



The history of the introduction of the giant river prawn, *Macrobrachium cf. rosenbergii* (Decapoda, Palaemonidae), in Brazil: New insights from molecular data

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Abstract

The giant river prawn, *Macrobrachium cf. rosenbergii*, is one of the most cultivated freshwater prawns in the world and has been introduced into more than 40 countries. In some countries, this prawn is considered an invasive species that requires close monitoring. Recent changes in the taxonomy of this species (separation of *M. rosenbergii* and *M. dacqueti*) require a re-evaluation of introduced taxa. In this work, molecular analyses were used to determine which of these two species was introduced into Brazil and to establish the geographic origin of the introduced populations that have invaded Amazonian coastal waters. The species introduced into Brazil was *M. dacqueti* through two introduction events involving prawns originating from Vietnam and either Bangladesh or Thailand. These origins differ from historical reports of the introductions and underline the need to confirm the origin of other exotic populations around the world. The invading populations in Amazonia require monitoring not only because the biodiversity of this region may be affected by the introduction, but also because admixture of different native haplotypes can increase the genetic variability and the likelihood of persistence of the invading species in new habitats.

Key words: Bioinvasion, exotic species, giant river prawn, *Macrobrachium dacqueti*, COI, 16S rRNA, Amazonia.

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Introduction

Crustaceans are among the most successful organisms in invading new habitats. Approximately 28% of the exotic species found on the coasts of North America are crustaceans (Ruiz *et al.*, 2000). A number of characteristics have contributed to the success of these animals, including their small size, especially in the juvenile stages, and their exoskeleton which protects from damage during transportation and other potential sources of physical injury (Ruiz *et al.*, 2000). Many species can also tolerate marked variations in temperature and salinity (MacIsaac *et al.*, 2001). In addition, the economic importance of many crustaceans has led to their deliberate introduction into several countries for commercial farming (Naylor *et al.*, 2001; Minchin, 2007).

The giant river prawn, traditionally classified as *Macrobrachium rosenbergii* de Man, 1879, is a freshwater species with many of the traits mentioned above and is a

classic example of a species that has become widespread because of its popularity in commercial aquaculture. Worldwide, the annual production of the giant river prawn surpasses 200,000 tons (FAO-FIGIS, 2009). This species occurs naturally in southern and southeastern Asia, parts of Oceania, and some Pacific islands (New, 2000), and has been recorded as an exotic species in more than 40 countries: the giant river prawn is now found in the wild in Taiwan, Panama and Russia (FAO-DIAS, 2009), Madagascar (Hanamura *et al.*, 2008), Venezuela (Pereira *et al.*, 1996), the United States (Woodley *et al.*, 2002), and Jamaica (Kairo *et al.*, 2003). In Brazil, specimens have been recorded in the wild in the states of Pará (Barros and Silva, 1997; Cintra *et al.*, 2003), Maranhão (I3N-Brazil, 2009), São Paulo (Magalhães *et al.*, 2005) and Paraná (Gazola-Silva *et al.*, 2007).

The worldwide expansion of giant river prawn farming is relatively well documented. Modern aquaculture of the species began in the 1960s. In 1961, Shao-Wen Ling, a researcher at the Marine Fisheries Research Institute in Penang, Malaysia, discovered that the larvae of this species required brackish water to survive. In 1965, Takuji Fuji-

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mura and his team took a number of specimens from Malaysia to the Anuenu Fisheries Research Center (AFRC) in Honolulu, Hawaii, where they embarked on research that made the production of post-larvae (PL) possible on a commercial scale. These advances, together with the results of other studies in larviculture, and the large-scale production of PLs from Hawaii, led to the introduction of this species into various countries, principally in the New World, but also in Africa and some parts of Europe (New, 2000).

In Brazil, the giant river prawn was first introduced in 1977 by the Oceanography Department of the Federal University of Pernambuco, which imported PL directly from the AFRC. However, major expansion of the prawn farming industry in this country took place in the 1990s, when there were 32 hatcheries for larviculture and more than 700 hectares of growout ponds (Valenti, 1993). Since the original importation, a number of other public and private institutions have conducted their own introductions (Cavalcanti, 1998). As a result, the exact geographic origin of most of the populations that now exist in Brazil is unknown.

The relatively ample natural distribution of the giant river prawn has resulted in variation in some characteristics, and this has generated considerable debate about the taxonomic status of this species. Originally, two subspecies were recognized, the eastern *M. rosenbergii rosenbergii*, and the western *M. rosenbergii dacqueti* (Johnson, 1973; Lindenfelser, 1984). However, a recent study by de Bruyn *et al.* (2004a), which analyzed the mitochondrial rRNA 16S gene, concluded that these two forms may in fact represent phylogenetically distinct species. Morphological analyses have not only corroborated these conclusions but have also shown that the two species can be easily distinguished on the basis of a few simple diagnostic traits (Wowor and Ng, 2007). Furthermore, *M. dacqueti*, and not *M. rosenbergii*, is the species farmed throughout the world (Wowor and Ng, 2007).

These findings raise a number of questions, the most obvious of which is the exact identity of the taxon that has been introduced for aquaculture in different countries, including Brazil. Are the historic data on the dissemination of the giant river prawn in Brazil and other parts of the world accurate? In other words, was Malaysia really the single geographic source of all the exotic populations of this prawn? In an attempt to answer these questions, we have used molecular tools to compare the populations of giant river prawn found in Brazil with native populations in their natural environment in southern Asia and the western Pacific.

Material and Methods

The prawns used in this study were collected from different bodies of freshwater along the coast of the Brazilian state of Pará (Amazon region), from Salvador in the state of Bahia, and from the Aquaculture Center

(CAUNESP) of the São Paulo State University, in Jaboticabal, São Paulo (Figure 1). Molecular identification of the species of giant prawn that occurs in Brazil was based on the analysis of mitochondrial rRNA 16S gene sequences that were compared with those obtained from native populations by de Bruyn *et al.* (2004a). For this analysis, *Macrobrachium australiense* and *Macrobrachium lar* were used as the outgroups (Table 1). To identify the geographic origin of the Brazilian populations, DNA sequences of cytochrome C oxidase subunit I (COI) gene were generated and compared with the sequences available for native populations of *M. rosenbergii* (de Bruyn *et al.*, 2004b) and *M. dacqueti* (de Bruyn *et al.*, 2005).

DNA extraction, amplification and sequencing

DNA was extracted in Eppendorf tubes using a rapid phenol-chloroform protocol adapted from the standard procedure of Sambrook *et al.* (1989). The mitochondrial rRNA 16S gene was amplified using the primers 16S L1987 and 16S H2609 (Palumbi *et al.*, 1991), while COI was amplified using the primers developed by Folmer *et al.* (1994). The PCR conditions were as follows: each 25 μ L reaction contained 4 μ L of deoxynucleotide triphosphate (dNTP; 1.25 mM), 2.5 μ L of 10 x buffer solution, 1 μ L of MgCl₂ solution (25 mM), 1-2 μ L of total DNA (200 ng/ μ L), 0.25 μ L of each primer (200 ng/ μ L), 0.20 μ L of Taq polymerase (5 U/ μ L; Invitrogen) and bidistilled water to complete the final volume.

For 16S rRNA, the PCR consisted of the following temperature cycles: initial denaturation for 3 min at 94 °C, followed by 30 cycles of 30 s at 94 °C for denaturation, 45 s at 50 °C for hybridization and 45 s at 72 °C for extension, with a final extension of 5 min at 72 °C. For COI, the conditions were: initial denaturation for 3 min at 94 °C, followed by 35 cycles of extension at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, with by a final extension at 72 °C for 5 min.

The quality of extracted DNA and the PCR products were assessed by electrophoresis in 1% agarose gels. The samples were then purified with the ExoSAP-IT enzyme (Amersham Pharmacia Biotech Inc.) and sequenced using a ABI BigDye® Terminator Mix (Applied Biosystems, Carlsbad, CA). The samples were sequenced in an ABI 3120 automatic sequencer (Applied Biosystems).

Phylogenetic and populational analyses

The DNA sequences were aligned with BioEdit 7.0.5.3 (Hall, 1999) and saturation of the database was evaluated with DAMBE 4.2.13 (Xia and Xie, 2001). The evolutionary model that best accounted for the observed variation in the database was selected using MODELTEST v. 3.7 (Posada and Crandall, 1998). Three procedures were used for the phylogenetic analyses, namely, the neighbor joining (NJ) and maximum parsimony (MP) methods that

Table 1 - Samples used for the molecular identification of the giant river prawn taxon that occurs in Brazil based on the analysis of 16S rRNA.

Collection site	Haplotype code	N	Form ¹	GenBank
Native ²				
Northwestern Malaysia	1M	1	Western	AY203912
Southwestern Malaysia	2M	1	Western	AY203915
Northeastern Malaysia	3M	1	Western	AY203904
Malaysia	4M	1	Western	AY203905
Vietnam	1V	1	Western	AY203914
Southern Vietnam	2V	1	Western	AY203907
Southwestern Thailand	1T	1	Western	AY203908
Southeastern Thailand	2T	1	Western	AY203911
Java, Indonesia	JA	1	Western	AY203913
Philippines	PH	1	Eastern	AY203910
Papua New Guinea	PN	1	Eastern	AY203906
Irian Jaya, Indonesia	IJ	1	Eastern	AY203909
Wenlock River, Australia	1A	1	Eastern	AY203918
Leichardt River, Australia	2A	1	Eastern	AY203919
Roper River, Australia	3A	1	Eastern	AY203920
McArthur River, Australia	4A	1	Eastern	AY203921
Katherine River, Australia	5A	1	Eastern	AY203917
Ord River, Australia	6A	1	Eastern	AY203916
Non-Native				
Augusto Corrêa, northern Brazil	1BR ³ , 2BR ³	4 (1BR: 3; 2BR: 1)	-	GQ985381; GQ985387
Colares, northern Brazil	1BR, 2BR	2 (1BR:1; 2BR: 1)	-	GQ985382; GQ985388
Irituia, northern Brazil	1BR	1	-	GQ985383
Capanema, northern Brazil	1BR	1	-	GQ985384
Tracuateua, northern Brazil	1BR	3	-	GQ985385
CAUNESP ⁴ , southeastern Brazil	1BR	2	-	GQ985386
Outgroups				
<i>Macrobrachium australiense</i> ²	Mau	1	-	AY203923
<i>Macrobrachium lar</i> ²	Mlar	1	-	AY203922

¹Western = *M. dacqueti*; Eastern = *M. rosenbergii*; ²Samples from de Bruyn *et al.* (2004a); ³1BR and 2BR refer to the haplotypes found in different regions; ⁴Specimens from the Aquaculture Center (CAUNESP) of São Paulo State University, in Jaboticabal, São Paulo.

were run in PAUP* 4b10 (Swofford, 2002) and the maximum likelihood (ML) method that was run using PHYML 2.4.4 (Guindon and Gascuel, 2003). The significance of the observed groupings was estimated by bootstrap analysis based on 1,000 pseudoreplicates. Genetic divergence was evaluated in matrices constructed using PAUP*, according to the evolutionary model suggested by the MODELTEST application.

The geographic origin of the populations was identified from a haplotype network that was constructed using Network 4.0 (Bandelt *et al.*, 1999) based on the median joining (MJ) method. The mean divergence between the observed haplotypes in the different populations was estimated and analysis of molecular variance (AMOVA) was run using Arlequin 3.11 (Excoffier *et al.*, 2005) to compare native and exotic populations.

Results

Molecular identification using 16S rRNA

The alignment obtained for 16S rRNA contained 472 bp, of which 42 were variable between *M. rosenbergii* and *M. dacqueti*. Most of the mutations were exclusive to one or the other of the species. The phylogenetic analyses revealed the formation of two clades supported by strong bootstrap values in all cases (Figure 2). Only two haplotypes were identified in the Brazilian samples (GenBank accession numbers: GQ985381-GQ985388), and both were typical of *M. dacqueti* (de Bruyn *et al.*, 2004a).

Genetic divergence between the eastern (*M. rosenbergii*) and western (*M. dacqueti*) forms varied from 4.9% to 5.9%. Within-group divergence varied from 0.0% to 0.8% in *M. rosenbergii* and from 0.0% to 0.6% in *M.*

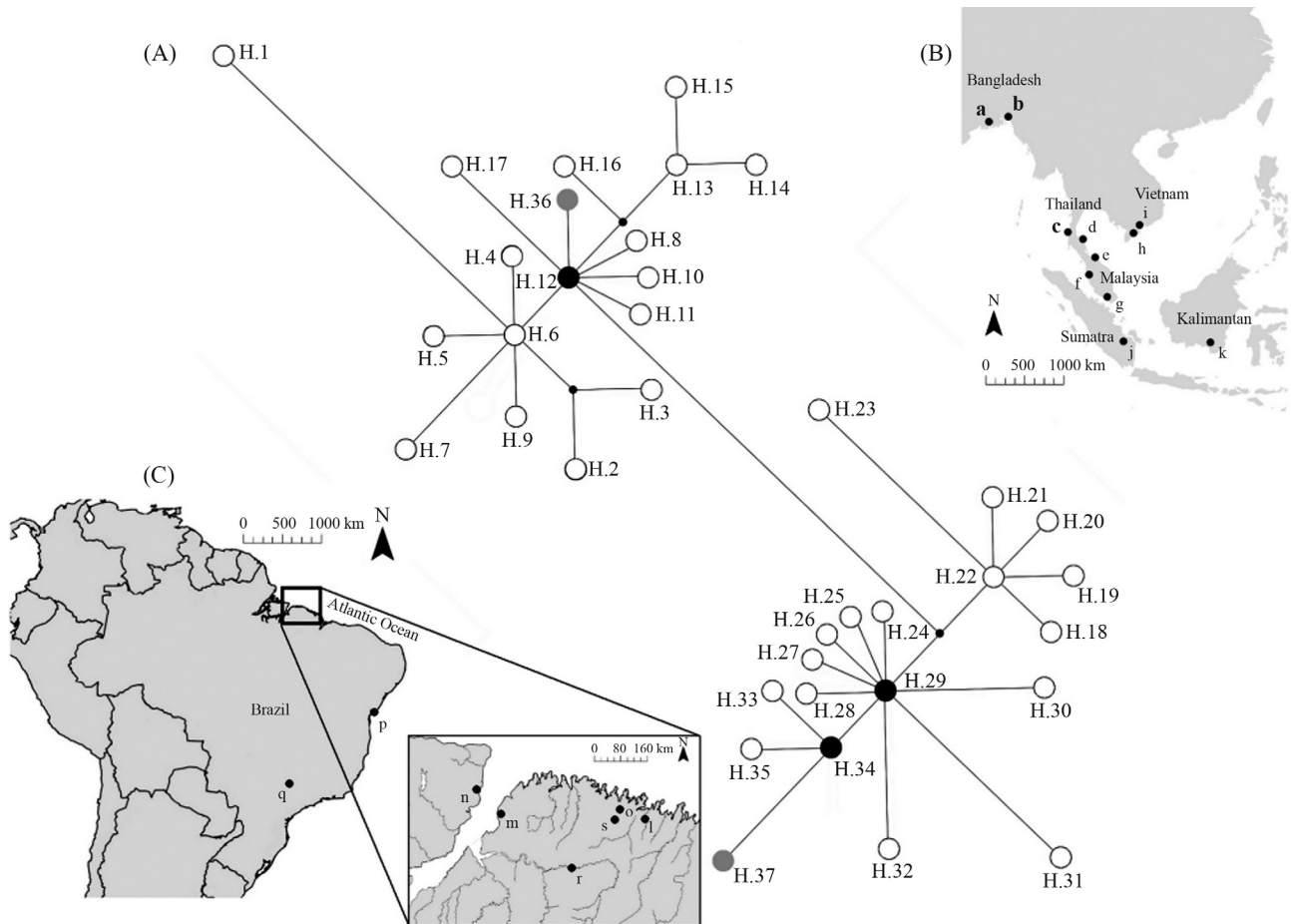


Figure 1 - (A) Network showing the relationships between the haplotypes found only in native populations of *M. dacqueti* (white), only in exotic populations (gray), and in native and exotic populations (black). (B) Locations of the native *M. dacqueti* populations sampled by de Bruyn *et al.* (2005): a – Raimangal, b – Meghna, c – Kraburi, d – Tapi, e – Setiu, f – Semenyih, g – Bahand, h – Dongnai, i – Mekong, j – Musi, k – Barito. The probable origins of the populations introduced into Brazil are highlighted in bold. (C) Exotic populations sampled in Brazil in this study. In detail, the coast of Pará (Amazon region): l – Augusto Corrêa, m – Colares, n – Soure, o – Tracuateua, p – Salvador (Bahia), q – CAUNESP (Aquaculture Center of São Paulo State University), São Paulo, r – Irituia and s – Capanema.

dacqueti (Table 2). The 1BR haplotype was by far the most frequent and was detected in all of the Brazilian regions sampled, whereas 2BR was found only in Pará (in the municipalities of Augusto Corrêa and Colares).

Origin of the Brazilian populations based on analysis of COI

The database used to identify the geographic origin of the Brazilian population included the samples used here and the 35 haplotypes recorded by de Bruyn *et al.* (2005) from 11 native populations of *M. dacqueti* (Table 3). Our results confirmed that the *Macrobrachium* species introduced into Brazil was *M. dacqueti*.

One hundred and seven sequences of COI were obtained from the Brazilian samples, with a total of 602 bp, 59 of which were variable. Five haplotypes were identified (GenBank accession numbers GQ995505-GQ995518), three of which were identical to those previously identified in native populations (haplotypes 12, 29, and 34). The other

two haplotypes (36 and 37) were recorded only in the Brazilian specimens. These five haplotypes formed two clades (Figure 1) with genetic divergences of 1.7-2.4%. Of the 11 natural populations analyzed by de Bruyn *et al.* (2005), eight (Raimangal, Meghna, Kraburi, Semenyih, Bahand, Dongnai, Mekong and Musi) may be the source of the Brazilian populations of *M. dacqueti*, as suggested by the presence of one of the three haplotypes. This relatively large number of possibilities reflected the presence of haplotypes 12 and 29 in some native populations (Table 3). However, the most probable geographic origin of these populations would be either Mekong in Vietnam, Raimangal or Meghna in Bangladesh, or Kraburi in Thailand (Table 4). Given the considerable geographic distances that separate these populations, it seems likely that the introduction of giant prawns into Brazil occurred on at least two different occasions.

The mean genetic divergence of the Brazilian populations (1.2-1.8%) was greater than that observed within native populations, which varied from 0.41% to 0.77%

Table 2 - Genetic distances between the 16S rRNA haplotypes analyzed in this study. The evolutionary model used in this analysis was HKY+G (G = 0.1502). Intraspecific divergence values for *M. rosenbergii* and *M. dacqueti* are marked in light gray and dark gray, respectively.

Haplotypes	Mlar	Mau	Mac	PH	PN	1A	2A	3A	4A	5A	6A	IJ	JA	1M	2M	3M	4M	1V	2V	1T	2T	1BR	2BR	
Mlar																								
Mau	9.3																							
Mac	13.6	13.0																						
PH	11.2	10.4	11.4																					
PN	11.4	10.1	11.2	0.2																				
1A	11.2	10.4	11.4	0.2	0.4																			
2A	11.0	10.1	11.2	0.2	0.4	0.2																		
3A	11.0	10.1	11.2	0.2	0.4	0.2	0.0																	
4A	11.2	10.1	11.2	0.4	0.6	0.4	0.2	0.2																
5A	11.2	10.4	11.4	0.4	0.6	0.4	0.2	0.2	0.4															
6A	11.4	10.4	11.7	0.6	0.8	0.6	0.4	0.4	0.6	0.2														
IJ	11.4	10.6	11.2	0.2	0.4	0.4	0.4	0.4	0.6	0.6	0.8													
JA	11.0	9.9	11.4	5.3	5.5	5.3	5.1	5.1	4.9	5.3	5.5	5.5												
1M	10.8	9.7	11.7	5.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2											
2M	10.8	9.7	11.7	5.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2	0.0										
3M	10.8	9.7	11.7	5.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2	0.0	0.0									
4M	11.2	0.1	11.7	5.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2	0.4	0.4	0.4								
1V	10.8	9.7	11.9	5.3	5.5	5.3	5.1	5.1	4.9	5.3	5.5	5.5	0.4	0.2	0.2	0.2	0.6							
2V	10.8	9.7	11.7	5.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2	0.0	0.0	0.0	0.4	0.2						
1T	11.0	9.9	11.9	5.7	5.9	5.7	5.5	5.5	5.3	5.7	5.9	5.9	0.4	0.2	0.2	0.2	0.6	0.4	0.2					
2T	10.8	9.7	11.7	5.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2	0.0	0.0	0.0	0.4	0.2	0.0	0.2				
1BR	10.8	09.7	11.7	05.5	5.7	5.5	5.3	5.3	5.1	5.5	5.7	5.7	0.2	0.0	0.0	0.0	0.4	0.2	0.0	0.2	0.0			
2BR	10.8	9.5	11.4	5.3	5.5	5.3	5.1	5.1	4.9	5.3	5.5	5.5	0.4	0.2	0.2	0.2	0.6	0.4	0.2	0.4	0.2	0.2		

(Table 4). This variation was due primarily to the divergence of approximately 2% observed between haplotypes 12 and 34, and indicated clearly that the Brazilian populations were derived from distinct native populations (Mekong and Raimangal, Meghna or Kraburi), with the exception of the captive CAUNESP population, which had a single origin (either Raimangal, Meghna or Kraburi; Table 4). AMOVA indicated significant genetic structuring of the native population, with haplotypic diversity divided primarily among populations rather than within them. In contrast, the introduced populations showed high intra- and inter-population diversity (Table 5), thus reaffirming the conclusion that they originated from genetically distinct native populations.

Discussion

According to the FAO Database on Introductions of Aquatic Species, the giant river prawn (identified as *M. rosenbergii*) has been introduced for aquaculture into at least 40 countries (FAO-DIAS, 2009). The results of the present study showed that the species introduced into Brazil was *M. dacqueti*, rather than *M. rosenbergii*. The available historical records indicate that Malaysia is the geographic origin of the *M. dacqueti* giant prawn populations farmed in a number of countries. Specimens were originally taken from the wild in Malaysia to the AFRC in Ho-

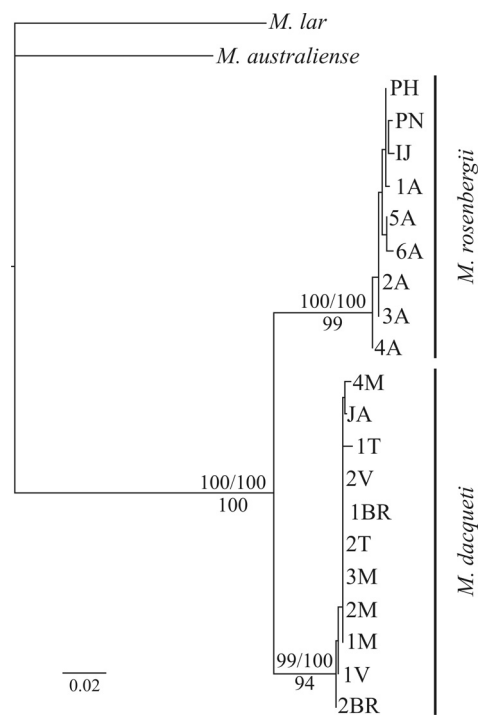


Figure 2 - Neighbor-joining tree showing the relationships among the haplotypes of *M. dacqueti* and *M. rosenbergii*. The values correspond to the neighbor-joining, maximum parsimony and maximum likelihood scores, respectively. The topology of the trees did not differ significantly among the three models.

Table 3 - Haplotypes of subunit I of the cytochrome oxidase gene (COI) used to determine the origin of the giant river prawn populations found in Brazil. Native populations were studied by de Bruyn *et al.* (2005).

Haplotype	Native						Non-Native										
	Bangladesh	Thailand	Malaysia	Vietnam	Indonesia	Brazil	Augusto Corrêa (l)	Colares (m)	Soure (n)	Tracueteua Salvador CAUNESP (p)	CAUNESP (q)						
1																	
2	4																
3	3																
4	2																
5	12																
6	1																
7	16																
8	1																
9		2															
10		1															
11	2	1															
12	10	11					8	3	3	2	22						
13		2															
14																	
15		1															
16		5															
17		1															
18																	
19			1				27										
20																	
21		26															
22			1														
23				17			1										
24				1													
25							1										
26																	
27		1															
28																	
29		1															
30			46														
31			40														
32		39															
33			14														
34																	
35																	
36																	
37																	
N	32	34	43	40	15	46	42	45	49	23	36	21	18	14	21	11	22

nolulu, Hawaii, where captive breeding techniques were first developed. This institution subsequently provided a number of other countries, including Brazil, with specimens (New, 2000, 2002). The data used here to reconstruct the history of the introduction of the giant river prawn into Brazil indicate different geographic origins (Bangladesh or Thailand and Vietnam). These data also indicate that there were at least two introductions involving specimens from different native stocks that may have favored their success during and following colonization.

Macrobrachium dacqueti is present not only in the Amazon region of northern Brazil, but also in the northeast (Salvador, Bahia) and in ponds of the CAUNESP facility in southern Brazil. In wild Amazonian populations, two 16S rRNA haplotypes (1BR and 2BR) were observed (with a molecular difference of only 0.2%), while only 1BR was recorded in the captive population from CAUNESP. This haplotype was 100% similar to those observed in Malaysia (1M, 2M and 3M), southern Vietnam (2V), and southeast Thailand (2T). Hanamura *et al.* (2008) found the same haplotypes in introduced individuals in Madagascar. The differences in relation to *M. rosenbergii* were > 5%, a level more characteristic of species rather than population divergence. These values are higher than those found, for example, among *M. lar*, *M. mamillocladactylus* and *M. latidactylus*, and between *M. acanthochirus* and *M. olfersii*, *M. crenulatum* and *M. zariqueyi*, and *M. latimanus* and *M. grandimanus* (Murphy and Austin, 2005; Liu *et al.*, 2007). In particular, these results further corroborate the detailed morphological analysis of Wowor and Ng (2007), who concluded that *M. dacqueti* (and not *M. rosenbergii*) was the species introduced worldwide for aquaculture. Currently,

Table 5 - Results of the analysis of molecular variance (AMOVA) for the native populations of *M. dacqueti* and those introduced into Brazil based on the sequences of subunit I of the cytochrome oxidase gene.

Source of variation	d.f.	Variance components	Variation* (%)
Native range			
Among	10	2.21809	73.19
Within	394	0.81262	26.81
Total	404	3.03072	
Introduced range			
Among	5	1.35118	46.01
Within	101	1.58548	53.99
Total	106	2.93666	

*p < 0.001 for all values.

M. rosenbergii refers to the eastern form, which occurs in eastern Indonesia, Australia and the Philippines. However, Wowor and Ng (2007) suggested suppressing the *M. rosenbergii* holotype by replacing it with that of *M. dacqueti*, thus making the two names objective synonyms and the former available for use. This would avoid much of the confusion that would be generated by renaming the widely-used aquaculture species, although it would also require a new holotype and name for the eastern form.

The history of the introduction of this species into Brazil is not well documented. There are few official records, and many have simply been lost following the deaths of the researchers involved in the first transfers. However, two of the early introductions are well-known. The first involved specimens obtained from Hawaii (originally from

Table 4 - Possible source localities of the *M. dacqueti* populations found in Brazil based on the COI sequences reported by de Bruyn *et al.* (2005), and the mean genetic divergence within each population.

Population	Mean within-population sequence divergence (%) - introduced	Number of source localities	Inferred source locality	Mean within-population sequence divergence (%) - source locality ¹
Northern Brazil				
Pará				
Augusto Corrêa	1.3	2	Mekong	0.41
Colares	1.2		Raimangal	0.62
Soure	1.3		Meghna	0.55
Tracuateua	1.4		Kraburi	0.77
Northeastern Brazil				
Bahia				
Salvador	1.8	2	Mekong, Raimangal, Meghna or Kraburi	
Southeastern Brazil				
São Paulo				
CAUNESP	0.0	1	Raimangal, Meghna or Kraburi	

¹Values estimated from the data of de Bruyn *et al.* (2005).

Malaysia) for research at the Federal University of Pernambuco in northeastern Brazil in 1977 or 1978 (Cavalcanti, 1998). The second involved specimens imported from Miami, USA, by the Agricultural Research Organization (PESAGRO) of the state of Rio de Janeiro, in southeastern Brazil, in 1981 (R.C. Martino, pers. commun.). It is nevertheless possible that a number of other institutions also imported the species at some time in the past (Cavalcanti, 1998). The CAUNESP center is currently Brazil's largest freshwater prawn research institute and still conducts studies on giant prawns derived from specimens obtained from PESAGRO.

The COI data used here to reconstruct the history of the introduction of the giant river prawn into Brazil indicated origins different from those reported previously (Cavalcanti, 1998; New, 2000, 2002). The presence of haplotypes 12 and 34 in Brazilian specimens confirmed that they did not originate from Malaysia. Haplotype 12 is found in native populations from Bangladesh (Raimangal and Meghna) and Thailand (Kraburi), while haplotype 34 occurs in the population from Mekong in Vietnam (de Bruyn *et al.*, 2005). Clearly, then, the Brazilian giant prawns are descended from native populations in Vietnam and either Bangladesh or Thailand.

Combining this finding with the historical records suggests that the first specimens introduced from Hawaii in 1977 and 1978 were derived from native Vietnamese populations. The subsequent importation of specimens from Miami, in 1981, appears to have involved a lineage originally from either Bangladesh (Raimangal or Meghna) or Thailand (Kraburi). It is nevertheless possible that the first introduction did in fact involve a Malaysian lineage, which would agree with the historical records given that haplotype 34 diverged by only a single transition (0.2%) from the most common haplotype (29) found in Malaysian populations. This haplotype was also recorded in a specimen from Colares (Pará). It is also possible that this population originated in Malaysia, given that haplotype 34 has been recorded in giant prawns, although this region was not sampled by de Bruyn *et al.* (2005) and therefore was not included in the present study. On the other hand, as Vietnam is the world's second largest producer of giant river prawn (New, 2005), we cannot rule out the possibility that specimens from this country were introduced into Brazil.

In the case of the original native populations, COI haplotypes 12 and 34 formed two distinct clades (a northern one and a southern one) that separated 4.5–5.5 million years ago, according to de Bruyn *et al.* (2005) following the formation of a marine barrier that isolated peninsular Malaysia from Thailand. The presence of both of these haplotypes in Brazil reinforces the conclusion that there were at least two introductions. These genetic lineages have since mixed in the Amazon region and in northeastern Brazil (Salvador, Bahia). The data on genetic divergence and the AMOVA results (Tables 4 and 5) are typical of exotic species that

have experienced multiple introduction events, *i.e.*, higher divergence values and more variation within than among populations (Kolbe *et al.*, 2004, 2007; Kelly *et al.*, 2006).

The admixture of individuals from genetically distinct native populations is typical of invasive species of vertebrates (Kolbe *et al.*, 2004; Lindholm *et al.*, 2005; Ficetola *et al.*, 2008) and invertebrates (Hänfling *et al.*, 2002; Meixner *et al.*, 2002; Kelly *et al.*, 2006; Ashton *et al.*, 2008; Audzijonyte *et al.*, 2009). This situation may be purely demographic (the occurrence of different genotypes within the same area) or genomic, with crossing and recombination resulting in novel genotypes (Keller and Taylor, 2008). Such events have two important effects that may contribute to the success of the introduction. One is the dilution of the founder effect, *i.e.*, a decrease in inbreeding depression and the maintenance of high levels of genetic variability (Hänfling, 2007), while another is the generation (by recombination) of new genotypes that are absent in the native populations (Lavergne and Molofsky, 2007). Although the present study focused on mitochondrial DNA, thereby restricting the analysis to the identification of demographic mixing, recombination cannot be ruled out. Indeed, haplotypes 36 and 37, which were only recorded in the Brazilian samples, may be the first signs of the invasion's success. Additional analyses with nuclear markers will be necessary to confirm admixture in Brazilian populations of the giant river prawn.

The first records of the occurrence of giant river prawns in the wild in the Amazon region (coast of Pará state) were from the municipalities of Belém and Bragança (Barros and Silva, 1997). Five years later, specimens (including ovigerous females) were collected in the wild in the municipalities of Colares and Salvaterra (Cintra *et al.*, 2003). More recently, Silva-Oliveira and coworkers (unpublished data) observed specimens (once again including ovigerous females) in a further eight municipalities, and developed models to show that the Amazon coastal zone is especially suitable for the development of the giant river prawn. There are also a number of records of the species at localities in the state of Maranhão (I3N-BRASIL, 2009), which borders Pará to the east. These records constitute a preoccupying scenario of establishment and expansion of *M. dacqueti* and indicate that the occurrence of this species in Brazil should be monitored carefully, particularly in the Amazon basin because of the region's high biodiversity.

Analysis of the correlation between molecular and historical data proved useful for validating events known to have occurred during the introduction process, in addition to helping in the identification of new variables. The species of giant river prawn introduced into Brazil was confirmed to be *M. dacqueti*, and the Brazilian populations were shown to have been derived from at least two introduction events that involved specimens from distinct native populations, none of which was cited in the historical records. This conclusion emphasizes the need to confirm the

origin of the giant river prawn populations introduced into other countries (FAO-DIAS, 2009), in particular the United States, Jamaica, Panama, Venezuela, Taiwan and Russia, where the prawns are known to have invaded natural environments (Pereira *et al.*, 1996; Woodley *et al.*, 2002; Kairo *et al.*, 2003; FAO-DIAS, 2009). Our findings also indicate an urgent need to monitor the potential impacts of wild populations of the giant river prawn in the coastal zone of the Amazon region.

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References

- Ashton GV, Stevens MI, Hart MC, Green DH, Burrows MT, Cook EJ and Willis KJ (2008) Mitochondrial DNA reveals multiple northern hemisphere introductions of *Caprella mutica* (Crustacea, Amphipoda). *Mol Ecol* 17:1293-1303.
- Audzijonyte A, Wittmann KJ, Ovcarenko I and Väinölä R (2009) Invasion phylogeography of the Ponto-Caspian crustacean *Limnomysis benedicti* dispersing across Europe. *Divers Distrib* 15:346-355.
- Bandelt HJ, Forster P and Rohlf A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37-48.
- Barros MP and Silva LMA (1997) Registro de introdução da espécie exótica *Macrobrachium rosenbergii* (De Man, 1879) (Crustacea, Decapoda, Palaemonidae) em águas do Estado do Pará. *Bol Museu Paraense Emílio Goeldi* 13:31-37.
- Cavalcanti LB (1998) Histórico. In: Valenti WC (ed) *Carcinicultura de Água Doce*. Instituto Brasileiro do Meio Ambiente e do Recursos Naturais Renováveis, Brasília, pp 17-20.
- Cintra IHA, Silva KCA and Muniz APM (2003) Ocorrência de *Macrobrachium rosenbergii* (de man, 1879) em áreas estuarinas do Estado do Pará (Crustacea, Decapoda, Palaemonidae). *Bol Tec Cient CEPNOR* 3:219-227.
- de Bruyn M, Wilson JA and Mather PB (2004a) Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Mol Phylogenet Evol* 30:251-257.
- de Bruyn M, Wilson JC and Mather PB (2004b) Reconciling geography and genealogy: Phylogeography of giant freshwater prawns from the Lake Carpentaria region. *Mol Ecol* 13:3515-3526.
- de Bruyn M, Nugroho E, Hossain MM, Wilson JC and Mather PB (2005) Phylogeographic evidence for the existence of an ancient biogeographic barrier: The Isthmus of Kra Seaway. *Heredity* 94:370-378.
- Excoffier L, Laval G and Schneider S (2005) Arlequin v. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47-50.
- Ficetola GF, Bonin A and Maud C (2008) Population genetics reveals origin and number of founders in a biological invasion. *Mol Ecol* 17:773-782.
- Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294-299.
- Gazola-Silva FF, Melo SG and Vitule JRS (2007) *Macrobrachium rosenbergii* (Decapoda, Palaemonidae): Possível introdução em um rio da planície litorânea paranaense (PR, Brasil). *Acta Biol Paranaense* 36:83-90.
- Guindon S and Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696-704.
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95-98.
- Hanamura Y, Koizumi N, Morioka S, Andriantsoa M and Rafalimanana T (2008) Occurrence of the non-indigenous freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) (Decapoda, Caridea, Palaemonidae) in Madagascar. *Crustaceana* 81:1143-1149.
- Hanfing B (2007) Understanding the establishment success of non-indigenous fishes: Lessons from population genetics. *J Fish Biol* 71:115-135.
- Hänfling B, Carvalho GR and Brandl R (2002) mt-DNA sequences and possible invasion pathways of the Chinese mitten crab. *Mar Ecol Prog Ser* 238:307-310.
- Johnson DS (1973) Notes on some species of the genus *Macrobrachium* (Crustacea, Decapoda, Caridea, Palaemonidae). *J Singapore Nat Acad Sci* 3:273-291.
- Kairo M, Ali B, Cheesman O, Haysom K and Murphy S (2003) Invasive Species Threats in the Caribbean Region. Report to the Nature Conservancy, Arlington, 63 pp.
- Keller SR and Taylor DR (2008) History, chance and adaptation during biological invasion: Separating stochastic phenotypic evolution from response to selection. *Ecol Lett* 11:852-866.
- Kelly DW, Muirhead JR, Heath DD and Macisaac HJ (2006) Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. *Mol Ecol* 15:3641-3653.
- Kolbe JJ, Glor RE, Rodriguez Schettino L, Lara AC, Larson A and Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431:177-181.
- Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A and Losos JB (2007) Multiple sources, admixture, and genetic aariation in introduced *Anolis* lizard populations. *Conserv Biol* 21:1612-1625.
- Lavergne SB and Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proc Natl Acad Sci USA* 104:3883-3888.
- Lindenfelser ME (1984) Morphometric and allozymic congruence: Evolution in the prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae). *Syst Zool* 33:195-204.
- Lindholm AK, Breden F, Alexander HJ, Chan W-K, Thakurta SG and Brooks R (2005) Invasion success and genetic diversity

- of introduced populations of guppies *Poecilia reticulata* in Australia. *Mol Ecol* 14:3671-3682.
- Liu M, Cai Y and Tzeng C (2007) Molecular systematics of the freshwater prawn genus *Macrobrachium* Bate, 1868 (Crustacea, Decapoda, Palaemonidae) inferred from mtDNA sequences, with emphasis on East Asian species. *Zool Stud* 46:272-289.
- MacIsaac H, Grigorovich I and Ricciardi A (2001) Reassessment of species invasions concepts: The Great Lakes basin as a model. *Biol Invasions* 3:405-416.
- Magalhães C, Bueno SLS, Bond-Buckup G, Valenti WC, Silva HLM, Kiyohara F, Mossolin EC and Rocha SS (2005) Exotic species of freshwater decapod crustaceans in the state of São Paulo, Brazil: Records and possible causes of their introduction. *Biodivers Conserv* 14:1929-1945.
- Meixner MD, McPheron BA, Silva JG, Gasparich GE and Shepard WS (2002) The Mediterranean fruit fly in California: Evidence for multiple introductions and persistent populations based on microsatellite and mitochondrial DNA variability. *Mol Ecol* 11:891-899.
- Minchin D (2007) Aquaculture and transport in a changing environment: Overlap and links in the spread of alien biota. *Mar Pollut Bull* 55:302-313.
- Murphy NP and Austin CM (2005) Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea, Decapoda, Palaemonidae): Biogeography, taxonomy and the convergent evolution of abbreviated larval development. *Zool Scr* 34:187-197.
- Naylor R, Williams SL and Strong DR (2001) Aquaculture – A gateway for exotic species. *Science* 294:1655-1656.
- New MB (2000) History and global status of freshwater prawn farming. In: New MB and Valenti WC (eds) *Freshwater Prawn Culture: The Farming of *Macrobrachium rosenbergii**. Blackwell Science, London, pp 1-10.
- New MB (2002) *Farming Freshwater Prawns: A Manual for the Culture of the Giant River Prawn (*Macrobrachium rosenbergii*)*. FAO Fisheries Technical Papers, Rome, 197 pp.
- New MB (2005) Freshwater prawn farming: Global status, recent research and a glance at the future. *Aquac Res* 36:210-230.
- Palumbi SR, Martin A, Romano S, Mcmillan WO, Stice L and Grabowski G (1991) *The Simple Fool's Guide to PCR*. University of Hawaii, Honolulu, 46 pp.
- Pereira G, Egáñez H and Monente JA (1996) First record of a reproductive wild population of *Macrobrachium rosenbergii* (De Mam) (Crustacea, Decapoda, Palaemonidae) in Venezuela. *Acta Biol Venezuelica* 16:93-95.
- Posada D and Crandall KA (1998) Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14:817-818.
- Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ and Hines AH (2000) Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. *Annu Rev Ecol Syst* 31:481-531.
- Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edition. Cold Spring Harbor Laboratory Press, New York, 1659 pp.
- Swofford DL (2002) PAUP*, *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, v. 4. Sinauer Associates, Sunderland.
- Valenti WC (1993) Freshwater prawn culture in Brazil. *J World Aquac Soc* 24:29-34.
- Woodley CM, Slack WT, Peterson MS and Vervaeke WC (2002) Occurrence of the non-indigenous giant Malaysian prawn, *Macrobrachium rosenbergii* (De Man, 1879) in Simmons Bayou, Mississippi, USA. *Crustaceana* 75:1025-1031.
- Wowor D and Ng PKL (2007) The giant freshwater prawns of the *Macrobrachium rosenbergii* species group (Crustacea, Decapoda, Caridea, Palaemonidae). *Raffles Bull Zool* 55:321-336.
- Xia X and Xie Z (2001) DAMBE: Data analysis in molecular biology and evolution. *J Hered* 92:371-373.

Internet Resources

- Food and Agriculture Organization of the United Nations, Database on Introductions of Aquatic Species (FAO-DIAS), <http://www.fao.org/fi/website/FIRetrieveAction.do?dom=collection&xml=dias.xml> (November 7, 2009).
- Food and Agriculture Organization of the United Nations, Fisheries Global Information System (FAO-FIGIS), http://www.fao.org/figis/servlet/TabLandArea?tb_ds=Aquaculture&tb_mode=TABLE&tb_act=SELECT&tb_grp=COUNTRY (November 7, 2009).
- Base de Dados I3N de Espécies Exóticas Invasoras do Brasil, <http://i3n.institutohorus.org.br/index.asp> (November 12, 2009).

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