Morphological and molecular characterization of *Corbicula* (Mollusca, Bilvalvia) at Rosana Resevoir, Brazil

Yara Moretto Bagatini[1*], Renata de Souza Panarari[1], Janet Higuti[1,2], Evanilde Benedito-Cecilio[1,2], Alberto José Prioli[1,2] and Sonia Maria Alves Pinto Prioli[2]

*[1]Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900 Maringá, Paraná, Brasil. [2]Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura (Nupélia), Universidade Estadual de Maringá. *Author for correspondence: e-mail: ymorettob@hotmail.com*

ABSTRACT. The purpose of the present study was to verify, using RAPD and ISSR molecular techniques associated with the morphological characterization of bivalve shells, if the morphologically distinct specimens of *Corbicula fluminea* sampled at Rosana Reservoir belong to different species. The foot muscle tissue of the three groups (G1, G2, and G3) of *Corbicula* was removed and frozen in liquid nitrogen to the DNA analyze. Morphometric variables of the shell indicated significant differences among the specimens of *Corbicula*. On the other hand, molecular analyses revealed genetic similarity among the specimens, showing the nonexistence of molecular polymorphism. The data confirmed that the two morphologically different groups also belong to the species *C. fluminea,* showing the occurrence of phenotypic plasticity, probably caused by environmental or biotic factors.

Key words: *Corbicula*, molecular techniques, phenotypic plasticity, environmental variation.

RESUMO. Caracterização morfológica e molecular de *Corbicula* (Mollusca, Bivalvia) no reservatório de Rosana, Brasil. O objetivo do presente estudo foi verificar através de técnicas moleculares, RAPD e ISSR aliadas à caracterização morfológica da concha dos bivalves, se os indivíduos morfologicamente distintos de *Corbicula fluminea* amostrados no reservatório de Rosana pertencem a espécies diferentes. O tecido muscular do pé dos três grupos (G1, G2 e G3) de *Corbicula* foi removido e congelado em nitrogênio líquido para a análise do DNA. As variáveis morfométricas das conchas indicaram diferenças significativas entre os indivíduos de *Corbicula*. Por outro lado, as análises moleculares revelaram similaridade genética, evidenciando a não existência de polimorfismo molecular. Os resultados confirmam que os dois grupos diferentes morfologicamente também pertencem a espécie *C. fluminea*, demonstrando a ocorrência de plasticidade fenotípica, provavelmente causada por fatores bióticos ou ambientais.

Palavras-chave: *Corbicula*, técnicas moleculares, plasticidade fenotípica, variação ambiental.

Introduction

The species *Corbicula fluminea* (Müller, 1774) is native from Southeast Asia and has r-strategic characteristics, clearly observed through its short life span and high rates of fecundity, dispersion, and growth (McMahon, 1982; Ortmann and Grieshaber, 2003). In recent decades, *C. fluminea* has invaded the drainage systems of North America, South America, and Europe (Park and Kim, 2003), mostly through ballast water and human consumption (McMahon, 1991). In Brazil, *C. fluminea* has been recorded since the early 1970s (Veitenheimer-Mendes, 1981; Callil and Mansur, 2002; Luz *et al.,* 2002) in different aquatic ecosystems.

Most of the mollusk studies about systematics have used shell morphology to differentiate species (Mansur, 1970; Ituarte, 1981; Mansur and Garces, 1988). However, some studies have revealed difficulty in distinguishing species based on only morphological characters (Parodiz and Hennings, 1965; Simison and Lindberg, 1999; Urabe, 2000; Huang *et al.*, 2002), mainly because of the great phenotypic plasticity observed in the Phylum Mollusca (Kristensen *et al.*, 1999; Wood *et al.*, 2003; Jordaens *et al.*, 2003). This also applies to *C. fluminea* because of its great variability in form, size, and shell color (some nomenclature mistakes have occurred) (Morton, 2000). Ituarte (1981) suggested a critical reconsideration of the characters used in Corbiculidae systematics, because there are

numerous species that are considered ecophenotypic variations resulting from allometric growth.

This morphological variation is probably caused by genetic and environmental factors that can affect the shell phenotype (Cook, 1991; Urabe, 2000). Individuals of the same species developed in different environments may differ in phenotypic characters (Via, 1994). Such phenotypic plasticity refers to the ability of a genotype to produce different phenotypes as a consequence of variation in the environment (Dzialowski *et al.*, 2003), which may be an important attribute of the success of introduced species in the colonization of different habitats (Barret and Richardson, 1986).

Shell morphological differences of *Corbicula* specimens at Rosana Reservoir create doubts about the existence of phenotypic plasticity or the occurrence of more than one species in sympatry. Thus, the use of morphological characters alone would not be sufficient to taxonomically distinguish these groups of *Corbicula*. In this study, RAPD and ISSR molecular markers, associated with morphological analyses of *C. fluminea* and the two different phenotypes of *Corbicula*, were used to verify whether they belong to different species.

Material and methods

Sampling and morphological analysis of biological material

Samples were collected on November 7*th* , 2003, in points located on the banks of Rosana Reservoir (22°35'24"S and 52°49'51"W), near the dam (Figure 1). Samples were obtained using a modified Petersen grab. Three groups of *Corbicula*, distinct morphologically, were analyzed: G1 (typical *C. fluminea*), G2 and G3. Ten individuals, from each collected group (n = 30), were kept alive in order to preserve their tissues. Foot muscle samples of each specimen were extracted and preserved in liquid nitrogen (-196°C).

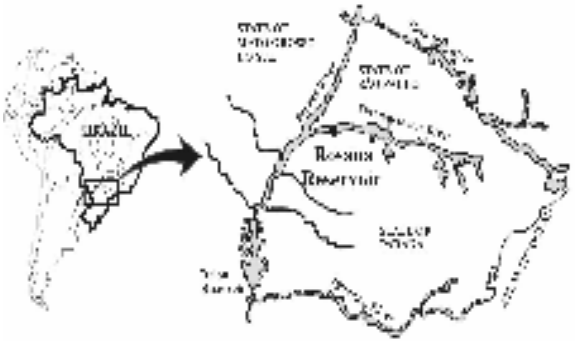

Figure 1. Localization of Rosana Reservoir at Paranapanema River.

In *C. fluminea* (G1), the shell is typically trigonal, with an isometric outline, and an inflated and prominent umbo, thus forming a triangle, in addition to showing widely-spaced concentric sulcations (Figure 2a). G2 has a shell with an isometric outline; however, it is flat, with an uninflated, usually eroded and unprominent umbo, with narrowly-spaced concentric shell sulcations (Figure 2b). In G3 the shell is also trigonal; however, it presents lateral expansion, a less inflated umbo and closely-spaced concentric striations, compared to G1 (Figure 2c).

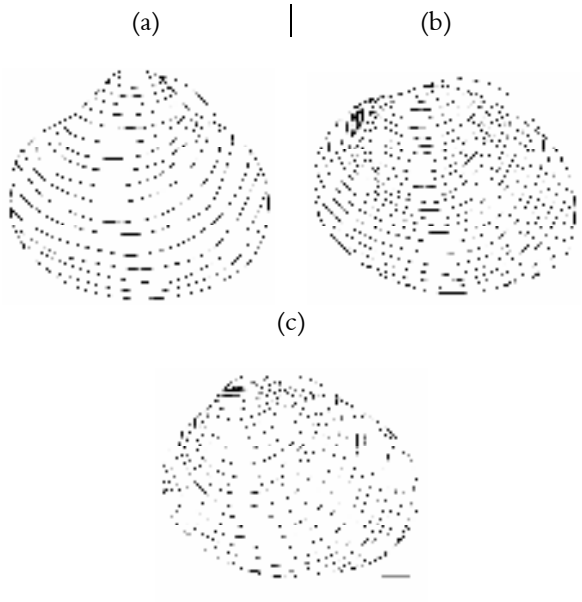

Figure 2. Dorsal external view of the right valve of (a) *C. fluminea* (G1), (b) G2, and (c) G3 (Size scaling bar is 3 mm long).

In order to show the morphological and morphometric differences among the three *Corbicula* groups, measurements of shell length, height, and width were taken. A null model ANOVA by Ecosim program (Gotelli and Entsminger, 2003) was performed to test differences among the studied groups. A Tukey post hoc test was applied to the shell measurements to verify which group had the highest morphological difference.

Molecular analysis

The same specimens used in the morphological analyses (i.e. ten individuals from each morphological group), were submitted to RAPD and ISSR molecular analyses. These techniques differ in relation to the target sequence of annealing primers. The RAPD (random amplified polymorphic DNA) technique is based on the amplification of genomic DNA using single primers of short and arbitrary nucleotide sequences

(Williams *et al.*, 1990; Welsh and McClelland, 1990). On the other hand, in the ISSR (inter-simple sequence repeat) technique, the primer is unique and consists of a few repeat internal units characteristic of a microsatellite. However, the ISSR technique is more advantageous than the RAPD technique because it results in greater reproducibility due to the use of longer primers, which allows the use of higher annealing temperatures and, therefore, higher stringency (Reddy *et al.*, 2002).

Extraction and DNA quantification

DNA extraction was performed according to Monesi *et al.* (1998). Samples of foot muscle tissue were macerated in liquid nitrogen. Then, they were homogenized in 500 µL of TH buffer (10 mM Tris-HCl, 60 mM NaCl, 10 mM EDTA, 5% sucrose, 0.15 mM spermine and 0.15 mM spermidine) plus 500 µL of PS buffer (0.2 M Tris-HCl, 30 mM EDTA, 2% SDS and 5% sucrose) and 5 µL of proteinase K (20 µg/µL). After shaking for 2 h at 37°C, the DNA was extracted using an equal volume of phenol/chloroform (1:1; v:v), followed by chloroform. DNA samples were precipitated using a NaCl solution and 100% cold ethanol, followed by overnight incubation at –20°C. After centrifugation, the precipitate was washed using 70 and 100% ethanol. The DNA pellet was resuspended in diluted TE buffer (0.1 mM Tris-HCl pH 8.0, 0.01 mM EDTA) containing (20 µg/mL) RNAse. Aliquots of each DNA sample were quantified by comparison with known quantities of phage λ DNA, in agarose gel (0.8%) stained with ethydium bromide (20 µg/100 mL).

Amplification and analysis of RAPD loci

PCR amplifications were initially tested with RAPD primers from kits OPA, OPX, and OPW (Operon Technologies Inc., Alameda, CA, USA). Primers OPA-02, OPX-01, OPX-02, OPX-07, and OPW-13 were used in RAPD amplifications and analysis. DNA amplification conditions were performed according to Bardakci and Skibinski (1994), and Mikhailova and Johannesson (1998). The PCR reaction mixture consisted of buffer Tris-KCl (20 mM Tris-HCl pH 8.4 and 50 mM KCl), 2 mM $MgCl_2$, 0.46-μM primer, 0.19 mM dNTP, 1 U/reaction of Taq-DNA polymerase (Gibco BRL), DNA (10 ng), and sterile deionized water in order to complete a total volume of 13 μL. The reaction mixture was heated at 92°C for 4min, followed by 40 cycles of 1min at 92°C, 1min 30 s at 40°C and 2min. at 72°C.

Immediately after the last amplification cycle, the reaction mixture was maintained at 72°C for 5min, and then, cooled at 20°C for 20min; after that, it was cooled at 4°C. Negative controls (absence of DNA) were included in each PCR amplification.

Aliquots (10 μL) of PCR reaction mixture, after amplification, were submitted to eletrophoresis in 1.4% agarose gel stained with (20 μg/100 mL) ethidium bromide, and then, separated by electrophoresis in TBE buffer (Tris-borate) at 5 $V.cm^{-1}$ for 3-4 hours. The electrophoretic profiles were visualized under UV radiation and photographed using the Kodak EDAS-290. Fragment sizes were estimated by comparison with standard Ladder 100 bp (Gibco BRL).

Amplification and analysis of ISSR loci

ISSR primers composed of tetranucleotide repeats $(GGAC)_3C$ and $(GGAC)_3T$ were selected for analysis. The PCR reaction mixture consisted of the same components described above for RAPD analysis, but the primer was replaced by one of the selected ISSR primers. Amplifications were done according to Albert *et al.* (1999), in 5 cycles of 45 s at 94°C, 1min at 51°C and 1min at 72°C, immediately followed by 30 cycles of 45 s at 94°C, 1min at 48°C and 1 min at 72°C. In each experiment, a reaction mixture without DNA template was included as a negative control. PCR amplified DNA fragments were separated by electrophoresis, and visualized and photographed using the procedure described above for RAPD.

Results

The mean values, standard deviation and variation amplitude of *Corbicula* shell measurements are shown in Table 1. The lowest mean values of shell length, height, and width were seen in G1. However, in G2, intermediary values were observed, while the highest values were observed in the G3 shell.

Table 1. Mean values, standard deviation, maximum and minimum amplitude of shell length, height and width of *Corbicula* (n=10).

Values (cm)	G1	G2	G3
Length	1.57 ± 0.24	1.77 ± 0.14	2.21 ± 0.11*
	1.22-1.90	1.52-2.03	2.11-2.42
Height	1.32 ± 0.17	1.42 ± 0.11	1.80 ± 0.17
	1.03-1.53	1.31-1.62	1.72-2.08
Width	0.93 ± 0.10	0.93 ± 0.07	1.18 ± 0.94*
	0.78-1.07	0.81-1.03	1.11-1.38

Tukey test: *$p<0.05$

Morphometric differences observed among typical *C. fluminea* and the other two groups (G2 and

G3) were significant for length ($F = 81.64$; $p < 0.05$) and width ($F = 9.41$; $p < 0.05$) (non-parametric ANOVA). A Tukey post hoc test was applied to the length and width measurements of the shell and indicated that G3 was the group with the highest morphological difference.

RAPD

A total of five primers (OPA-02, OPX-01, OPX-02, OPX-07, and OPW-13) were selected for RAPD analysis. The number of sharp reproducible bands generated by each primer in all *Corbicula* groups ranged from 6 to 11, totaling 46 loci (Table 2).

Table 2. Approximate size, in numbers of base pairs (bp), of *Corbicula* DNA fragments from Rosana Reservoir, amplified by PCR with RAPD primers.

Primers	Bands (~bp)										
RAPD	a	b	c	d	e	f	g	h	i	j	k
OPA02	1800	1550	1300	1150	1000	520					
OPX01	2070	1600	1500	1300	1200	1150	950	870	850	650	350
OPX02	3100	2100	1800	1700	1150	1000	950	720	680		
OPX07	1350	1200	1150	1100	950	800	750	700	550	500	
OPW13	1900	1700	1300	1220	1090	900	750	700	620	550	

The size of the amplified fragments ranged between 350 and 3100 base pairs (bp). Electrophoretic profiles of the *Corbicula* phenotypes, obtained with the OPX-07 RAPD primer, are shown in Figure 3. The electrophoretic profile of the specimens (2nd G2 in the first part of the Figure 3 and 7th G3 in the second part of Figure 3) did not present the same monomorphic standard because a DNA excess occurred in these loci, which did not influence the result. Thus, all amplified loci were monomorphic for the three *Corbicula* phenotypes.

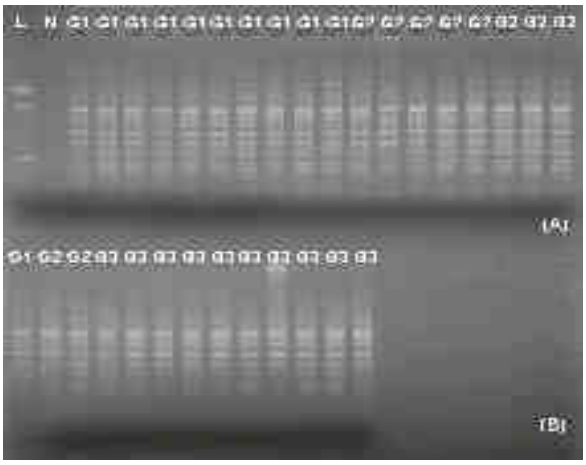

Figure 3. RAPD electrophoretic profiles of *Corbicula* phenotypes, using the OPX-07 primer. A- DNA molecular weight markers (L) (Ladder 100 bp, Gibco BRL); Negative control (N), *C. fluminea* (G1) and G2 specimens; B- PCR replicate of *C. fluminea* (G1); G2 and G3 specimens.

ISSR

The same specimens analyzed using RAPD molecular markers were used in ISSR analysis. ISSR primers composed of tetranucleotide repeats $(GGAC)_3C$ and $(GGAC)_3T$ were selected for analyses because no other primer tested presented polymorphism among *C. fluminea* and the other two morphologically distinct groups of *Corbicula*. ISSR primers $(GGAC)_3C$ and $(GGAC)_3T$ produced approximately 10 bands, totaling 18 loci (Table 3).

Table 3. Approximate size, in numbers of base pairs (bp), of *Corbicula* DNA fragments from Rosana Reservoir, amplified by PCR with ISSR primers.

Primers	Bands (~bp)									
ISSR	a	b	c	d	e	f	g	h	i	j
GGAC3C	1500	1350	1300	950	900	750	700	580		
GGAC3T	1900	1700	1150	1000	900	850	750	650	550	350

The size of the amplified fragments ranged between 350 and 1900 base pairs (bp). According to the electrophoretic profiles, all amplified loci were monomorphic for the three *Corbicula* phenotypes (Figure 4).

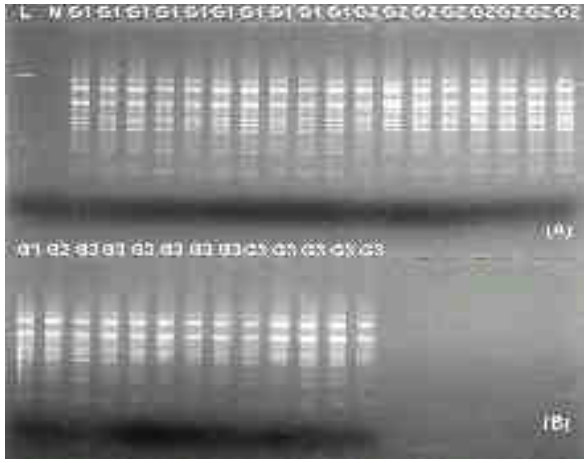

Figure 4: ISSR electrophoretic profiles of *Corbicula* phenotypes, using the $(GGAC)_3T$ primer. A- DNA molecular weight markers (L) (Ladder 100 bp, Gibco BRL); Negative control (N), *C. fluminea* (G1) and G2 specimens (C1); B- PCR replicate of *C. fluminea* (G1); G2 and G3 specimens.

Discussion

Regarding organisms with a high tendency for morphological differentiation, studies that consider both molecular and morphological characters are relevant and have been frequent (McLeod, 1986; Maas *et al.*, 1999; Jordaens *et al.*, 2003). The use of electrophoresis techniques to study *Corbicula* from North America did not reveal polymorphism in the analyzed populations (Smith *et al.*, 1979). However,

subsequent works that studied the same genus, with other populations from North America (McLeod, 1986; Siripattrawan *et al.*, 2000), Asia (Smith *et al.*, 1979; Park and Kim, 2003) and Europe (Pfenninger *et al.*, 2002), detected polymorphism, thus, suggesting the existence of more than one species.

In relation to *C. fluminea* species, problems concerning systematics have also been common in South America (Parodiz and Hennings, 1965; Ituarte, 1981, 1994; Mansur *et al.*, 2004). However, morphological and morphometric studies alone may be not sufficient to identify phenotypically distinct individuals (Maas *et al.*, 1999). This is because morphological characters are rarely constant within a species (Travis, 1994) and the variations associated with body form are the most difficult to be quantified (Parsons *et al.*, 2003).

The same genotype can be expressed in different phenotypes, according to the environmental variations. Thus, in genetically homogeneous individuals, most of the variation is caused by the environment (Futuyma, 1997), as inferred by the RAPD and ISSR markers, the genetic similarity among *C. fluminea* typicall and the other two studied groups is high, showing that they belong to the species *C. fluminea*. Therefore, the molecular data, associated with the shell morphology results, suggest the occurrence of high phenotypic plasticity among these organisms, characterizing three different phenotypes, while the molecular analysis identifies them as genetically similar.

Shell alterations, as observed in *C. fluminea* specimens, may have been induced by environmental factors. Variations in the shell morphology of mollusks have been recorded and are usually correlated with exposure to waves (O'loughlin and Aldrich, 1987), substratum types (Edwards, 1988), predation (Thomas and Himmelman, 1988), geographical variation (Olabarria and Thurston, 2004), and chemical composition of the water (Chambers, 1980). Ituarte (1994) reported the occurrence of alterations in *Corbicula* shell form, especially in its outline, and also attributed the variations to environmental factors such as the composition of the substratum, hydrodynamic forces and hydrological characteristics. However, the three different phenotypes found at Rosana Reservoir were observed in sandy substratum. Consequently, the distinction observed can be associated with other abiotic or even biotic factors.

In addition to birds, some benthonic fish are the main predators of *Corbicula* (McMahon, 1999). This species of fish can differ morphologically to increase its efficiency in the capture of prey (Hjelm *et al.*, 2000). Likewise, changes can also occur in the prey. Delgado *et al.* (2002), when studying the sea gastropod *Strombus gigas*, observed that predation could induce changes in its behavior and shell morphology. Other studies also indicated that predation could cause morphological changes in the prey (Wainwright *et al.*, 1991; Walls *et al.*, 1997; Dzialowski *et al.*, 2003). In the case of *C. fluminea*, the morphological differentiation can be the development of a defense mechanism to make its capture more difficult. According to McMahon (1991), high growth rates make the specimens of *C. fluminea* and other bivalves less susceptible to predation. In this way, the development of a lateral expansion in G3 could be favoring its own defense.

According to Johannesson *et al.* (1993), and Johannesson and Johannesson (1996), the morphological variability among populations of the same species can have a genetic base, as a response to predation pressure. However, the molecular markers used in the present study did not detect any genetic variability in the specimens of *C. fluminea*, which can suggest morphological changes induced by predation (fishes, mammals, or birds), or some other biotic or abiotic factors. Nevertheless, this hypothesis cannot be rejected without further studies aimed at conclude if different lineages of *C. fluminea* demonstrate morphological changes as a result of genotypic factors or as a result of predation.

The ecological success of such species can be related to their capacity for expressing different phenotypes, in addition to the clonal reproductive mode (Siripattrawan *et al.*, 2000). In this way, another explanation for the genetic homogeneity among the specimens of *C. fluminea* can be self-fertilization, considering that cross-fertilizations, in a determined population, are not frequent (McLeod, 1986). Due to this hermaphroditic characteristic (self-fertilization), the introduction of a single individual into the environment can induce the growth of a new population, as suggested by McMahon (1991) for some populations of North America. Thus, it can be assumed that a single introduction of a few specimens of *C. fluminea* into Rosana Reservoir constituted a new population, or that several introductions occurred, but with individuals coming from the same place. This factor could explain, in part, the genetic homogeneity verified in these species, characterizing a highly endogamic and unique population of *C. fluminea*.

In spite of wide ecological success, the systematic and the biogeography of *Corbicula* are poorly understood (Park and Kim, 2003), possibly, due to

its high morphological variation. Therefore, the explanation for the biological success of such exotic species depends on understanding the factors involved in the high morphological variability of these organisms. Thus, more evidence is required to understand the factors involved in the morphological differentiation process of *C. fluminea* and to know how such factors can affect its adaptability to different habitats. Consequently, more detailed studies are necessary to elucidate this issue. The results of such researches could provide information for the establishment of effective measures to control these exotic species, which have been widely introduced in aquatic freshwater ecosystems.

Acknowledgements

Dr. Cláudia Callil for the confirmation of some *C. fluminea* specimens; Dr. Izabel de Fátima Andrian, Dr. Rosemara Fugi, and Dr. Luiz Felipe Machado Velho for their valuable suggestions; the designer Jaime Lopes Pereira for the map; Rodrigo Fernandez for the statistical support, and Gisele Novakowfki for the boards; to CNPq-Brazil for the financial and to Nupélia for the logistic support.

References

ALBERT, J.S. *et al.* New species of *Gymnotus* (Gymnotiformes, Teleostei) from Southeastern Brazil: towards the deconstruction of *Gymnotus carapo*. *Copeia*, Lawrence, n. 2, p. 410-421, 1999.

BARDAKCI, F.; SKIBINSKI, D.O.F. Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity*, Oxford, v.73, p. 117-123, 1994.

BARRET, S.C.H.; RICHARDSON, B.J. Genetic attributes of invading species. *In:* GROVES, R.H. & BURDON, J.J. (Ed.). *Ecology of biological invasions.* Cambridge: University Press, New York. 1986, Cap. 1, p. 21-33.

CALLIL, C.T.; MANSUR, M.C.D. Corbiculidae in the Pantanal: history of invasion in southeast and central South America and biometrical data. *Amazoniana*, Kiel, v. 17, p. 153-167, 2002.

CHAMBERS, S.T. Genetic divergence between populations of *Goniobasis* (Pleuroceridae) occupying different drainage systems. *Malacologia*, Philadelphia, v. 20, p. 63-81, 1980.

COOK, L.M. *Genetic and ecological diversity:* the sport of nature. London: Chapman & Hall, 1991.

DELGADO, G.A. *et al.* Predator-induced behavorial and morphological plasticity in the tropical marine gastropoda *Strombus gigas*. *Biol. Bull*., Woods Hole, v. 203, p. 112-119, 2002.

DZIALOWSKI, A.R. *et al.* Predator-induced phenotypic plasticity in the exotic cladoceran *Daphinia lumholtzi*. *Freshw. Biol.*, Oxford, v. 48, p. 1593-1602, 2003.

EDWARDS, A.L. Latitudinal clines in shell morphologies of *Busycon carica* (Gmelin 1791). *J. Shellfish. Res.*, Southampton, v. 7, p. 461-466, 1988.

FUTUYMA, D.J. *Evolutionary Biology*. 2nd ed., Sunderland: Sinauer Associated Inc., 1997.

LUZ, K.D.G. *et al.* Alterations in the *Pterodoras granulosus* (Valenciennes, 1833) (Osteichthyes, Doradidae) diet due to the abundance variation of a bivalve invader species in the Itaipu reservoir, Brazil. *Acta Scientiarum*, Maringá, v. 24, n. 2, p. 427-432, 2002.

GOTELLI, N.J.; ENTSMINGER., G.L. *EcoSim: null models software for ecology*. Version 7.44, 2001. Disponível em: < *http://together.net/~gentsmin/ecosim.htm* > Acesso em: 14 mar. 2003.

HUANG, Y., *et al.* Testing the relationships of Chinese freshwater Unionidae (Bivalvia) based on analysis of partial mitocondrial 16S rRNA sequences. *J. Moll. Stud*., London, v. 68, p. 359-363, 2002.

HJELM, J. *et al.* Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia*, Berlin, v. 122, p. 190-199, 2000.

ITUARTE, C.F. Primera Noticia acerca de la introducción de pelecipodos asiáticos en el area Rioplatense. *Neotropica*, La Plata, v. 27, n. 77, p. 79-82, 1981.

ITUARTE, C.F. *Corbicula* and *Neocorbicula* (Bivalvia: Corbiculidae) in the Paraná, Uruguay, and Río de La Plata Basins. *Nautilus*, Sanibel, v. 107, p. 129-135, 1994.

JOHANNESSON K. *et al.* Morphological-differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail *Littorina-saxatilis*. *Evolution*, Lawrence, v. 47, n. 6, p. 1770-1787, 1993.

JOHANNESSON, B.; JOHANNESSON, K. Population differences in behavior and morphology in the snail *Littorina saxatilis*: Phenotypic plasticity or genetic differentiation. *J. Zool.*, Cambridge, v. 240, n. 3, p. 475-493, 1996.

JORDAENS, K. *et al.* Molecular and morphological discrimination between the pulmonate land snails *Zonitoides nitidus* and *Z. excavatus*. *J. Moll. Stud*. London, v. 69, p. 295-300, 2003.

KRISTENSEN, T.K. *et al.* Molecular characterization of *Biomphalaria* spp in Egypt. *J. Moll. Stud.*, London, v. 65, p. 133-136, 1999.

MAAS, P.A. *et al.* Genetic and morphometric characterization of mussels (Bivalvia: Mytilidae) from Mid-Atlantic Hydrothermal Vents. *Biol. Bull.*, Woods Hole, v. 196, p. 265-272, 1999.

MANSUR, M.C.D. Lista dos moluscos bivalves das famílias Hyriidae e Mycetopodidae para o estado do Rio Grande do Sul. *Iheringia*, *ser. Zool*., Porto Alegre, v. 39, p. 33-95, 1970.

MANSUR, M.C.D.; GARCES, L.M.M.P. Ocorrência e densidade de *Corbicula fluminea* (Mueller, 1774) e *Neocorbicula limosa* (Maton, 1811) na Estação Ecológica do

Taim e áreas adjacentes, Rio Grande do Sul, Brasil (Mollusca, Bivalvia, Corbiculidae). *Iheringia, ser. Zool*., Porto Alegre, v. 68, p. 99-115, 1988.

MANSUR, M.C.D. *et al.* Uma retrospectiva e mapeamento da invasão de espécies de *Corbicula* (Mollusca, Bivalvia, Verenoida, Corbiculidae) oriundas do sudeste Asiático, na América do Sul. *In:* SILVA, J.S.V.; SOUZA, R.C.C.L. (Ed.). *Água de lastro e bioinvasão.* Rio de Janeiro: Editora Interciência, 2004, p. 39-58.

MCLEOD, M.J. Eletrophoretic variation in North American *Corbicula*. *In:* BRITTON, J.C. & PREZANT, R.S. (Ed.). International *Corbicula* Symposium, *Am. Malacol. Bull., Special Edition*, New York, v. 2, p. 125-132, 1986.

MCMAHON, R.F. The occurrence and spread of the introduced Asiatic freshwater clam, *Corbicula fluminea* (Müller), in North America: 1924-1982. *Nautilus*, Sanibel, v. 96, p. 134-141, 1982.

MCMAHON, R.F. Mollusca: Bivalvia. *In:* THORP, J.H.; COVICH, A.P. (Ed.). *Ecology and Classification of North American Freshwater Invertebrates*. London: Academic Press, Inc. 1991, p. 315-399.

MCMAHON, R.F. Invasive Characteristics of the Freshwater Bivalve, *Corbicula fluminea*. *In:* CLAUDI, R.; LEACH, J.H. (Ed.). *Nonindigenous Freshwater organisms: vectors, biology, and impacts.* Washington, D.C. Lewis Publisher. 1999, p. 315-343.

MIKHAILOVA, N.; JOHANNESSON, K. A comparison of different protocols for RAPD analysis of *Littorina*. *Hydrobiologia*, Dordrecht, v. 378, p. 33-42, 1998.

MONESI, N. *et al.* The DNA puff gene BhC4-1 of *Bradysia hygida* is specifically transcribed early prepupal salivary glands of *Drosophila melanogaster*. *Chromosoma*, Berlin, v. 107, p. 559-569, 1998.

MORTON, B. *Corbicula* in Asia-an update synthesis. *In:* BRITTON, J.C.; PREZANT, R.S. (Ed.). International *Corbicula* Symposium, *Am. Malacol. Bull., Special Edition*, New York, v. 2, p. 113-124, 2000.

OLLABARRIA, C.; THURSTON, M.H. Patterns of morphological variation of the deep-sea gastropod *Troschelia Berniciensis* (King, 1846) (Buccinidae) from the Northeastern Atlantic Ocean. *J. Moll. Stud*., London, v. 70, p. 59-66, 2004.

O'LOUGHLIN, E.F.M.; ALDRICH, J.C. An analysis of shell shape variation in the painted top shell *Calliostoma zizyphinum* (L.) (Prosobranchia: Trochidae). *J. Moll. Stud.*, London, v. 53, p. 62-68, 1987.

ORTMANN, C.; GRIESHABER, M.K. Energy and valve closure behavior in the Asian clam *Corbicula fluminea*. *J. Exper. Biol*., Cambridge, v. 206, p. 4167-4178, 2003.

PARK, J.K.; KIM, W. Two *Corbicula* (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment. *Mol. Phyl. Evol.*, Orlando, v. 29, n. 3, p. 529-539, 2003.

PARODIZ, J.J.; HENNINGS, L. The *Neocorbicula* (Mollusca, Pelecypoda) of the Parana-Uruguay Basin, South America. *Ann. Carnegie Mus*., Pittsburgh, v. 38, p. 69-96, 1965.

PARSONS, K.J. *et al.* Getting into a shape: an empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to new world cichlids. *Environm*. *Biol. Fishes*, Dordrecht, v. 67, p. 417-431, 2003.

PFENNINGER, M., *et al.* Evidence for cryptic hybridization between different evolutionary lineages of the invasive clam genus *Corbicula* (Verenoida, Bivalvia). *J.Evol. Biol*., Paris, v. 15, n. 5, p. 818-829, 2002.

REDDY, M.P. *et al.* Inter simple repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, Dordrecht, v. 128, p. 9-17, 2002.

SIMISON, W.B.; LINDBERG, D.R. Morphological and molecular resolution of a putative cryptic species complex: a case study of *Notoacmea fasciculares* (Menke, 1851) (Gastropoda: Patellogastropoda). *J. Moll. Stud.*, London, v. 65, p. 99-109, 1999.

SIRIPATTRAWAN, S. *et al.* Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. *J. Moll. Stud.*, London, v. 66, p. 423-429, 2000.

SMITH, M.H. *et al.* Genetic variability in *Corbicula*, and invading species. *In:* BRITTON, J.C. (Ed.). *Proceedings, First International Corbicula Simposium*. Texas Christian: University Research Foundation, Forth Worth, 1979, p. 244-248.

THOMAS, M.L.H.; HIMMELMAN, J.H. Influence of predation on shell morphology of *Buccinum undatum* L. on Atlantic Coast of Canada. *J. Exp. Mar. Biol. Ecol.*, Amsterdam, v. 115, p. 221-236, 1988.

TRAVIS, J. Evaluating the adaptive role of morphological plasticity. *In:* WAINWRIGTH, P.C.; REILLY, S.M. (Ed.). *Ecological Morphology: integrative organismal biology.* Chicago: The University of Chicago Press, 1994, p. 99-122.

URABE, M. Phenotypic modulation by the substratum of shell sculpture in *Semisulcospira reiniana* (Prosobranchia: Pleuroceridae). *J. Moll. Stud*., London, v. 66, p. 53-59, 2000.

VIA, S. The evolution of phenotypic plasticity: what do we really know? *In:* REAL, L. A. (Ed.). *Ecological Genetics*,.New Jersey: Princeton University Press, 1994, p. 35-57.

VEITENHEIMER-MENDES, I.L. *Corbicula manilenssis* (Philippi, 1844), molusco asiático, na bacia do Jacuí e do Guaíba, Rio Grande do Sul, Brasil (Bivalvia, Corbiculidae). *Iheringia, ser. Zool*., Porto Alegre, v. 60, p. 63-74, 1981.

WAINWRIGHT, P.C. *et al.* Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus): effects of environment on ontogeny. *Func. Ecol.*, Oxford, v. 5, p. 40-45, 1991.

WALLS, M. *et al.* Phenotypic plasticity of *Daphnia* life history traits: the roles of predation, food level and toxic cyanobacteria. *Freshw. Biol.*, Oxford, v. 38, p. 353-364, 1997.

WELSH, J.; MCCLELLAND, M. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res*., Oxford, v. 18, p. 7213-7218, 1990.

WILLIAMS, J.G.K. *et al.* DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res*., Oxford, v. 18, p. 6531-6535, 1990.

WOOD, A.R. *et al.* Analysis of a nuclear-DNA marker for species identification of adults and larvae in the *Mytilus edulis* complex. *J. Moll. Stud*., London, v. 69, p. 61-66, 2003.

Received on April 04, 2005.
Accepted on December 15, 2005.