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., 2000; 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-
mura and his team took a number of specimens from Malaysia to the Anuenue 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, 1991), while COI was amplified using the primers developed by Folmer et al. (1994). The PCR conditions were as follows: each 25 µL reaction contained 4 µL of deoxynucleotide triphosphate (dNTP; 1.25 mM), 2.5 µL of 10 x buffer solution, 1 µL of MgCl₂ solution (25 mM), 1-2 µL of total DNA (200 ng/µL), 0.25 µL of each primer (200 ng/µL), 0.20 µL of Taq polymerase (5 U/µL; 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 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 an 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
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. dacqueti*.
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 *Macrobrachium 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, Tapi, Setiu, 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 |
|------------|------|-----|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Mlar       | 9.3  |     |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Mau        | 13.6 | 13.0|     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Mac        | 11.2 | 10.4| 11.4|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| PH         | 11.4 | 10.1| 11.2| 0.2|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| PN         | 11.2 | 10.4| 11.4| 0.2| 0.4|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 1A         | 11.0 | 10.1| 11.2| 0.2| 0.4| 0.2|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2A         | 11.0 | 10.1| 11.2| 0.2| 0.4| 0.2| 0.0|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 3A         | 11.0 | 10.1| 11.2| 0.4| 0.6| 0.4| 0.2| 0.2|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 4A         | 11.0 | 10.1| 11.2| 0.4| 0.6| 0.4| 0.2| 0.2| 0.2|    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5A         | 11.2 | 10.4| 11.4| 0.4| 0.6| 0.4| 0.2| 0.2| 0.2| 0.2|    |    |    |    |    |    |    |    |    |    |    |    |
| 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| 0.4| 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| 5.3| 5.5| 5.5| 5.5| 5.5| 5.5| 5.5| 5.5| 5.5| 5.5| 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| 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| 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| 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| 0.2| 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| 0.0| 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| 0.2| 0.4| 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| 0.0| 0.4| 0.2| 0.0| 0.2|
| 1BR        | 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| 0.0| 0.4| 0.2| 0.0| 0.2|
| 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| 0.2| 0.6| 0.4| 0.2| 0.2|

(continued)
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 | Bangladesh | Thailand | Malaysia | Vietnam | Indonesia | Brazil |
|-----------|------------|----------|----------|---------|-----------|--------|
|           | Raimangal (a) | Krabun (c) | Tap (d) | Setiu (e) | Semenyih (f) | Bahand (g) | Dongnai (h) | Mekong (i) | Musi (j) | Barito (k) | Augusto Corrêa (l) | Colares (m) | Soure (n) | Tracuateua (o) | Salvador (p) | CAUNESP (q) |
| 1         | 4          | 1         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 2         | 3          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 3         | 2          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 4         | 12         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 5         | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 6         | 16         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 7         | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 8         | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 9         | 2          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 10        | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 11        | 2          | 1         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 12        | 10         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 13        | 11         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 14        | 2          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 15        | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 16        | 5          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 17        | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 18        | 2          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 19        | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 20        | 1          | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 21        | 26         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 22        | 26         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 23        | 14         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 24        | 14         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 25        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 26        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 27        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 28        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 29        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 30        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 31        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 32        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 33        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 34        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 35        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 36        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |
| 37        | 15         | 2         | 16       | 1       | 17        | 8       | 3          | 3          | 3        | 2          | 22       |

N 32 34 43 40 15 46 42 49 45 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. mammillodactylus* 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, *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...
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 (Krabi), 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 (Krabi). 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; Audijzonyte 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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