates that IGU is

directly connected with ARG2 population. However, our analysis is limited and prevents to infer if the gene flow is coming from IGU to ARG2 or vice versa. In addition, GUA also exhibited a private 112 allele at A02 locus but it is not unique across *Corbicula* spp. around the world. This finding clearly shows us that the hypothesis of multiple introductions of these clams in South America is true. It is probably based on the presence of those private alleles that PCoA analyzing only SA genotypes exhibited a different pattern than when were included all IN and NAT genotypes. Thus, our results clearly indicated, even with 8.54% of null alleles (with no amplification), homogeneous genetic pattern across all SA populations, except in ARG2, IGU and GUA.

Multiple introductions and admixture

We postulate that South American *C.* sp. form A/R populations present low/lack of genetic diversity, which is probably consequence of extensive bottlenecks during the introduction and dispersion processes in South American continental waters. Distinct rare alleles detected in ARG2, IGU and GUA suggest multiple introduction events in South America. Indeed, the distinct allele in GUA population is shared with others invasive (AB1 and S1) and native (BA, KMT, Fu, Jp, Vt, CR', EHM and CS) populations, strongly indicating invasion by different propagules in South America from several invasive/native areas. Our results revealed a closer relationship of all SA population with Jp population. Since, we postulated that SA populations are resulted of "egg parasitism" between nDNA of *C. japonica* (Jp) with mtDNA FW5 lineage (probably *C. leana*), and SA populations received propagules directly from native populations, the genetic source of *Corbicula* sp. propagules to SA populations could be originated both/either from KMT and Vt populations.

Indeed, first-generation of migrants were detected between Japanese KMT (*C. leana*) and Brazilian MAT populations. MAT site is close to Patos Lagoon, Porto Alegre city, southern Brazil (Fig. 1) and the detection of these migrants indicates that MAT is receiving new propagules from the mtDNA FW5 *C. leana* lineage of native range. Recently, Ludwig (unpublished data) detected 'hybrid' populations of *Corbicula* sp. in Guaíba Lake, Barra do Ribeiro city and, in Jacuí River, Agudo city, which are located even closer to Patos Lagoon than MAT. Those 'hybrid' populations presented cytonuclear mismatch between *Corbicula* form A/R and form B lineages, indicating that in this region both lineages coexist with probable gene flow between them. Thus, with these recently finding, Patos Lagoon is likely another introduction point of *Corbicula* spp. in South America. In addition, first migrant was also detected between ARG2 and Hw populations, indicating that ARG2 may be providing genetic material to Hw population, probably by human aided dispersion through stored propagules in ballast water of merchant ships, that travel to that site (Seebens *et al.*, 2013); both populations share various alleles, especially 192 at A03 locus and 278 at D12 locus.

First migrant was detected, also, between Vt and ARG2 populations, indicating that ARG2 is receiving new propagules from *C.* sp. likely from Vietnam populations. The last first migrant detected in our data was found between Jp and ITU populations. Consequently, ITU appears to be receiving new propagules from the native range of *C. japonica*, most probably through the port of Belém, Pará state, northern Brazil through ballast water of merchant ships. The ITU site is located at 427 km far from Belém (Fig. 1), through the Tocantins River. Thus, most probably, propagules of *Corbicula* spp. dispersed upstream by human aided or by larval natural dispersal. This finding indicates that Belém is also another point of invasion of *Corbicula* spp. in South America, as postulated previously (Ludwig unpublished data). Additionally, RJ site is located in Southeast Atlantic hydrographic basin and, this basin is not connected with any other South American basin, thus, most probably, *Corbicula* RJ propagules came from a distinct introduction point than others SA populations. Unfortunately, the geographical limitation of the populations sampled herein made it difficult to evaluate other putative points of introduction of *Corbicula* spp. in Brazil, such as the port of Santos, São Paulo state in Brazil.

Dispersal patterns within South American rivers

Biological invasions provide unique chances to evaluate invasion dynamics linked with expanding populations (e.g. Betancur-R *et al.*, 2011). An expanding population can therefore produce valuable insight into mechanisms associated with successful invasion and later range expansion (e.g. Bronnenhuber *et al.*, 2011). In this study, was observed a wide range of dispersal distances, suggesting a combination of short-and long-distance dispersal vectors. Long-distance dispersal usually happens accidently between continents by the transfer of new propagules transported in ballast water tanks of merchant ships, which are discharged in port regions. Additionally, human vectors (e.g. small recreation boats, construction waste, and plastic bottles) can also promote short-distance faster, longer and/or ‘jump’ dispersions between neighboring tributaries, rivers, and hydrographic basins, as reported by Zhan *et al*., (2013) for *Limnoperna fortunei* (Dunker, 1857) in South America. Another possibility could be construction and transportation of sand as vector of dispersion by human, which has been suggested by Belz et al. (2012) for *L. fortunei*. This mechanism could also be an important dispersion vector for *Corbicula* spp., because these clams are benthic organisms and usually are buried into the substrate (with sand). Sand dredging can be easily remove and transport these clams with sand to other regions.. Furthermore, fish may also represent important vectors for the dispersion of invasive mollusk species, because fishes ingest bivalves and these may survive the transport through the gastrointestinal tract by closing their valves, as reported for *C. fluminea* (Cantanhêde et al. 2007) and, for *L. fortunei* (Belz et al. 2012) in South American rivers.

Migrants' assignment tests detected first migrants between ARG2 and GO populations, indicating that few individuals likely dispersed upstream in the Brazilian Paraná River from the Argentinian Rio de La Plata most likely by human mediated "jump" dispersal, as previously suggested for *L. fortunei* (Zhan *et al.,* 2013). *Corbicula* larvae can naturally disperse at least 1.2 km/year upstream without human aid (Voelz *et al.,* 1998), thus we cannot rule out the hypothesis that *Corbicula* larvae are naturally dispersing into South American continental waters. However, the hydrodynamic flow regime of rivers can also limit dispersal of adult's animals that is capable solely of realizing few slow movements, required to hold their buried position in the substrate, especially in high flow environments such as the Río de La Plata. Thus, characterization of the dispersal mechanisms during colonization of *C.* sp. form A/R, has led to an increase in studies arguing that human aided dispersion is a facilitator for secondary range expansion (e.g. Zhan *et al.,* 2013). Given the importance of the Prata basin, which includes the Rio de La Plata as an important commercial harbor for many ships originating from the Eurasia (Seebens *et al.,* 2011), we predict that the local discharge of ballast water will continue to play an main role in the introducing of new *Corbicula* propagules in the aquatic system of South America.

Phenotypic plasticity in South American populations

The results of morphological analyses indicated that most of the morphological variance of South American *C.* sp. form A/R shell in each population could be reduced to a few principal components displaying common patterns of variations in shape and size. In general, the PC1 accounts for most of the variance among populations (56.93%) which discriminated the shape within populations and, the PC2 discriminated the size within populations. Furthermore, the analysis also suggested that, there is some influence of temperature variation in size and, the latitude distribution influence the variation in shape of South American *C.* sp. form A/R's shells. Such variations are moderately correlated with the lack of genetic variability, as demonstrated in this study. Significant intraspecific variation among populations with respect to linear measures was observed among populations. Similar findings have been reported in other bivalve invasive species, *Crassostrea gigas* (Thunberg, 1793) and *L. fortunei* (Paolucci *et al.,* 2014). Morphological variation thus may be associated with the success in colonization by invasive mollusks and most likely represent a phenotypic plasticity in *Corbicula,* for instance. Morphologic plasticity may facilitate the establishment and spread into new environments, including those exhibiting substantial environmental gradients.

According to Paolucci *et al.,* (2014), the shell shapes *of L. fortunei,* particularly the L/W and H/W ratios, may represent adaptive responses to different South American environmental conditions. Despite the apparent complexity of the interaction between shell shape and abiotic variables, recently, Crespo *et al.,* (2015) correlated that *C. fluminea*

colonization and adaptation into new environments are strongly influenced by changing temperatures. Indeed, the temperature is considered as one of the most significant abiotic factor influencing the growth rate and producing distinct morphotypes in *D. rostiformis bugensis* (Peyer *et al.*, 2010). In Portuguese *C. fluminea* (Sousa *et al.*, 2007), previous morphological studies showed that there is considerable shell plasticity (Sousa *et al.*, 2007), which is resulted from local adaptation during the establishment process in a new environment. Alternatively, based in our data, we postulate that distinct morphotypes found in SA populations can also be a consequence of post-colonization adaptation into new environment and admixture of propagules coming from different parts of the world. However, additional studies should address and test such hypothesis.

Invasion history of C. sp. form A/R in South America

The genetic structure of SA populations of *C.* sp. form A/R is apparently strongly linked to clonal propagation as well as to it invasion history. Although the number of populations sampled in our study is small, the genetic variation detected by microsatellites data allowed some insights into the invasion history of *Corbicula* sp. form A/R and its relation to clonal propagation and human aided dispersion into South America.

Herein, we postulate the following scenario. New propagules with high genetic diversity, from Jp (*C. japonica*), Vt (*Corbicula* sp.), both likely from Asia, and KMT (*C. leana*) from Japan, were introduced into South American through discharge of ballast water. Most probably, the main introductions points of those propagules detected herein are the regions of Belém, Patos Lagoon and Río de La Plata River, which are present commercial ports.

The first introduction reported of *Corbicula* clams into South America was around 1975, through Rio de La Plata in Argentina (Ituarte, 1981) and, probably during the same period in Brazil, through the Patos Lagoon, Southern Brazil (Veitenheimer-Mendes, 1981). Most likely, the propagules were subjected to massive bottlenecks, decreasing the number of the founder population and the genetic diversity of the remaining individuals (e.g. Golani *et al.*, 2007). Subsequently of the introduction, few surviving individuals, which are capable of self-fertilization by clonal reproduction, became accommodated in the new local environment (event called as Lag-time; see Cox, 2004). Once established, clonal individuals slowly started a new population subsequently abruptly accelerating the population growth rate – *Corbicula* spp. are capable of generating up to 90.000 descendants in a single reproductive season by solely one individual - reaching an asymptotic invasion velocity (McMahon 1999). Dispersion into nearby areas follows. Some years later, *C.* sp. form A/R was reported in high densities in the area of the Jacuí and Guaíba Rivers, close to Porto Alegre city, around 1978 (Ituarte, 1994). Since then, *Corbicula* specimens have been detected across South American

hydrographic basins (Ludwig unpublished data), indicating established and in expansion populations.

Historical publications points to initial colonization by *Corbicula* spp. in Río de La Plata estuary system in Argentina (Ituarte, 1994), and a subsequently range expansion both north and east across South America, reaching upper Paraguay River (Callil & Mansur, 2002) and recently the southeast of Brazil (Maroneze *et al.,* 2011). In contrast, the pattern emerging from our study strongly suggests that there are/were at least four introductions points (Fig. 1) of *Corbicula* spp. in South America, Río de La Plata in Argentina, Patos Lagoon in Southern Brazil, and Amazon Delta in Northern Brazil. Thus, once *C.* sp. form A/R is established in the new environment, its genetic diversity (which is similar to other invasive individuals) might only be maintained through admixture during multiple introductions of new propagules. Based on our data, we postulate that, once, new propagules were introduced in South American rivers, the migrant individuals exchange genetic material with the established populations increasing the allelic frequency. However, the small propagule pressure and high genetic drift decrease the genetic variability and, in parallel, the clonal reproduction maintains the genetic diversity of the genitor. Even with hard bottleneck decreasing the genetic diversity of *C.* sp. form A/R, multiple introductions and admixture in ARG might maintain the genetic diversity pool, since this site was postulated in this study as the main genetic source to SA populations. Indeed, the presence of private alleles in ARG2 and IGU indicates that high migration rate between these sites is maintaining the diversity and that the genetic drift did not reduce the invasiveness of those populations.

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References

Araújo, R., Moreno, D. & Ramos, M.A. (1993) The asiatic clam *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. *American Malacological Bulletin,* **10,** 39-49.

Arnaud-Haond., S.O.P.H.I.E., & Belkhir, K. (2007) GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. Molecular Ecology Notes, **7**, 15-17.

Belz, C.E., Darrigran, G, Netto, O.S.M., Boeger, W.A. & Ribeiro, P.J. (2012) Analysis of four dispersion vectors in inland waters: the case of the invading bivalves in South America. Journal of Shellfish Research, **31**, 777-784.

Bengtsoon, B. O. (2003) Genetic variation in organisms with sexual and asexual reproduction. Journal od Evolutionary Biology, **16**, 189-199.

Betancur-R, R., Hines, A., Acero, A.P., Ortí, G., Wilbur, A.E. and Freshwater, D.W. (2011) Reconstructing the lionfish invasion: insights into Greater Caribbean biogeography. Journal of Biogeography, 38: 1281-1293. http://dx.doi.org/10.1111/j.1365-2699.2011.02496.x

Bronnernhuber, J.E., Dufour, B.A., Higgs, D.M. and Health, D.D. (2011) Dispersal strategies, secondary range expansion and invasion genetics of the nonindigenous round goby, *Neogobius melanostomus*, in Great Lakes tributaries. Molecular Ecology, 20: 1845-1859. http://dx.doi.org/10.1111/j.1365-294X.2011.05030.x

Callil, C.T. and Mansur, M.C.D. (2002) Corbiculidae (Mollusca, Bivalvia) in Pantanal of Mato Grosso, high Paraguay Basin, Brazil: distribution and population density. *Amazoniana*, **17,** 1-15.

Cantanhêde, G., Hahn, N.S., Gubiani, E.A. & Fugi, R. (2008) Invasive molluscs in the diet of Pterodoras granulosus (Valenciennes, 1821) (Pisces, Doradidae) in the upper Paraná River floodplain, Brazil. *Ecology of Freshwater Fish*, **17**, 47-53.

Counts, C.L. (1981) *Corbicula fluminea* (Bivalvia: Sphaeriacea) in British Columbia. *Nautilus*, **95**, 12-13.

Crespo, D., Dolbeth, M., Leston, S., Sousa, R. and Pardal, M.A. (2015) Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. Biol Invasions, http:dx.doi.org/10.1007/s10530-015-0862.y

Cristescu, M. E. (2015) Genetic reconstructions of invasion history. Molecular Ecology, http://dx.doi.org/10.1111/mec.13117

Cox, G.W. (2004) Alien species and evolution: The evolutionary ecology of exotic plants, animals, microbes, and interacting native species. Island Press (eds.), Washington, Covelo, London.

Darling, J.A., Bagley, M.J., Roman, J., Tepolt, C.K. & Geller, J.B. (2008) Genetic patterns across multiple introductions of the globally invasive crabs *Carcinus. Molecular Ecology*, **17**, 4992-5007.

Darrigran, G. (2002) Potential impact of filter-feeding invaders on temperate inland fresh water environments. *Biological Invasions*, **4**, 145-156.

Folmer, O., Black, M., Hoeh, W. et al (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Mar Biology and Biotechnology*, **3**, 294-299.

Frisch, D., Havel, J.E., and Weider, L.J. (2013) The invasion history of the exotic freshwater zooplankter *Daphnia lumholtzi* (Cladocera, Crustacea) in North America: a genetic analysis. *Biological invasions*, **15,** 817-828.

Garcia-Vallve, S., & Puigbo, P.E.R.E. (2009) DendroUPGMA: a dendrogram construction utility. Universitat Rovira I Virgili.

Gatlin, M.R., Shoup, D.E. & Long, J.M. (2012) Invasive zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) survive gut passage of migratory fish species: implications for dispersal. *Biological Invasions*, **15**, 1195-1200.

Glaubrecht, M., Féher, Z. & Köhler, F. (2007) Inventorizing an invader: anootated type catalogue of Corbiculidae Gray, 1847 (Bivalvia, Heterodonta, Veneroidea), including old World limnic *Corbicula* in the Natural History Museum Berlin. *Malacologia*, **49**, 243-272.

Golani, D., Azurro, E., Corisni-FokaM., Falautano, M., Andaloro, F. and Bernardi, G. (2007) Genetic bottlenecks and successful biological invasions: the case of a recente Lessepsian migrant. Biology Letters, **3**, 541-545. http://dx.doi.org/10.1098/rsbl.2007.0308

Hakenkamp, C.C. & Palmer, M.A. (1999) Introduced bivalves in freshwater ecosystems: the impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia*, **119**, 445-451.

Hammer, O., Harper, D.A.T. & Ryan, P.D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, **4**, 9.

Hedtke, S.M., Stanger-Hall, K., Baker, R.J. & Hillis, D.M. (2008) All-male asexuality: origin and maintenance of androgenesis in the Asian clam *Corbicula*. *Evolution*, **62**, 1119-1136.

Hedtke, S.M., Glaubrecht, M. & Hillis, D.M. (2011) Rare gene capture in predominantly androgenetic species. *PNAS*, **108**, 9520-9524.

Houki, S., Yamada, M., Honda, T. & Komaru, A. (2011) Origin and Possible role of males in hermaphroditic androgenetic *Corbicula* clams. *Zoological Science*, **28**, 526-531.

Ishibashi, R. Ookubo, K., Aoki, M., Utaki, M., Komaru, A., Kawamura, K. (2003) Androgenetic reproduction in a freshwater diploid clam Corbicula fluminea (Bivalvia: Corbiculidae). Zool Sci, **20**, 727-732.

Ituarte, C.F. (1981) Primera noticia acerca de la introduccion de pelecipodos asiaticos en el area rioplatense**.** *Neotropica*, **27**, 79-82

Ituarte, C.F. (1994) *Corbicula* and *NeoCorbicula* (Bivalvia: Corbiculidae) in the Parana, Uruguay, and Rio de La Plata Basins**.** *Nautilus* **107,**129-135.

Jombart, T. (2008) *adegenet*: a R package for the multivare analysis for genetic markers. Bioinformatics, **24,** 1403-1405.

Jombart, T., Devillard, S. and Balloux, F. (2010) Discriminat analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics, **11,** 1-15.

Keller, S.R. & Taylor, D.R. (2010) Genomic admixture increases fitness during a biological invasion. *Journal of Evolutionary Biology*, **23**, 1720-1731.

Komaru, A., Konishi, K., Nakayama, I., Kobayashi, T., Sakai, H. & Kawamura, K. (1997) Hermaphroditic freshwater clams in the genus *Corbicula* produce non-reductional spermatozoa with somatic DNA content. *The Biological Bulletin*, **193**, 320-323.

Komaru, A., Kawagishi, T., Konishi, K. (1998) Cytological evidence of spontaneous androgenesis in the freshwater clam Corbicula leana Prime. Dev Genes Evol, **208,** 46-50.

Komaru, A. Kumamoto, A. Ishibashi, R. (2001) Possible elevation of ploidy levels by acidental formation off emale pronucleus in androgenetic clam Corbicula leana. Dev Biol, **18**, 87.

Komaru, A., Kumamoto, A. Kato, T., Ishibashi, R., Obata, M. Nemoto, T. (2006) A hypothesis of ploidy elevation by formation of a female pronucleus in the androgenetic clam Corbicula flumínea in the Tone River Estuary, Japan. Zool Sci, **23**, 529-532.

Lee, T., Siripattrawan, S., Ituarte, C. & Ó Foighil, D. (2005) Invasion of the clonal clams: *Corbicula* lineages in the New World. *American Malacological Bulletin*, **20**, 113-122.

Mantel, N.A. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209-220.

Maroneze, D.M., Coscarelli, D., Vidigal, T.H.D.A., Callisto, M. (2011) First record of *Corbicula fluminea* (Muller, 1774) in the drainage basin of the Araguari River, Minas Gerais, Brazil. *Brazilian Journal of Biology* 71: 221-222, http://dx.doi.org/10.1590/S1519-69842011000100033

Martínez, R. (1987) *Corbicula manilensis* molusco introducido en Venezuela. *Acta Cientifica Venezolana*, **38**, 384-385.

McMahon, R.F. (1999) Invasive characteristics of the freshwater bivalve Corbicula fluminea. In: Claudi R, Leach JH (eds). Nonindigenous Freshwater Organisms Vectors, Biology and Impact. Lewis Press, Washington, DC, pp 315-343.

McMahon, R.F. (2002) Evolutionary and physiological adaptations of aquatic invasive animals: *r* selection versus resistance. *Canadian Journal of Fisheries and Aquatic Sciences*, **59,** 1235 – 1244.

Paetkau, D., Slade, R., Burden, M. and Estoup, A. (2004) Genetic assignment methods fot the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology, **13**, 55-65.

Paolucci, E.M., Sardiña, P., Sylvester, F. Perepelizin, P.V., Zhan, A., Ghabooli, S. et al. (2014) Morphological and genetic variability in an alien invasive mussel across an environmental gradient in South America. Limnology and Oceanography, **59**, 400-412. http://.dx.doi.org/10.4319/lo.2014.59.2.0400

Park, J.K. and Kim, W. (2003) Two Corbicula (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment. Molecular Phylogenetics and Evolution, **29,** 529-539.

Park, J.K., Lee, J.S. & Kim, W. (2002) A single mitochondrial lineage is shared by morphologically and allozymatically distinct freshwater *Corbicula* clams. *Molecules and cells*, **14**, 318-322.

Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, **28**, 2537-2539.

Peyer, S.M., Hermanson, J.C., & Lee, C.E. (2010) Developmental plasticity of shell morphology of quagga mussels from shallow and deep-water habitats of the Great Lakes. The Journal of experimental biology, **213**, 2602-2609.

Pigneur, L.M., Marescaux, J., Roland, K., Etoundi, E., Descy, J.P. & Van Doninck, K. (2011a) Phylogeny and androgenesis in the invasive *Corbicula* clams (Bivalvia, Corbiculidae) in Western Europe. *BMC Evolutioniary Biology*, **11**, 147.

Pigneur, L.M., Risterucci, A.M., Dauchot, N., Li, X. & Van Doninck, K. (2011b) Development of novel microsatellite markers to identify the different invasive lineages in the *Corbicula* complex and to assess androgenesis. *Molecular Ecology*, **11**, 573-577

Pigneur, L.M., Hedtke, S.M., Etoundi, E., Van Doninck, K. (2012) Androgenesis: a review through the study of the shelfish shellfish *Corbicula* spp. Heredity, **108**, 581-591. http://dx.doi.org/10.1038/hdy.2012.3

Pigneur, L.M, Etoundi, E., Aldridge, D.C, Marescaux, J., Yasuda, N. and Van Doninck, K. (2014a) Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. Molecular Ecology, **23**, 5102-5116. http://dx.doi.org/10.1111/mec.12912

Pigneur, L.M., Falisse, E., Roland, K., Everbecq, E., Deliége, J.F., Smitz, J. S., Van Doninck, K. & Descy, J.P. (2014b) Impacts of invasive Asian clams, *Corbicula* spp., on a large river ecosystem. *Freshwater Biology*, **59**, 573-583.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L and Stoup A (2004) GENECLASS2: a software for genetic assignment and fisrt-generation migrant detection. Journal of Heredity, 95: 536-539. http://dx.doi.org/10.1093/jhered/esh074

Rambaut, A. (2009) FigTree v1.3.1: Tree figure drawing tool. Website: http://tree.bio.ed.ac.uk/software/figtree.

Rannala, B. and Mountain, J.L. (1997) Detecting immigration by using multilocus genotypes. Proceedings of Natural Academy of Sciences, 94: 9197-9201.

Renard, E., Bachmann, V., Cariou, M.L. & Moreteau, C. (2000) Morphological and molecular differentiation of invasive freshwater species of the genus *Corbicula* (Bivalvia, Corbiculidea) suggest the presence of three taxa in French rivers. *Molecular Ecology*, **9**, 2009-2016.

Sax, D.F. & Brown, J.H. (2001) The Paradox of invasion. *Global Ecology & Biogeography*, **9**, 363-371.

Seebens, H., Gastner, M.T. and Blasius, B. (2013) The risk of marine bioinvasion caused by global shipping. Ecology Letters, http://dx.doi.org/10.1111/ele.12111

Siripattrawan, S., Park, J.K. & Ó Foighil, D. (2000) Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. *Journal of Molluscan Studies*, **66**, 423-429.

Sousa, R., Freire, R., Rufino, M., Méndez, J., Gaspar, M., Antunes, C. & Guilhermino, L. (2007) Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries. *Estuarine, Coastal and Shelf Science*, **74**, 166-174.

Sousa, R., Nogueira, R.A.J.A, Gaspar, M.B., Antunes, C. & Guilhermino, L. (2008) Growth and extremely high production of the non-indigenous invasive species Corbicula

fluminea (Müller, 1774): Possible implications for ecosystem functioning. *Estuarine, Coastal and Shelf Science*, **80**, 289-295.

Strayer, D.L. (2008) Effects of alien species on freshwater mollusks in North America. *Journal of the North American Benthological Society*: 74-98.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P. (2004) MICRO-CHECKER: software for identifying and correcting genotyping erros in microsatellite data. Mol Ecol, **4,** 535-538. http://dx.doi.org/10.1111/j.1471-8286.2004.00684.x

Voelz, N.J. McArthur, J.V. & Rader, R.B. (1998) Upstream mobility of the Asian clam *Corbicula fluminea*: identifying potential dispersal agentsa. Journal of Freshwater Ecology, **13**, 39-45.

Veitenheimer-Mendes, I.L. (1981) *Corbicula manilenssis*, (Philippi, 1844) molusco asiático, na bacia do Jacuí e do Guaíba, Rio Grande do Sul, Brasil (Bivalvia, Corbiculidae). *Ilheringia Serie Zoologica*, **60**, 63-74.

Zhan, A. Perepelizin, P.V., Ghabooli, S., Paloucci, E., Sylvester, F., Sardina, et al. (2012) Scale-dependent post-establishment spread and genetic diversity in an invading mollusk in South America. Diversity and Distributions, **18**, 1042-1055. http://dx.doi.org/10.1111/j.1472-4642.2012.00894.x

Zhang, L., Shen, Q., Hu, H., Shao, S. & Fan, C. (2011) Impacts of *Corbicula fluminea* on oxygen uptake and nutrient fluxes across the sediment-water interface. *Water, Air, & Soil Pollution*, **220**, 399-411.

Supporting Information 1. Microsatellite data of *Corbicula* sp. Form A/R from South America and from invasive and native range, with their respective coordinates. This set of data was used to build a matrix of genetic and geographic distances to Mantel test.

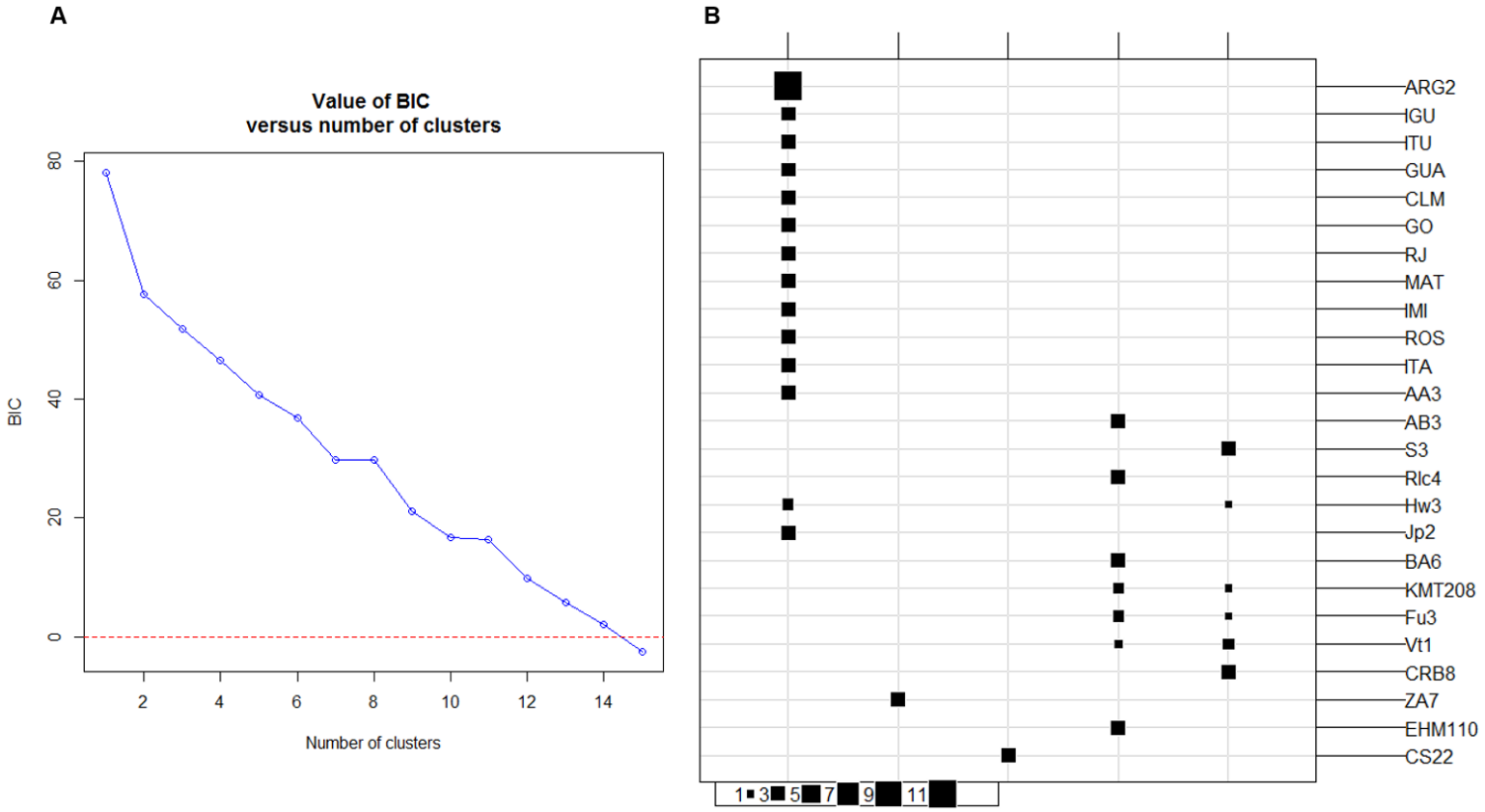
Pop. code	Ind. code	Loci										Coordinates (decimal)	
		ClA01	ClA02	ClCA03	ClB03	ClB11	ClC01	ClC12	ClD06	ClE01	ClD12	Latitude	Longitude
1	1	184198	000000	000000	233239	311311	175179	226226	199207	213213	27427	34,916	57,950
1	2	198198	000000	192192	233239	311311	175179	226226	199207	213213	27427	34,916	57,950
1	14	184198	000000	192192	233239	000000	175179	226226	199207	213213	27427	34,916	57,950
1	16	184198	110114	192192	233239	311311	175179	226226	199207	213213	27427	34,916	57,950
1	17	198198	110114	192192	233239	000000	175179	226226	000000	213213	27427	34,916	57,950
1	20	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	34,916	57,950
1	47	184198	110114	192192	233239	311311	175179	226226	000000	213213	27427	34,916	57,950
1	48	198198	110114	000000	233239	311311	175179	226226	199207	213213	27427	34,916	57,950
1	49	184198	110114	192192	233239	000000	175179	226226	000000	000000	27427	34,916	57,950
1	50	184198	110114	000000	233239	311311	175179	226226	199207	000000	27427	34,916	57,950
1	51	198198	110114	192192	233239	311311	175179	226226	199207	000000	27427	34,916	57,950
2	202	184198	000000	192192	233239	311311	175179	226226	199207	213213	27427	25,446	54,504
2	203	184198	000000	192192	233239	311311	175179	226226	199207	213213	27427	5,133	49,166
2	205	184198	000000	192192	233239	311311	175179	226226	199207	213213	27427	5,133	49,166
3	374	198198	110114	192192	233239	311311	175179	226226	000000	213213	000000	5,133	49,166
3	375	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	24,066	54,25
3	377	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	24,066	54,25
4	529	198198	110112	192192	233239	311311	175179	226226	199207	213213	27427	24,066	54,25
4	533	198198	110112	192192	233239	311311	175179	226226	199207	213213	000000	25,543	53,49
4	535	198198	110112	192192	233239	311311	175179	226226	000000	213213	27427	25,543	53,49
5	184	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	25,543	53,49
5	185	198198	110114	192192	233239	311311	000000	226226	199207	213213	27427	17,95	51,72
5	186	198198	110114	000000	233239	311311	175179	226226	199207	213213	27427	17,95	51,72
6	141	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	17,95	51,72
6	142	198198	110114	192192	000000	311311	175179	226226	199207	213213	27427	22,84	43,6
6	143	198198	110114	192192	233239	311311	000000	226226	199207	213213	27427	22,84	43,6

7	281	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	22,84	43,6
7	285	198198	110114	192192	233239	311311	175179	226226	199207	000000	27427	29,579	54,42
7	286	198198	000000	192192	233239	311311	175179	226226	199207	213213	27427	29,579	54,42
8	349	198198	110114	192192	233239	311311	175179	000000	199207	213213	27427	29,579	54,42
8	350	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	23,73	54,55
8	351	198198	000000	192192	233239	311311	175179	226226	199207	213213	27427	23,73	54,55
9	440	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	23,73	54,55
9	441	198198	110114	192192	000000	311311	175179	226226	199207	213213	27427	22,35	52,7
9	442	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	22,35	52,7
10	523	198198	110114	192192	233239	311311	000000	226226	199207	213213	27427	22,35	52,7
10	524	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	27,26	52,36
10	525	198198	110114	000000	233239	311311	175179	226226	199207	213213	27427	27,26	52,36
11	488	198198	000000	192192	233239	311311	175179	226226	199207	213213	27427	27,26	52,36
11	489	198198	110114	192192	233239	311311	175179	226226	199207	213213	27427	30,633	97,684
11	490	198198	110114	192192	233239	311311	175179	226226	199207	000000	27427	30,633	97,684
12	AA1	198198	110114	190194	233239	311311	175179	226226	199207	213213	27427	30,633	97,684
12	AA2	198198	110114	190194	233239	311311	175179	226226	199207	213213	27427	30,633	97,684
12	AA3	198198	110114	190194	233239	311311	175179	226226	199207	213213	27427	30,633	97,684
13	AB1	198198	112114	188188	233239	311311	173175	226228	199211	000000	27427	30,633	97,684
13	AB2	198198	112114	188188	233239	311311	173175	226228	199211	000000	27427	47,561	7,632
13	AB3	198198	112114	188188	233239	311311	173175	226228	199211	000000	27428	47,561	7,632
14	S1	000000	112116	000000	233239	311313	173175	226228	237237	209209	26427	47,561	7,632
14	S2	000000	112117	000000	233239	311313	173175	226228	237237	209209	26427	43,956	4,2719
14	S3	000000	112118	000000	233239	311313	173175	226228	237237	209209	26427	43,956	4,2719
15	Rlc4	196196	112116	190190	239239	311313	173175	230230	000000	000000	27427	43,956	4,2719

15	Rlc5	196196	112116	190190	239239	311313	173175	230231	000000	000000	27427	22,285	114,157
15	Rlc6	196196	112116	190190	239239	311313	173175	230232	000000	000000	27427	22,285	114,157
16	BA1	198198	112112	188188	233233	311311	175175	224226	203211	213213	26427	22,285	114,157
16	BA5	000000	112112	188188	233233	000000	175175	224226	199207	000000	26427	32,715	130,802
16	BA6	198198	112116	188188	233233	311311	175175	000000	199207	213213	26427	32,715	130,802
17	KMT201	000000	112116	000000	233239	313313	175179	000000	199199	207207	27427	32,715	130,802
17	KMT203	196196	112116	188192	233239	313313	175179	226226	207207	213213	27427	22,285	114,157
17	KMT208	196196	112116	000000	233239	311311	000000	224240	199207	207207	27427	22,285	114,157
18	Fu1	000000	112116	188188	000000	311313	000000	224224	000000	209209	264274	22,285	114,157
18	Fu2	000000	112116	188188	000000	311313	000000	224224	000000	209209	264274	19,068	155,765
18	Fu3	000000	112114	000000	233235	311313	175175	226228	000000	209209	274274	19,068	155,765
19	Hw10	198198	110114	190194	000000	311311	175179	226226	199207	213213	274278	19,068	155,765
19	Hw2	200200	110114	192192	000000	319319	175177	000000	000000	203205	000000	35,689	139,69
19	Hw3	198198	114114	000000	233239	311311	175179	226226	000000	000000	274278	35,689	139,69
20	Jp1	198198	110112	190190	239239	311311	175179	226226	207207	213213	274274	35,689	139,69
20	Jp10	198198	102114	190194	233239	311311	175179	226226	199207	213213	274278	21,024	105,841
20	Jp2	198198	114114	190194	233239	311317	175179	226226	199207	213213	274278	21,024	105,841
21	Vt7	198198	110112	190194	233239	307311	173175	226226	199207	205205	278278	21,024	105,841
21	Vt16	196206	110114	162162	239239	309311	173175	212222	207207	213217	000000	21,024	105,841
21	Vt1	198198	112112	000000	239243	311313	173175	222222	237237	205205	274274	21,024	105,841
22	CRB6	226226	112114	000000	239245	307311	175175	222226	237237	205205	274274	21,024	105,841
22	CRB7	198198	112114	000000	239245	311311	175175	226226	207237	205213	274278	20,688	27,098
22	CRB8	198198	112116	000000	229239	311311	173175	226226	199207	209213	274278	20,688	27,098
23	ZA5	000000	116150	000000	249249	193311	161173	222222	000000	189189	000000	20,688	27,098
23	ZA6	000000	116150	000000	249249	193311	161173	222222	000000	213213	000000	33,784	32,861

23	ZA7	000000	116150	000000	249249	193311	161173	222222	000000	189213	000000	33,784	32,861
24	EHM108	192206	112116	188188	233239	311311	171173	228228	199199	213213	274274	33,784	32,861
24	EHM109	192206	112116	188188	233239	311311	171173	228228	207207	213213	274274	35,156	135,943
24	EHM110	192206	112112	188188	233239	307311	171173	228228	207207	213213	274274	35,156	135,943
25	CS19	196196	112112	192194	235235	311311	161173	226240	207207	207207	268282	35,156	135,943
25	CS21	196196	112116	190194	235235	311311	161173	226226	207207	207207	268282	35,156	135,943
25	CS22	182182	112116	192192	235235	311311	173173	226240	207207	207207	268282	35,156	135,943

Supporting Information 2. Discriminant Analysis of Principal Components results. (A) Bayesian Information Criterion (BIC) to *find.clusters* function. (B) Clusters selected by *find.clusters* function of DAPC and plotted through *table.value* function.



Supporting information 3. First migrants detected between *Corbicula* spp. populations across South America, invasive range and native range. Gray color indicates the migrants in α=0.05 probability.

			Populations ('-log(L))												
Assigned sample	Score	Probability	ARG2	IGU	ITU	GUA	CLM	GO	RJ	MAT	IMI	ROS	ITA	AA3	AB3
/1	0.000	0.582	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/2	0.000	0.578	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/14	0.000	0.528	1.232	2.075	2.075	2.075	2.173	2.283	2.075	2.226	2.185	2.173	2.075	2.075	3.124
/16	0.000	0.586	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/17	0.000	0.526	1.232	2.075	2.075	2.075	2.173	2.283	2.075	2.226	2.185	2.173	2.075	2.075	3.124
/20	0.000	0.583	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/47	0.000	0.579	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/48	0.000	0.579	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/49	0.000	0.527	1.232	2.075	2.075	2.075	2.173	2.283	2.075	2.226	2.185	2.173	2.075	2.075	3.124
/50	0.000	0.582	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/ARG2	0.000	0.580	1.517	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/202	1.637	0.218	1.441	3.078	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/203	1.637	0.238	1.441	3.078	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/IGU	1.637	0.247	1.441	3.078	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/374	1.637	0.232	1.441	2.587	3.078	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/375	1.637	0.236	1.441	2.587	3.078	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/ITU	1.637	0.242	1.441	2.587	3.078	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/529	1.637	0.238	1.441	2.587	2.587	3.078	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/533	1.637	0.239	1.441	2.587	2.587	3.078	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/GUA	1.637	0.227	1.441	2.587	2.587	3.078	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/184	1.805	0.112	1.441	2.587	2.587	2.587	3.246	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/185	1.301	0.162	0.991	1.899	1.899	1.899	2.292	2.009	1.899	2.050	2.009	1.899	1.899	1.899	2.346

/CLM	1.805	0.144	1.441	2.587	2.587	2.587	3.246	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/141	1.991	0.000	1.441	2.587	2.587	2.587	2.685	3.432	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/142	1.420	0.110	0.961	1.832	1.832	1.832	1.930	2.381	1.832	1.983	1.832	1.930	1.832	1.832	2.881
/GO	1.487	0.131	0.991	1.899	1.899	1.899	1.899	2.478	1.899	2.050	2.009	1.899	1.899	1.899	2.346
/281	1.637	0.227	1.441	2.587	2.587	2.587	2.685	2.795	3.078	2.738	2.697	2.685	2.587	2.587	3.636
/285	1.637	0.250	1.441	2.587	2.587	2.587	2.685	2.795	3.078	2.738	2.697	2.685	2.587	2.587	3.636
/RJ	1.637	0.222	1.441	2.587	2.587	2.587	2.685	2.795	3.078	2.738	2.697	2.685	2.587	2.587	3.636
/349	1.108	0.250	1.187	1.955	1.955	1.955	2.053	2.163	1.955	2.295	2.065	2.053	1.955	1.955	2.557
/350	1.895	0.136	1.441	2.587	2.587	2.587	2.685	2.795	2.587	3.336	2.697	2.685	2.587	2.587	3.636
/MAT	1.895	0.125	1.441	2.587	2.587	2.587	2.685	2.795	2.587	3.336	2.697	2.685	2.587	2.587	3.636
/440	1.823	0.114	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	3.264	2.685	2.587	2.587	3.636
/441	1.252	0.155	0.961	1.832	1.832	1.832	1.930	1.930	1.832	1.983	2.213	1.930	1.832	1.832	2.881
/IMI	1.823	0.129	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	3.264	2.685	2.587	2.587	3.636
/523	1.301	0.134	0.991	1.899	1.899	1.899	1.899	2.009	1.899	2.050	2.009	2.292	1.899	1.899	2.346
/524	1.805	0.129	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	3.246	2.587	2.587	3.636
/ROS	1.805	0.129	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	3.246	2.587	2.587	3.636
/488	1.637	0.225	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	3.078	2.587	3.636
/489	1.637	0.232	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	3.078	2.587	3.636
/ITA	1.637	0.242	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	3.078	2.587	3.636
/AA1	1.637	0.220	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	3.078	3.636
/AA2	1.637	0.225	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	3.078	3.636
/AA3	1.637	0.216	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	3.078	3.636
/AB1	0.023	0.758	3.599	3.791	3.791	3.791	3.764	3.875	3.791	3.817	3.901	3.764	3.791	3.791	3.300
/AB2	0.023	0.780	3.599	3.791	3.791	3.791	3.764	3.875	3.791	3.817	3.901	3.764	3.791	3.791	3.300
/AB3	0.023	0.782	3.599	3.791	3.791	3.791	3.764	3.875	3.791	3.817	3.901	3.764	3.791	3.791	3.300
/S1	0.090	0.669	4.554	4.393	4.393	4.393	4.366	4.477	4.393	4.419	4.503	4.366	4.393	4.393	3.432

/S2	0.090	0.636	4.554	4.393	4.393	4.393	4.366	4.477	4.393	4.419	4.503	4.366	4.393	4.393	3.432
/S3	0.090	0.656	4.554	4.393	4.393	4.393	4.366	4.477	4.393	4.419	4.503	4.366	4.393	4.393	3.432
/Rlc1	0.000	0.563	6.182	5.442	5.442	5.442	5.415	5.498	5.442	5.322	5.525	5.415	5.442	5.442	4.840
/Rlc2	0.000	0.531	6.182	5.442	5.442	5.442	5.415	5.498	5.442	5.322	5.525	5.415	5.442	5.442	4.840
/Rlc4	0.000	0.532	6.182	5.442	5.442	5.442	5.415	5.498	5.442	5.322	5.525	5.415	5.442	5.442	4.840
/BA1	0.247	0.387	3.053	3.597	3.597	3.597	3.667	3.750	3.597	3.623	3.679	3.667	3.597	3.597	3.840
/BA5	0.000	0.671	2.797	3.085	3.085	3.085	3.155	3.238	3.085	3.111	3.168	3.155	3.085	3.085	3.328
/BA6	0.364	0.194	1.719	2.363	2.363	2.363	2.433	2.515	2.363	2.363	2.445	2.433	2.363	2.363	2.363
/KMT201	0.000	0.946	3.371	3.402	3.402	3.402	3.500	3.610	3.402	3.402	3.512	3.500	3.402	3.402	4.004
/KMT203	1.055	0.069	3.626	4.034	4.034	4.034	4.132	4.242	4.034	4.185	4.144	4.132	4.034	4.034	5.083
/KMT208	1.279	0.002	3.432	3.346	3.346	3.346	3.346	3.456	3.346	3.226	3.456	3.346	3.346	3.346	3.346
/Fu1	0.000	0.813	3.906	3.193	3.193	3.193	3.193	3.193	3.193	3.073	3.193	3.193	3.193	3.193	3.193
/Fu2	0.000	0.819	3.906	3.193	3.193	3.193	3.193	3.193	3.193	3.073	3.193	3.193	3.193	3.193	3.193
/Fu3	1.715	0.180	4.820	4.597	4.597	4.597	4.667	4.653	4.597	4.623	4.583	4.667	4.597	4.597	4.238
/Hw10	2.084	0.107	0.961	1.832	1.832	1.832	1.930	1.930	1.832	1.983	1.832	1.930	1.832	1.832	2.881
/Hw2	0.216	0.971	3.970	3.249	3.249	3.249	3.222	3.222	3.249	3.249	3.249	3.222	3.249	3.249	3.249
/Hw3	3.051	0.016	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/Jp1	1.723	0.209	1.707	2.791	2.791	2.791	2.889	2.971	2.791	2.942	2.873	2.889	2.791	2.791	3.840
/Jp10	1.864	0.034	1.441	2.587	2.587	2.587	2.685	2.795	2.587	2.738	2.697	2.685	2.587	2.587	3.636
/Jp2	1.211	0.570	2.395	3.189	3.189	3.189	3.287	3.397	3.189	3.340	3.299	3.287	3.189	3.189	4.238
/Vt7	1.831	0.000	3.475	3.791	3.791	3.791	3.764	3.875	3.791	3.942	3.901	3.764	3.791	3.791	3.636
/Vt16	0.016	0.601	6.182	5.442	5.442	5.442	5.415	5.498	5.442	5.322	5.525	5.415	5.442	5.442	4.840
/Vt1	0.192	0.502	6.995	5.840	5.840	5.840	5.813	5.799	5.840	5.720	5.826	5.813	5.840	5.840	5.238
/CRB6	0.000	0.813	4.820	4.597	4.597	4.597	4.667	4.653	4.597	4.623	4.583	4.667	4.597	4.597	4.840
/CRB7	0.774	0.190	2.787	3.393	3.393	3.393	3.463	3.449	3.393	3.544	3.378	3.463	3.393	3.393	3.840
/CRB8	0.563	0.194	3.599	3.791	3.791	3.791	3.764	3.750	3.791	3.942	3.776	3.764	3.791	3.791	3.636

/ZA5	0.000	0.501	9.153	7.044	7.044	7.044	6.893	6.753	7.044	6.924	6.905	6.893	7.044	7.044	6.442
/ZA6	0.000	0.500	9.153	7.044	7.044	7.044	6.893	6.753	7.044	6.924	6.905	6.893	7.044	7.044	6.442
/ZA7	0.000	0.500	9.153	7.044	7.044	7.044	6.893	6.753	7.044	6.924	6.905	6.893	7.044	7.044	6.442
/EHM108	0.000	0.604	6.040	5.238	5.238	5.238	5.086	5.197	5.238	5.118	5.348	5.086	5.238	5.238	3.636
/EHM109	0.000	0.595	6.040	5.238	5.238	5.238	5.086	5.197	5.238	5.118	5.348	5.086	5.238	5.238	3.636
/EHM110	0.000	0.584	6.995	5.840	5.840	5.840	5.688	5.799	5.840	5.720	5.951	5.688	5.840	5.840	4.238
/CS19	0.000	0.500	6.837	5.597	5.597	5.597	5.445	5.306	5.597	5.623	5.458	5.445	5.597	5.597	5.238
/CS21	0.000	0.500	5.758	4.995	4.995	4.995	4.843	4.704	4.995	5.146	4.856	4.843	4.995	4.995	4.840
/CS22	0.000	0.501	6.837	5.597	5.597	5.597	5.445	5.306	5.597	5.623	5.458	5.445	5.597	5.597	4.840

Continuation

			Populations ('-log(L))											
Assigned sample	Score	Probability	S3	Rlc4	BA6	KM T208	Fu3	Hw3	Jp2	Vt1	CRB8	ZA7	EHM1 10	CS22
/1	0.000	0.582	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/2	0.000	0.578	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/14	0.000	0.528	3.124	4.483	3.342	2.722	4.033	2.647	2.103	3.550	3.228	5.930	4.726	4.754
/16	0.000	0.586	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/17	0.000	0.526	3.124	4.483	3.342	2.722	4.033	2.647	2.103	3.550	3.228	5.930	4.726	4.754
/20	0.000	0.583	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/47	0.000	0.579	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/48	0.000	0.579	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/49	0.000	0.527	3.124	4.483	3.342	2.722	4.033	2.647	2.103	3.550	3.228	5.930	4.726	4.754
/50	0.000	0.582	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/ARG2	0.000	0.580	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/202	1.637	0.218	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/203	1.637	0.238	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266

/IGU	1.637	0.247	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/374	1.637	0.232	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/375	1.637	0.236	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/ITU	1.637	0.242	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/529	1.637	0.238	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/533	1.637	0.239	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/GUA	1.637	0.227	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/184	1.805	0.112	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/185	1.301	0.162	2.793	4.152	2.938	3.117	3.913	2.617	2.052	3.219	2.751	4.997	3.471	3.374
/CLM	1.805	0.144	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/141	1.991	0.000	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/142	1.420	0.110	3.328	4.328	2.871	3.148	3.640	2.379	1.957	3.550	2.508	4.930	4.608	3.307
/GO	1.487	0.131	2.793	4.152	2.938	3.117	3.913	2.617	2.052	3.219	2.751	4.997	3.471	3.374
/281	1.637	0.227	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/285	1.637	0.250	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/RJ	1.637	0.222	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/349	1.108	0.250	3.004	3.363	2.804	2.722	3.390	2.647	2.108	3.208	3.108	4.810	3.284	4.363
/350	1.895	0.136	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/MAT	1.895	0.125	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/440	1.823	0.114	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/441	1.252	0.155	3.328	4.328	2.871	3.148	3.640	2.379	1.957	3.550	2.508	4.930	4.608	3.307
/IMI	1.823	0.129	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/523	1.301	0.134	2.793	4.152	2.938	3.117	3.913	2.617	2.052	3.219	2.751	4.997	3.471	3.374
/524	1.805	0.129	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/ROS	1.805	0.129	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/488	1.637	0.225	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266

/489	1.637	0.232	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/ITA	1.637	0.242	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/AA1	1.637	0.220	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/AA2	1.637	0.225	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/AA3	1.637	0.216	4.083	5.442	3.985	3.903	4.992	3.430	2.740	4.509	3.865	6.889	5.363	5.266
/AB1	0.023	0.758	3.277	4.840	4.286	4.681	4.868	4.384	3.944	4.208	4.108	6.287	3.916	5.044
/AB2	0.023	0.780	3.277	4.840	4.286	4.681	4.868	4.384	3.944	4.208	4.108	6.287	3.916	5.044
/AB3	0.023	0.782	3.277	4.840	4.286	4.681	4.868	4.384	3.944	4.208	4.108	6.287	3.916	5.044
/S1	0.090	0.669	3.522	4.636	4.763	4.283	4.663	4.861	4.488	4.305	4.652	6.685	4.460	5.646
/S2	0.090	0.636	3.522	4.636	4.763	4.283	4.663	4.861	4.488	4.305	4.652	6.685	4.460	5.646
/S3	0.090	0.656	3.522	4.636	4.763	4.283	4.663	4.861	4.488	4.305	4.652	6.685	4.460	5.646
/Rlc1	0.000	0.563	4.481	3.476	6.086	4.964	5.566	5.685	5.333	4.606	5.032	6.685	5.509	6.345
/Rlc2	0.000	0.531	4.481	3.652	6.086	4.964	5.566	5.685	5.333	4.606	5.032	6.685	5.509	6.345
/Rlc4	0.000	0.532	4.481	3.652	6.086	4.964	5.566	5.685	5.333	4.606	5.032	6.685	5.509	6.345
/BA1	0.247	0.387	4.287	5.889	3.300	4.283	3.992	4.111	3.944	4.935	4.467	6.889	5.567	5.743
/BA5	0.000	0.671	3.328	4.930	2.425	3.102	3.033	3.328	3.307	3.976	3.830	5.930	4.930	5.231
/BA6	0.364	0.194	2.810	3.810	2.083	3.102	2.913	2.851	2.710	3.333	3.166	4.810	3.488	4.363
/KMT201	0.000	0.946	3.004	3.363	3.980	2.861	3.390	3.823	3.430	3.731	4.430	5.810	4.606	5.810
/KMT203	1.055	0.069	4.083	5.442	5.161	4.680	4.992	4.606	4.062	5.032	5.187	7.889	6.685	6.713
/KMT208	1.279	0.002	3.793	4.152	3.239	4.504	3.691	3.793	3.499	3.997	4.073	4.997	3.471	4.073
/Fu1	0.000	0.813	2.834	2.834	2.301	2.265	2.046	3.219	3.260	3.135	3.260	3.436	3.260	3.193
/Fu2	0.000	0.819	2.834	2.834	2.301	2.265	2.046	3.219	3.260	3.135	3.260	3.436	3.260	3.193
/Fu3	1.715	0.180	3.879	5.685	4.161	4.584	5.594	4.764	4.789	5.208	5.011	7.287	5.664	5.500
/Hw10	2.084	0.107	3.328	4.328	2.871	3.148	3.640	3.045	1.957	3.550	2.508	4.930	4.608	3.307
/Hw2	0.216	0.971	3.249	3.249	2.867	3.222	3.038	3.083	3.249	3.249	3.073	3.851	3.851	3.851
/Hw3	3.051	0.016	4.083	5.442	3.985	3.903	4.992	4.492	2.740	4.509	3.865	6.889	5.363	5.266

/Jp1	1.723	0.209	4.287	4.840	4.830	4.107	5.293	3.555	3.430	4.333	3.467	6.889	5.567	5.266
/Jp10	1.864	0.034	4.083	5.442	3.985	3.903	4.992	3.430	3.305	4.509	3.865	6.889	5.363	5.266
/Jp2	1.211	0.570	4.481	5.840	4.462	4.204	5.390	3.907	3.606	4.907	4.409	7.287	5.907	5.868
/Vt7	1.831	0.000	3.879	5.238	4.462	4.681	5.390	4.384	3.886	5.305	3.807	6.685	5.004	5.169
/Vt16	0.016	0.601	5.083	4.636	6.086	5.663	6.169	5.685	5.333	4.652	4.731	5.840	5.509	6.345
/Vt1	0.192	0.502	4.879	4.636	6.086	5.362	5.566	5.861	5.810	4.828	4.953	5.238	5.907	6.345
/CRB6	0.000	0.813	5.083	5.442	5.006	5.283	5.566	4.764	4.567	4.208	4.162	6.442	6.208	6.345
/CRB7	0.774	0.190	4.287	5.044	4.228	4.681	4.992	3.810	3.421	4.412	3.560	6.889	5.965	5.266
/CRB8	0.563	0.194	4.083	4.840	4.830	4.982	5.293	4.208	3.819	4.208	4.162	6.287	5.363	4.567
/ZA5	0.000	0.501	6.685	6.685	6.931	7.140	6.646	6.764	7.111	5.685	6.333	3.078	6.509	5.868
/ZA6	0.000	0.500	6.685	6.685	6.931	7.140	6.646	6.764	7.111	5.685	6.333	3.078	6.509	5.868
/ZA7	0.000	0.500	6.685	6.685	6.931	7.140	6.646	6.764	7.111	5.685	6.333	3.078	6.509	5.868
/EHM108	0.000	0.604	4.083	5.442	5.609	5.635	5.470	5.685	5.391	5.287	5.664	6.287	3.254	5.743
/EHM109	0.000	0.595	4.083	5.442	5.609	5.635	5.470	5.685	5.391	5.287	5.664	6.287	3.254	5.743
/EHM110	0.000	0.584	4.481	5.840	6.086	5.936	5.868	6.162	5.935	5.384	5.907	6.685	3.555	6.345
/CS19	0.000	0.500	5.685	6.287	5.977	6.061	5.470	5.588	5.722	5.810	5.488	5.685	5.965	3.180
/CS21	0.000	0.500	5.287	6.287	5.675	6.061	5.293	5.111	5.120	5.509	4.944	5.685	5.965	3.305
/CS22	0.000	0.501	5.287	5.889	5.977	6.061	5.470	5.588	5.722	5.412	5.312	5.889	5.567	3.305

CAPÍTULO IV

Looking for a needle in a haystack: molecular detection of larvae of invasive *Corbicula* clams[1]

Abstract

The invasive bivalves *Corbicula* spp. and *Limnoperna fortunei* predominate in South American rivers. They can be sympatric in distribution, and because their larval stages are morphologically similar, monitoring them in zooplankton using microscopy protocols is often inefficient, producing ambiguous results. We designed a pair of primers to amplify a fragment of the mtDNA cytochrome c oxidase subunit I of *Corbicula* species. A multiplex reaction, containing the specific primer pair and a pair of universal primers (to control for the quality of the DNA in the sample) was tested with regards to specificity and ability to detect *Corbicula* spp. larvae in plankton samples that also contain other species in different proportions. Our molecular protocol allows for fast and accurate detection of *Corbicula* spp. even when concentrations of these species are low in samples, which is useful when examining large volumes of ballast/piped water. Further, the protocol is valuable for the monitoring/prospecting of early stages of the life cycle of *Corbicula* spp. in watersheds that have been invaded, or which are considered at risk of invasion by these species.

Keywords: mtDNA; Asian clam; molecular markers; zooplankton; prospecting; larvae.

Running title: Molecular detection of invasive *Corbicula* larvae

[1] Este capítulo está publicado na Management of Biological Invasions (see Supporting Information 1).

Introduction

Invasive species are often associated with loss of biodiversity (Rosa et al. 2011; Sousa et al. 2013; Pigneur et al. 2014), changes in native communities (Schlaepfer et al. 2005) and even accelerated extinction of native species (Clavero and Garcia-Berthou 2005). Additionally, some invasive species damage artificial structures and impact economic activities (Rosa et al. 2011). Successful invasive organisms, for instance the South American bivalves *Corbicula fluminea* (Müller 1774) and *Limnoperna fortunei* (Dunker 1857), often disperse efficiently using a combination of natural and human-mediated mechanisms (Cox 2004).

Corbicula clams and *L. fortunei* (the "golden mussel") were accidentally introduced to South America, most likely by ballast water (Darrigran and Pastorino 1993). These invaders often occur sympatrically (Darrigran 2002) and are still expanding their distribution in this continent (Oliveira et al. 2010). According to Pigneur et al. (2014), *Corbicula* spp. are particularly efficient invaders of river systems, reaching densities of up to several thousands of individuals per square metre in the Rio Paraná, Argentina. *Corbicula* spp. clams can reduce phytoplankton density and compete with native bivalve species of Mycetopodidae and Hyriidae (Santos et al. 2012). The golden mussel, on the other hand, has caused many problems for South American hydroelectric power plants by fouling in cooling ducts (Darrigran and Damborenea 2009; Belz et al. 2012). Management and control strategies need to be implemented for these species where they are present, and should include continuous evaluations of propagule pressure in new habitats (Darling & Blum 2007).

Active search for individuals is frequently used to monitor invasive bivalves. Adult specimens can be found in the substrate, and larval stages present in plankton are detected using optical microscopy (Pestana et al. 2008; Lopes and Vieira 2012). However, zooplankton monitoring is often inefficient (Mansur et al. 2012a). According to Darrigran et al. (2009), this is in part because larvae of *Corbicula* clams and the golden mussel are very similar (Figure 1), which makes species determination under the microscope difficult, repetitive and tedious. One (untested) strategy that is often adopted by surveyors is to assume that all free-living bivalve larvae found in freshwater plankton samples are golden mussels. This strategy is based on the premise that all larval stages of native bivalves are exclusively parasitic (glochidia of Mycetopodidae and Hyriidae species; Mansur et al. 2012b; Gatlin et al., 2013) and/or that some species of *Corbicula* incubate their initial larval stages in the gills of their parents (Martins et al. 2006; Houki et al. 2011; Mansur et al. 2012b).

A molecular protocol for monitoring golden mussel larvae (Pie et al., 2006) has been widely used in hydroelectric power plants in Southern Brazil (Boeger et al., 2007). Combined with microscopic procedures, this protocol assists with larval identification and informs decision-makers regarding the need for management interventions such as the chemical control of larval settlements in the cooling system of turbines. However, this molecular protocol has failed to detect *L. fortunei* larvae in zooplankton samples in the past, even when large numbers of bivalve larvae were detected under the microscope. These results have prompted us to ask whether the early developmental stages present in these samples were in fact larvae of *Corbicula* spp., since no other

freshwater species of bivalves in the freshwater environs of South America release larvae in the plankton.

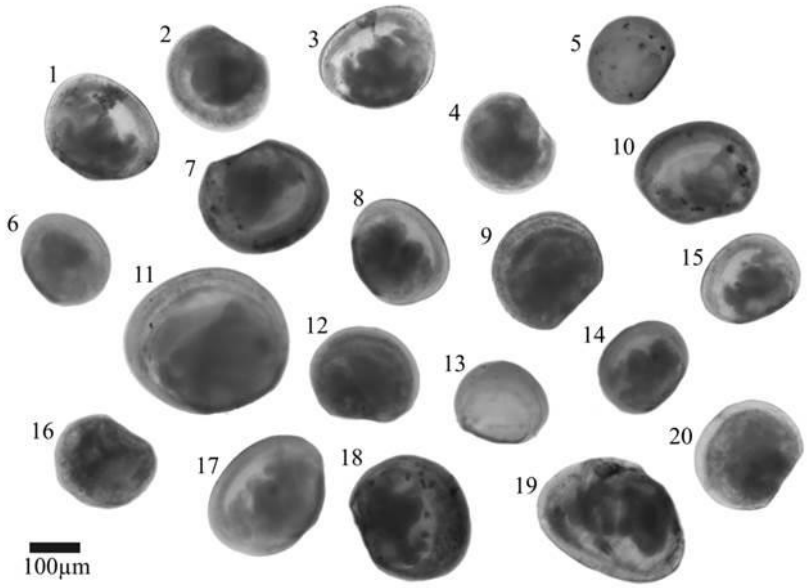


Fig. 1 Larvae showing how similar *Corbicula* spp. (2, 4, 6, 7, 9, 10, 11, 12, 14, 16, 18, 20) and *L. fortunei* (1, 3, 5, 8, 13, 15, 19) are at these stages in their life cycle.

We believe that markers are needed for species of *Corbicula* because the taxonomy of the genus is uncertain and species determination is difficult due to morphological plasticity/variability (Lee et al. 2005; Pigneur et al. 2011). As a consequence, the species composition of invasive *Corbicula* clams in South America and in other continents is largely questionable (Pfenninger et al. 2002; Lee et al. 2005; Hedtke et al. 2008; Pigneur et al. 2011). We therefore developed a molecular protocol for the detection of *Corbicula* species, similar to the one available for *L. fortunei* (Pie et al., 2006), with the following goals in mind: (i) to provide a tool to monitor the temporal and spatial availability of bivalve larvae; (ii) to facilitate identification of adults and larvae; (iii) to investigate whether free early larval stages of *Corbicula* (outside their parents' gills) are common in plankton samples, and (iv) to ascertain whether *Corbicula* spp. occur sympatrically with *L. fortunei* larvae in South America.

Materials and Methods

Sampling and DNA extraction

Zooplankton samples and adult specimens of *Corbicula* spp. and *L. fortunei* were collected from reservoirs and Hydroelectric power stations (UHE) in southern Brazil (Table 1). Zooplankton samples were collected by filtering 4,000 L of water through a plankton net (64 µm mesh size), following Tschá et al. (2012). Two independent zooplankton samples were obtained from each collecting point and were preserved in 96% ethanol and taken to the laboratory. One zooplankton sample from

each collecting point was processed under the dissecting scope and each identified bivalve larva was transferred to a microscope slide. Larval stage determination, based on Santos et al. (2005), was performed under a light microscope. Whole DNA extracts of zooplankton samples (from the second zooplankton sample per collection point) and DNA extracts from individual larvae were subjected to molecular protocols for the identification of *Corbicula* spp. and *L. fortunei*.

Total genomic DNA was extracted from the mantle tissue of adult *Corbicula* spp. (n=10 specimens) and one specimen of each *Crassostrea gigas* (Thunberg 1793), *Modiolus brasiliensis* Chemnitz 1795, *Thais* sp. (Röding 1798), *Melanoides tuberculatus* (Müller 1774), and from each bivalve larvae isolated from the zooplankton samples, using the EZ-DNA kit (Biosystems, Brazil), following the manufacturer's instructions. The concentration of all DNA products was measured using a NanoDrop 3300 (Thermo Scientific).

Design of specific COI primers for Corbicula *spp.*

A mtDNA fragment (700 bp approx.) from the cytochrome oxidase subunit I (COI) gene was amplified from the DNA of adult specimens of *Corbicula spp.* using a universal primer pair (LCO and HCO, Table 2). DNA was amplified in 25 µL reactions with 2-3 ng/µL of template DNA, 2 mM of $MgCl_2$, 0.4 mM of dNTPs, 1X buffer, 1.25 U of AmpliTaq DNA Polymerase and 0.5-1 mM of each primer. The following program protocol was used to obtain products: initial denaturation at 95°C for 5 min, followed by 35 cycles of 30s at 92°C, 30s at 48-51°C, 30s at 68°C, and final extension at 68°C, for 2 min. Amplified fragments were sequenced in laboratory, in both directions, using Applied Biosystems 3130 automatic sequencer and the same amplification primers. Sequences were assembled, edited and a consensus was generated using Geneious® 6.1.2 (Biomatters; Available at http://www.geneious.com/).

We compiled 25 COI sequences (600 bp approx. after trimming) derived from ten adults of *Corbicula* spp., as well as sequences from closely related species available on GenBank (Table 3), and aligned all these sequences based on the frequency of mismatches between them. Transversions and gaps were given more weight. Based on this alignment, unique regions were identified in the COI sequences of *Corbicula* spp., and a primer pair was designed to amplify 400 bp of their mtDNA (Table 2). Primer sequences were tested with GenBank's Basic Local Alignment Search Tool (BLASTn) (at https://www.ncbi.nlm.nih.gov/) to ensure that the designed primers would match only COI sequences of *Corbicula* spp.

Development of a Multiplex PCR assay

A multiplex PCR assay was developed using a pair of invertebrate universal primers that amplify an 800 bp fragment of nuclear 18S rDNA (Table 2) in addition to the specific COI marker that we developed for *Corbicula* spp. The universal 18S rDNA primer pair serves as positive control to account for variable DNA quality (Pie et al., 2006; King et al. 2009; Ludwig et al. 2011) and inhibition of the PCR of each individual sample. The Multiplex reaction was optimized by changing primer concentrations, DNA template concentration, and annealing temperature and time.

Specificity tests were conducted by testing the designed primers against samples of other mollusk species found in South America: *L. fortunei*, *C. gigas*, *M. brasiliensis*, *Thais* sp. and *M. tuberculatus* (some of these species were chosen also to test against species that could be found in ballast water). These tests ensured that the designed primers were specific to *Corbicula* spp., and that DNA from other species in a multiplex reaction would not result in cross-amplification.

In addition, a sensitivity and specificity test was performed by adding the equivalent of the DNA content of one, two, four and sixteen bivalve larvae to total DNA aliquots derived from a zooplankton sample that did not contain *Corbicula* spp. (confirmed by the absence of the specific band in the application of the multiplex PCR designed herein). The DNA content of a single larva of *Corbicula* spp. was estimated from larvae of *L. fortunei* (Pie, 2006), since they have similar size (approximately 28.5 ng of DNA per larva). The equivalent volume of the simulated number of larvae was added to an extract of 50µL of the full genomic DNA of the zooplankton sample (500 ng/µL). This plankton sample was primarily composed of cyclopoid copepods, bivalve larvae, insect larvae, cladocerans, tardigrades, nauplii larvae, and mites.

An additional test involved the use of environmental plankton samples obtained from distinct regions of Brazil (see Table 1). In order to identify each larva collected to the species level, and to evaluate the presence of the species in plankton samples, molecular markers specific for *L. fortunei* (e.g. Pie et al. 2006) were used in parallel with the molecular markers for *Corbicula* spp. developed in this study (Table 2).

Results

We designed a pair of primers to amplify a 400 bp fragment of the mitochondrial DNA COI gene of *Corbicula* spp. (Table 3). The optimized conditions for the Multiplex PCR assay are the following: initial denaturation of 5 min at 95°C, 35 cycles of 94°C for 30s, 44°C for 30s and 72°C for 40s, and final extension of 5 min at 72°C. The amplification reaction (25 µL) consisted of 3 mM of $MgCl_2$, 0.4 mM of dNTPs, 1X buffer, 2.5 U of AmpliTaq DNA Polymerase, and 4 mM of specific primers. PCR products were analyzed using electrophoresis in 1.5% agarose gel to compare the size of each amplified fragment with a marker of known size (Ladder 1Kb Invitrogen®). All sequences obtained by us from these fragments matched 100% with *Corbicula* spp. sequences in BLASTn (at https://www.ncbi.nlm.nih.gov/).

The specificity test revealed that the designed primers did not amplify the DNA of any other taxon tested (Fig. 2A). The combined tests of sensitivity and specificity with plankton samples spiked with specific amounts of *Corbicula* spp. DNA, demonstrating that the protocol above is capable of detecting the DNA of a single larva when this DNA is pooled with DNA extracted from a plankton sample (Fig. 2B).

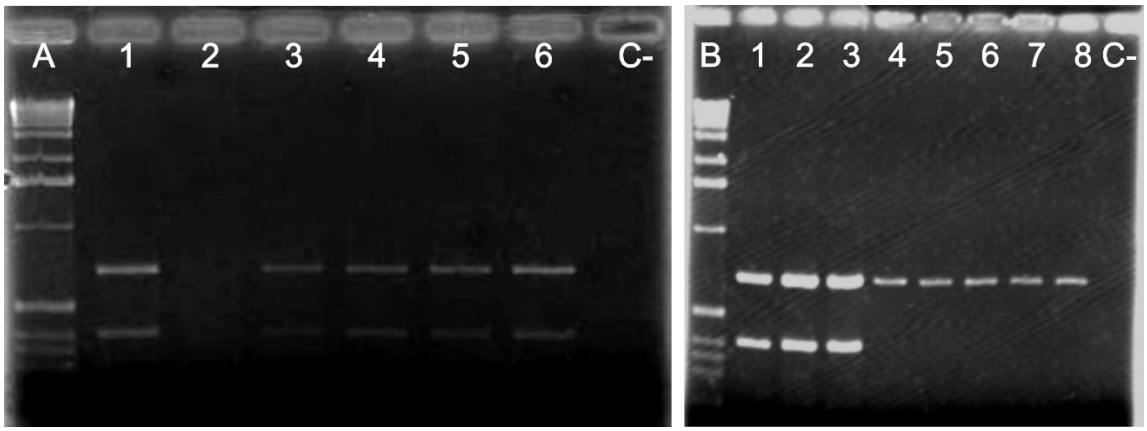


Fig. 2 Performance of the *Corbicula* spp. markers. **A)** Larval detection test: (1) Extract of *Corbicula fluminea* adult; (2) Extract of plankton sample without *Corbicula* spp. DNA; (3) Plankton sample with DNA of one *Corbicula* larva; (4) Plankton sample with DNA of two *Corbicula* larvae*;* (5) Plankton sample with DNA of four *Corbicula* larvae*;* (6) Plankton sample with DNA of sixteen *Corbicula* larvae*;* (C-) Negative control. **B)** Specificity test: (1) Extract of *Corbicula fluminea* adult, (2) Extract of *Corbicula largillierti* adult, (3) Extract of *Corbicula* sp. adult, (4) Extract of *Limnoperna fortunei* adult, (5) Extract of *Crassostrea gigas* adult, (6) Extract of *Modiolus brasiliensis* adult, (7) Extract of *Thais* adult; (8) Extract of *Melanoides tuberculatus,* (C-) Negative control. For both tests, the upper band is the quality control markers and the lower band is the specific molecular markers of *Corbicula* spp.

As a whole, the quality of the DNA in the environmental plankton samples processed in this study was adequate (non-degraded), as indicated by the positive amplification of the 18S rDNA fragment (Figure 2A). After identifying the early bivalve larval stages in the zooplankton of Southern Brazilian rivers (Table 1) we separated 160 larvae for molecular identification, of which only 129 had adequate amounts of DNA for molecular analysis. After application of the molecular identification protocol on both species, 49 larvae were identified as *Corbicula* spp. and 80 as *L. fortunei* (Table 1). D-shaped larva (see Santos et al., 2005) was the most common stage found for both species. Larvae of *Corbicula* and *L. fortunei* were detected in sympatry by the Multiplex reaction applied to zooplankton samples and to individual larvae in one location, UHE Jauru (Table 1).

Table 1 Sampling sites for the collection of zooplankton and specimens of *Corbicula* spp. and *L. fortunei*. The use of specific molecular protocols (this work; Pie et al., 2006) determined the presence (+) or absence (-) of larvae of both species in each location.

Sites	**Coordinates**	**Collection Date**	**Plankton Results**		**Larvae Results**	
			L. fortunei	***Corbicula* spp.**	***L. fortunei***	***Corbicula* spp.**
UHE[1] Caxias	25°38'8"S 53°20'43"W	May 2011	+	-	24	-
Uruguay River	29°70'42"S 56°33'28"W	April 13th, 2011	-	+	-	19
UHE Jauru	15°12'51"S 58°43'45"W	October 2010	+	+	56	2
UHE Mauá	24°03'48"S 50°42'05"W	December 2011	-	+	-	27
Ibicuí River	29°35'50.82"S 55°28'54.86"W	April 14th, 2011	-	+	-	1
Das Antas River	29°05'16"S 51°42'59"W	April 14th, 2011	-	-	-	-
Jacuí River	30°03'48"S 52°53'39"W	April 15th, 2011	-	-	-	-
				Total	**80**	**49**

Table 2 List of primers used in the development of the molecular detection protocol for *Corbicula* spp.

Primer	**Sequence (5'- 3')**	**Gene**	**Primer type**	**Reference**
LCO	GGTCAACAAATCATAAAGATATTGG	COI	Universal	Folmer et al.(1994)
HCO	TAAACTTCAGGGTGACCAAAAATCA			
CorbF2	GCTATTCCAGGGACTTTA	COI	Specific	This study
CorbR2	GCTCCAGGACGCATACAA			
7F	GCCCTATCAACTTACGATGGTA	18S	Universal	Modified from Telford (2000)
1100R	GATCGTCTTCGAACCTCTG			
Limno. COIR1	TCCAACCAGTCCCTACTCCACCCTCTA	COI	Specific	Pie et al. (2006)
Limno. COIF1	TTTAGAGTTAGCACGTCCTGGTAGGTT			

Table 3 Species list and their GenBank accession numbers used in the development of *Corbicula* spp. molecular markers.

Species	Location	GenBank number
C. fluminea	Korea	AF196269
C. fluminalis	Netherlands	AF269096-8
C. sandai	Japan	AF196273
C. fluminea	USA	U47647
C. japonica	Japan	AF196271
C. sp (form C)	Argentina	AF519512
C. sp (form A)	USA	AF519495-507
C. fluminea	France	AF269094
C. fluminea	Thailand	AF196270
Polymesoda caroliniana	USA	AF196276
Mya arenaria	USA	AF120668
Batissa violacea	Germany	DQ837726
Achatinella mustelina	USA	AY044338
Limnoperna fortunei	Brazil	DQ264395
Neocorbicula limosa	Argentina	AF196277

Discussion

Implementation of the specific molecular protocol to detect/identify *Corbicula* spp. larvae in environmental samples demonstrated that one or more species of this genus were present in the plankton of 4 out of 7 locations sampled. Since the current protocol cannot distinguish among *Corbicula* species, positive results may indicate the presence of one or more of the species recorded from Brazil (*C. fluminea*, *C. largillierti* and *C.* cf. *fluminalis*) (see Martins et al. 2004; Mansur et al. 2012a). Efforts are currently being made in our laboratory to improve this molecular protocol in order to differentiate among all species of *Corbicula* that occur in South America.

Our results contradict the notion that all species of *Corbicula* incubate in the demi-gills (e.g. Cataldo & Boltovskoy 2000; Martins et al. 2006) and suggest that their larval stages occur in sympatry with early larvae of *L. fortunei* (Table 1). Therefore, the widespread strategy currently used in South America for the microscopic detection and quantification of *L. fortunei* larvae, which assumes that all free-living bivalve larvae found in freshwater plankton samples are golden mussels, is inappropriate. While most freshwater *Corbicula* are hermaphrodites and ovoviviparous, with incubation in the maternal gill (e.g. Glaubrecht et al. 2006), some species do employ different reproduction modes, as reported by Byrne et al. (2000) for *Corbicula australis* (Deshayes 1830). *Corbicula australis* is dioecious and incubates veliger to pediveliger larvae in the inner demibranchs. There are published records of *C. fluminea* being hermaphroditic, incubating juveniles in outer demibranchs and releasing planktonic

veliger larvae (McMahon 2002). *Corbicula fluminalis* is not known to incubate larvae in its gills (e.g. Korniushin 2004).

Although the molecular detection protocol designed herein reveals only the presence/absence of *Corbicula* spp. larvae in plankton, this information can aid in studies on propagule pressure, which will allow for rapid effective management response and preparedness (e.g. Darling and Blum 2007). However, in order to increase the usefulness of the results derived from this method, the present protocol needs to be improved to allow quantification of larvae by real-time PCR, as proposed by Endo et al. (2009). Quantification of larvae of *L. fortunei* and *Corbicula* spp. is fundamental to guide methods of control, especially in the definition of dosages of anti-fouling and/or molluscicide products usually applied to semi-closed water systems such as cooling systems of hydroelectric power plants.

Early detection, which allows for rapid response, is crucial for integrated programs of management and control of invasive species (e.g. Molnar et al. 2008). However, early detection is often difficult when the invasive organism is small, inconspicuous and/or difficult to identify. Detecting invasive species during the first phases of an invasion, when they are still in low concentrations, is important for successful intervention. Therefore, our protocol represents an important tool to monitor and understand the biology and larval dispersal capacity of *Corbicula* species in continental waters. Similar monitoring has been applied systematically to *L. fortunei* since 2006 by technicians of the Instituto Lactec (http://www.institutoslactec.org.br/), COPEL (http://www.copel.com), ELEJOR (http://www.elejor.com.br/), Eletronorte (http://www.eln.gov.br), Tractebel (http://www.tractebelenergia.com.br/wps/portal/internet) and CEMIG (http://www.cemig.com.br) (unpublished information). We suggest that the molecular protocol to detect *Corbicula* spp. larvae is applied together with the protocol for the detection of *L. fortunei*. By doing so, technicians can decide on control measures to be adopted based on the propagules of the prevailing species detected.

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References

Belz CE, Darrigran G, Netto OSM, Boeger WA and Junior PJR (2012) Analysis of four dispersion vectors in Inland Waters: the case of the invading bivalves in South America. J Shell Res 31: 777-784. http://dx.doi.org/10.2983/035.031.0322

Byrne M, Phelps H, Church T, Adair V et al. (2000) Reproduction and development of the freshwater clam *Corbicula australis* in southeast Australia. Hydrobiologia 418: 185 – 197. http://dx.doi.org/10.1023/A:1003986512832

Boeger WA, Pie MR, Falleiros RM et al (2007) Testing a molecular protocol to monitor the presence of golden mussel larvae (*Limnoperna fortunei*) in plankton samples**.** J Plankton Res 29: 1015-1019. http://dx.doi.org/10.1093/plankt/fbm075

Cataldo D and Boltovskoy D (2000) Yearly reproductive activity of *Limnoperna fortunei* (Bivalvia) as inferred from the occurrence of its larvae in the plankton of the lower Paraná river and the Río de la Plata estuary (Argentina). Aquat Ecol 34: 307-317.

Clavero M and Garcia-Berthou E (2005) Invasive species are a leading cause of animal extinction. Trends Ecol Evol 20: 110. http://dx.doi.org/10.1016/j.tree.2005.01.003

Cox GW (2004) Alien species and evolution: the evolutionary ecology of exotic plants, animals, microbes, and interacting native species. Island Press, Washington.

Darling JA and Blum MJ (2007) DNA-based methods for monitoring invasive species: a review and prospectus. Biol Invasions 9: 751-765. http://dx.doi.org/10.1007/s10530-006-9079-4

Darrigran G and Pastorino G (1993) Bivalvos invasores en el Rio de Plata, Argentina. Com Soc Malac 64: 64 – 65.

Darrigran G (2002) Potential impact of filter-feeding invaders on temperate inland fresh water environments. Biol Invasions 4: 145-156. http://dx.doi.org/10.1023/A:1020521811416

Darrigran G and Damborenea C (2009) Características da espécie. In: Introdução a Biologia das Invasões. O mexilhão-dourado na América do Sul: biologia, dispersão, impacto, prevenção e controle, São Carlos, Cubo Editora, pp 246.

Darrigran G, Boeger W, Damborenea C, Maronãs M (2009) Evaluation of sampling and analysis techniques for early detection of *Limnoperna fortunei* (Mytilidae) in limit areas of its distribution. Braz J Biol 69: 979-980. http://dx.doi.org/10.1590/S1519-69842009000400029

Endo N, Sato K, Nogata Y (2009) Molecular based method for the detection and quantification of larvae of the golden mussel *Limnoperna fortunei* using real-time PCR. Plankton Benthos Res 4: 125-128. http://dx.doi.org/10.3800/pbr.4.125

Folmer O, Black M, Hoeh W et al (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294-299.

Gatlin MR, Shoup DE and Long JM (2013) Invasive zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) survive gut passage of migratory fish species: implications for dispersal. Biol Invasions 15: 1195 – 1200. http://dx.doi.org/10.1007/s10530-012-0372-0

Glaubrecht M, Fehér Z and von Rintelen T (2006) Brooding in *Corbicula madagascariensis* (Bivalvia, Corbiculidae) and the repeated evolution of viviparity in corbiculids. Zoologica Scripta 35: 641 – 654. http://dx.doi.org/10.1111/j.1463-6409.2006.00252.x

Hedtke SM, Stanger-Hall K, Baker RJ, Hillis DM (2008) All-male asexuality: origin and maintenance of androgenesis in the Asian clam *Corbicula*. *Evolution* 62: 1119-1136, http://dx.doi.org/10.1111/j.1558-5646.2008.00344.x

Houki S, Yamada M, Honda T and Komaru A (2011) Origin and posible role of males in hermafroditic androgentic *Corbicula* clams. Zool Sci 28: 526-531. http://dx.doi.org/10.2108/zsj.28.526

King CE, Debruyne R, Kuch M, Schwarz C and Poinar HN (2009) A quantitative approach to detect and overcome PCR inhibition in ancient DNA extracts. BioTechniques 47: 941-949. http://dx.doi.org/10.2144/000113244

Korniushin AV (2004) A revision of some Asian and African freshwater clams assigned to *Corbicula fluminalis* (Müller, 1774) (Mollusca: Bivalvia: Corbiculidae), with a

review of anatomical characters and reproductive features based on museum collections. Hydrobiologia 529: 251 – 270.

Lee T, Siripattrawan S, Ituarte CF and Ó Foighil D (2005) Invasion of the clonal clams: *Corbicula* lineages in the New World. *American Malacological Bulletin* 20:113-122.

Lopes M and Vieira J (2012) Space-time variation of the relative abundance of *Limnoperna fortunei* in deep zones of Gonçalo Channel, Rio Grande do Sul, Brazil. Iheringia, Sér Zool 112: 370 – 374. http://dx.doi.org/10.1590/S0073-47212012005000006

Ludwig S, Patella R, Stoiev S et al (2011) A molecular method to detect and identify the native species of southwestern Atlantic *Crassostrea* (Mollusca: Ostreidae). Zoologia 28: 420-426. http://dx.doi.org/10.1590/S1984-46702011000400002

Mansur MCD, Santos CP, Nehrke MV (2012a) Corbiculidae na América do Sul: Espécies nativas e invasoras, dispersão e a situação das pesquisas no Brasil (Mollusca: Bivalvia). In: Ecos do XIX Encontro Brasileiro de Malacologia, Rio de Janeiro, Technical Books, pp 467.

Mansur MCD, Pimpão DM, Bergonci PEA, et al (2012b) Morfologia e ciclo larval comparados de bivalves límnicos invasores e nativos. In: Ecos do XIX Encontro Brasileiro de Malacologia, Rio de Janeiro, Technical Books, pp 467.

Martins DS, Veitenheimer-Mendes IL, Faccioni-Heuser M (2004) *Corbicula* (Bivalvia, Corbiculidae) em simpatria no Lago Guaíba, Rio Grande do Sul, Brasil. Biociências 12: 129-138.

Martins DS, Veitenheimer-Mendes IL, Faccioni-Heuser MC (2006) Aspectos morfológicos e incubação em três espécies de Corbicula Mühlfeld, no lago Guaíba, Rio Grande do Sul, Brasil (Bivalvia, Corbiculidae). Biota Neotrop 6: 2-15. http://dx.doi.org/10.1590/S1676-06032006000200016

McMahon RF (2002) Evolutionary and physiological adaptations of aquatic invasive animals: *r* selection versus resistance. Can J Fish Aquat Sci 59: 1235 – 1244. http://dx.doi.org/10.1139/F02-105

Molnar JL, Gamboa RL, Revenga C and Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front Eco Environ 6: 485-492. http://dx.doi.org/10.1890/070064

Oliveira MD, Hamilton SK and Jacobi CM (2010) Forescasting the expansion of the invasive golden mussel *Limnoperna fortunei* in Brazilian and North American rivers based on its occurrence in the Paraguay River and Pantanal wetland of Brazil. Aquatic Invasions 5: 59-73. http://dox.doi.org/10.3391/ai.2010.5.1.8

Pestana D, Pie MR, Ostrensky A, Boeger WA et al (2008) Seasonal variation in larval density of Limnoperna fortunei (Bivalvia, Mytilidae) in the Iguaçu and Paraná rivers, in the region of Foz do Iguaçu, Paraná, Southern Brazil. Braz Arch Biol Technol 51: 607 – 612.

Pie MR, Boeger WA, Patella L, Falleiros RM (2006) A fast and accurate molecular method for the detection of larvae of the golden mussel *Limnoperna fortunei* (Mollusca: Mytilidae) in plankton samples. J Mollusc Stud 72: 218-219. http://dox.doi.org/10.1093/mollus/eyi070

Pfenninger M, Reinhardt F, Streit B (2002) Evidence for cryptic hybridization between different evolutionary lineages of the invasive clam genus *Corbicula* (Veneroida, Bivalvia). *Journal of Evolutionary Biology* 15: 818-829, http://dx.doi.org/10.1046/j.1420-9101.2002.00440.x

Pigneur LM, Marescaux J, Roland K, Etoundi E, Descy JP and Van Doninck K (2011) Phylogeny and androgenesis in the invasive *Corbicula* clams (Bivalvia, Corbiculidae) in Western Europe. *BMC Evolutionary Biology* 11:147, http://dx.doi.org/10.1186/1471-2148-11-147

Pigneur LM, Falisse E, Roland K et al (2014) Impact of invasive Asian clams, *Corbicula* spp., on a large river ecosystem. Fresh Biol 59: 573 – 583.

Rosa IC, Pereira JL, Gomes J et al. (2011) The Asian clam *Corbicula fluminea* in the European freshwater-dependent industry: A latent threat or a friendly enemy? Ecological Economics 70: 1805-1813. http://dx.doi.org/10.1016/j.ecolecon.2011.05.006

Santos CP, Würdig NL, Mansur MCD (2005) Fases larvais do mexilhão dourado *Limnoperna fortunei* (Dunker) (Mollusca, Bivalvia, Mytilidae) na Bacia do Guaíba, Rio Grande do Sul, Brasil. Rev Bras Zool 22: 702-708. http://dx.doi.org/10.1590/S0101-81752005000300029

Santos SB, Thiengo SC, Fernandez MA et al (2012) Espécies de moluscos límnicos invasores no Brasil. In: Moluscos límnicos invasores do Brasil: biologia, prevenção e controle, Porto Alegre, Redes Editora, pp 412.

Schlaepfer MA, Sherman PW, Blossey B and Runge MC (2005) Introduced species as evolutionary traps. Ecol Letters 8: 241-246. http://dx.doi.org/10.1111/j.1461-0248.2005.00730.x

Sousa R, Novais A, Costa R and Strayer DL (2013) Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. Hydrobiologia, 1-19. http://dx.doi.org/10.1007/s10750-012-1409-1

Telford MJ (2000) Embryology and developmental genes as clues to flatworm relationships. In: Interrelationships of the Platyhelminthes, London, Taylor & Francis, pp 356.

Tschá MK, Patella R, Ostrensky A and Boeger WA (2012) O método molecular de prospecção do mexilhão-dourado. In: Moluscos límnicos invasores do Brasil: biologia, prevenção e controle, Porto Alegre, Redes Editora, pp 412.

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Research Article

Looking for a needle in a haystack: molecular detection of larvae of invasive *Corbicula* clams

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Abstract

The invasive bivalves *Corbicula* spp. and *Limnoperna fortunei* predominate in South American rivers. They can be syntpatric in distribution, and because their larval stages are morphologically similar, monitoring them in zooplankton using microscopy protocols is often inefficient, producing ambiguous results. We designed a pair of primers to amplify a fragment of the mtDNA cytochrome c oxidase subunit I of *Corbicula* species. A multiplex reaction, containing the specific primer pair and a pair of universal primers (to control for the quality of the DNA in the sample) was tested with regards to specificity and ability to detect *Corbicula* spp. larvae in plankton samples that also contain other species in different proportions. Our molecular protocol allows for fast and accurate detection of *Corbicula* spp. even when concentrations of these species are low in samples, which is useful when examining large volumes of ballast/piped water. Further, the protocol is valuable for the monitoring/prospecting of early stages of the life cycle of *Corbicula* spp. in watersheds that have been invaded, or which are considered at risk of invasion by these species.

Key words: mtDNA, Asian clam, molecular markers, zooplankton, prospecting, larvae

Introduction

Invasive species are often associated with loss of biodiversity (Rosa et al. 2011; Sousa et al. 2013; Pigneur et al. 2014), changes in native communities (Schlaepfer et al. 2005) and even accelerated extinction of native species (Clavero and Garcia-Berthou 2005). Additionally, some invasive species damage artificial structures and impact economic activities (Rosa et al. 2011). Successful invasive organisms, for instance the South American bivalves *Corbicula fluminea* (Müller, 1774) and *Limnoperna fortunei* (Dunker, 1857), often disperse efficiently using a combination of natural and human-mediated mechanisms (Cox 2004).

Corbicula clams and *L. fortunei* (the "golden mussel") were accidentally introduced to South America, most likely by ballast water (Darrigran and Pastorino 1993). These invasives often occur sympatrically (Darrigran 2002) and are still expanding their distribution in this continent (Oliveira et al. 2010). According to Pigneur et al. (2014), *Corbicula* spp. are particularly efficient invaders of river systems, reaching densities of up to several thousands of individuals per square metre in the Rio Paraná, Argentina. *Corbicula* spp. clams can reduce phytoplankton density and compete with native bivalve species of Mycetopodidae and Hyriidae (Santos et al. 2012). The golden mussel, on the other hand, has caused many problems for South American hydroelectric power plants by fouling in cooling ducts (Darrigran and Damborenea 2009; Belz et al. 2012). Management and control strategies need to be implemented for these species where they are present, and should include continuous evaluations of propagule pressure in new habitats (Darling and Blum 2007).

Active search for individuals is frequently used to monitor invasive bivalves. Adult specimens can be found in the substrate, and larval stages present in plankton are detected using optical

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A partir dos dados coletados e apresentados, esta tese fornece informações morfológicas e genéticas sobre as linhagens invasoras de *Corbicula* presentes na América do Sul, assim como, sua atual distribuição. Além disso, fica evidente que a linhagem *Corbicula* sp. form A/R é a mais amplamente distribuída em diversas bacias hidrográficas da América do Sul. A partir do dados obtidos, fica claro que a pouca (ou nenhuma) variabilidade genética encontrada nas populações de *Corbicula* spp. está diretamente relacionada ao modo reprodução androgênico desses moluscos e, muito provavelmente, a propagação clonal mantém a baixa variabilidade genética dentro da área invadida. Em contrapartida, as populações 'híbridas' detectadas em Porto União (PU), Agudo (JAC) e Barra do Ribeira (BAR) não apresentaram um padrão de reprodução clonal e, por isso, estudos futuros devem ser desenvolvidos objetivando detalhar o padrão genético dessas populações e, ainda, detectar suas linhagens parentais, uma vez que este estudo não foi possível identifica-las.

Uma vez que essas linhagens clonais de *Corbicula* já estão estabelecidas e em expansão, faz-se necessário uma metodologia de detecção rápida, eficiente e de baixo custo visando monitorar os estágios larvais dessas espécies, seja em amostras de plâncton e/ou água de lastro. Na verdade, programas de prevenção precisam ser desenvolvidos de modo a evitar/retardar a introdução de novas populações desses moluscos através da água de lastro nas regiões portuárias; uma vez que a água de lastro é possivelmente o principal meio de introdução desses moluscos na América do Sul. Através do método molecular de detecção de larvas de *Corbicula* spp. proposto neste estudo, é possível desenvolver, aliado a este, protocolos eficientes de monitoramento que visam auxiliar no entendimento dos processos de introdução e dispersão dessas espécies em um novo ambiente. Dessa forma, evitando novas introduções de pool genético que podem potencializar o sucesso de invasão dessas espécies invasoras na América do Sul.