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DISTRIBUTION AND GENETIC DIFFERENTIATION OF *MACROBRACHIUM JELSKII* (MIERS, 1877) (NATANTIA: PALAEMONIDAE) IN BRAZIL REVEAL EVIDENCE OF NON-NATURAL INTRODUCTION AND CRYPTIC ALLOPATRIC SPECIATION

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ABSTRACT

Macrobrachium jelskii (Miers, 1877) occurs in all major Brazilian drainage basins. Considering its wide distribution and the supposed isolation of certain populations, we evaluated phylogenetic relationships among populations from several river basins. Analyses of molecular data using the mitochondrial COI and 16S genes suggest the division of the species into two clades, "Amazonian" and "coastal." The genetic divergence between these two groups is greater than the genetic divergence within the respective groups. Based on the time of divergence and the probable natural distribution of the species, we suggest two evolutionary scenarios to explain the origin of the coastal clade. The first scenario considers the capacity of *M. jelskii* to tolerate saline environments. The second is based on Pliocene climate changes that would have caused river confluence from sea level regression. The two clades occur sympatrically but this is a recent condition. The wide geographic distribution and low genetic divergence of the Amazonian clade allows us to propose that the current distribution of *M. jelskii* is partly due to human activity. Pending future analysis, the absence of a consistent morphological synapomorphy precludes the description of a new cryptic taxonomic entity for *M. jelskii*.

KEY WORDS: 16S rRNA, Caridea, COI, freshwater shrimps, human activities

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INTRODUCTION

Macrobrachium Spence Bate, 1868, placed in the family Palaemonidae Rafinesque, 1815, is one of the most species-rich freshwater shrimp groups, being distributed in tropical and subtropical areas around the world (Holthuis, 1980). There are 19 species of *Macrobrachium* found in Brazil, 17 are native and two were introduced (Magalhães et al., 2005; Maciel et al., 2011; Pileggi and Mantelatto, 2012). Two strongly marked characters shown by several species, low interspecific morphological variability, and high intraspecific variability have been raising doubts about the taxonomic status of some species (Pileggi and Mantelatto, 2010). Molecular data have therefore become an important tool for the elucidation of complex taxonomic, phylogenetic, and phylogeographic relationships in this very diverse taxon, as analyses of morphological characters alone are not enough (Liu et al., 2007; Baker et al., 2008; Pileggi and Mantelatto, 2010; Vergamini et al., 2011; Rossi and Mantelatto, 2013).

Macrobrachium jelskii (Miers, 1877) is a species endemic to South America, occurring in Venezuela, Trinidad, Suriname, the Guianas, Peru, Bolivia, Argentina, and Brazil. It occurs in all major river basins in Brazil: the Amazon, Tocantins/Araguaia, São Francisco, and Paraná/Paraguay river basins (Magalhães et al., 2005; Boos et al., 2012; Pileggi et al., 2013) as well as several coastal basins. *Macrobrachium jelskii* has abbreviated larval development, and therefore its dispersion capacity is considered to be limited compared to other species with extended larval development (Gamba, 1984; Jalihal et al., 1993; Magalhães, 2002). *Macrobrachium jelskii* is an important component of the limnic trophic web being exclusively found and abundant in freshwater environments. It is also economically important in the aquarium trade, artisanal fishing bait, and protein source for humans and other animals (Soares, 2008; Ottoni et al., 2011; Barros-Alves et al., 2012). Recreational fishing, storage of shrimps for aquaculture, the release of live bait, and the existence of illegal crops can also be considered causes for the introduction of this species in non-native areas (Eno et al., 1997; Gazola-Silva et al., 2007; Gozlan, 2008), which suggests human activity could be partly responsible for the known records of *M. jelskii*.

Its wide distribution (being natural or introduced) and the characteristics of its life cycle (with low capacity of dispersion) allow us to hypothesize that the morphological and molecular variability among populations of *M. jelskii* along Brazilian drainages are compatible with interspecific variations due to allopatric speciation. The identification of in-

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traspecific groups has already been reported in Brazil for some species of *Macrobrachium*, such as *M. amazonicum* (Heller, 1862) (Vergamini et al., 2011) and *Macrobrachium potiuna* (Müller, 1880) (Carvalho et al., 2013). These examples reinforce the possibility that a similar situation could happen to *M. jelskii*, once its natural distribution reaches that of *M. amazonicum* (Magalhães et al., 2005; Vergamini et al., 2011). These populations have not been evaluated from a molecular and phylogenetic perspective.

The objective of this study was to describe the genetic variability of *M. jelskii* from Brazilian river basins and evaluate the existence of cryptic, phylogenetically related species not yet described in order to explain the current distribution of the species.

Materials and Methods

Sampling and Morphological Analysis

Specimens were obtained from field collections over almost the complete distribution of the species in Brazil (Fig. 1) and loans and donations from different collections. The collections complied with current applicable state and federal Brazilian laws (FLC authorization from ICMBio No. 25329 and permanent license to FLM for the collection of Zoological Material No. 11777-1 MMA/IBAMA).

We performed prior identification of the material based on diagnostic morphological characteristics of the species (Holthuis, 1952; Melo, 2003). Morphological analyses for clade differentiation within *M. jelskii* were performed based on diagnostic characters traditionally used for the family (Holthuis, 1952; Pereira, 1997; De Grave and Ashelby, 2013; Carvalho et al., 2014).

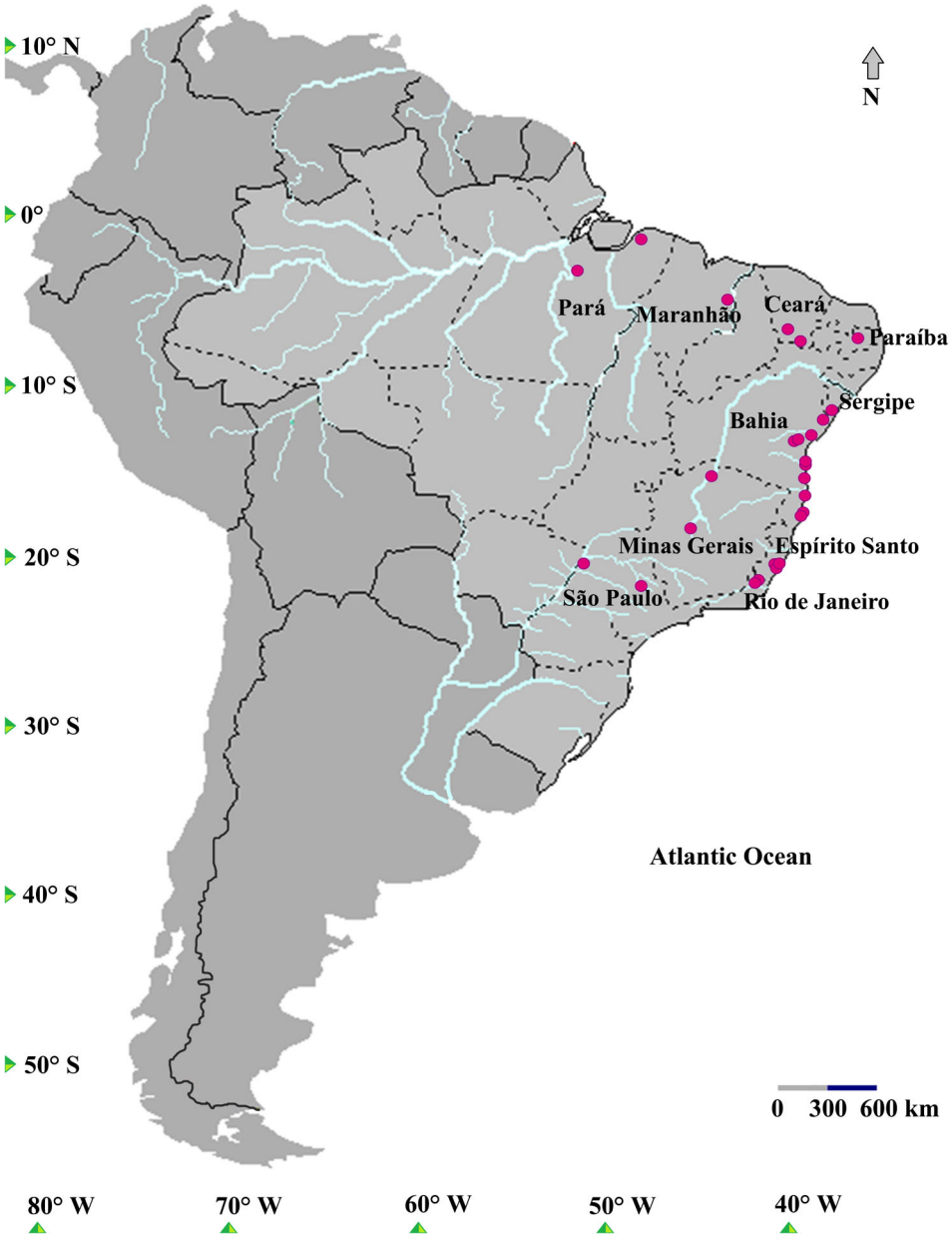

Fig. 1. Localities where the specimens of *Macrobrachium jelskii* used in the study were collected. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

Molecular Data

Nucleotide sequences were obtained based on protocols described by Schubart et al. (2000), modified by Mantelatto et al. (2007) and Pileggi and Mantelatto (2010). Some modifications were done to adapt to our material.

DNA Extraction

Adult individuals were selected whenever possible. Genetic vouchers, which were extracted from tissue samples for analyses, were deposited at Coleção de Crustáceos do Departamento de Biologia (CCDB), Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP) (Table 1).

Genomic DNA was extracted from muscle tissue of the abdomen of selected individuals. The tissue was incubated for 24 h in 600 μl of lysis buffer at 55°C with the addition of 200 μl of proteinase K (approximately 500 μg/ml; Thermo Scientific, Rockford, IL, USA), followed by 200 μl of ammonium acetate (7.5 M), and the sample was centrifuged at 14,000 rpm for 10 minutes at 18°C. The supernatant was collected and transferred to 600 μl of isopropanol. The resulting sample was centrifuged at 14,000 rpm for 10 minutes at 18°C and kept in a freezer at −20°C. After 24 to 48 h the sample was centrifuged again at 14,000 rpm for 10 minutes at 18°C and the supernatant discarded. The resulting residue (pellet) was washed with ethanol 70%, dried, and resuspended in 30 μl of TE 1× buffer. The extracted DNA concentration was measured by spectrophotometer (NanoDrop® 2000/2000c, Wilmington, DE, USA).

DNA Amplification

The regions of interest (fragments of the mitochondrial genes 16S rRNA and cytochrome *c* oxidase subunit I (COI)) were amplified by PCR (Polymerase Chain Reaction). The PCR products were obtained in reactions of 25 μl containing 6.5 μl of deionized water, 5 μl of 5 M betaine, 3 μl of 10× PCR buffer, 3 μl of 25 mM $MgCl_2$, 4 μl of 0.25 μM each DNTP, 2 μl of primer (1 μl of each, 16S-1472 4.59 nmol/OD (AGATAGAAAC CAACCTGG), 16S-L2 4.44 nmol/OD (TGCCTGTTTATCAAAAACAT), HCOI 3.18 nmol/OD (TAAACTTCAGGGTGACCAAAAAATCA), and LCOI 3.41 nmol/OD (GGTCAACAAATCATAAAGATATTGG) (Folmer et al., 1994; Crandall and Fitzpatrick, 1996; Schubart et al., 2002)), 0.5 μl of Taq DNA recombinant polymerase (5 U/μl, Thermo Scientific, Waltham, MA, USA), and 1 μl of DNA at 10 ng/μl for 16S and 80 ng/μl for COI.

The amplification was performed in an Applied Biosystems (Foster City, CA, USA) Veriti 96 Well Thermal Cycler® (thermal cycles: initial denaturing for 5 min at 95°C; pairing for 40 cycles: 45 s at 95°C, 45 s at 52°C, 1 min at 72°C; final extension 3 min at 72°C for 16S rRNA; and initial denaturing for 2 min at 94°C; pairing for 40 cycles: 30 s at 94°C, 30 s at 42-46°C, 1 min at 72°C; final extension 10 min at 72°C for COI). Electrophoresis was performed using 1.5% agarose gel with PCR product. We photographed the results with a digital camera C-7070 Olympus® in a transilluminator UV M20 UVP®.

Table 1. Specimens of *Macrobrachium jelskii* used in phylogenetic analyses, sampling locality, and CCDB (Coleção de Crustáceos do Departamento de Biologia, Universidade de São Paulo, Brazil) catalog numbers. Notations "II," "III" and "IV" were assigned to specimens with the same catalog number to allow individuals to be distinguished; *Pileggi and Mantelatto (2010); **Carvalho et al. (2013).

Species	Locality	Catalog number	GenBank accession number	
			16S	COI
M. jelskii	Córrego na rodovia, Castanhal, Pará	CCDB 4337	KP054355	–
	Igarapé, Senador José Porfírio, Pará	CCDB 4992	KP054349	KP054337
	Riacho do Brejinho, Caxias, Maranhão	CCDB 1578	KP054340	–
	Açude Bengue, Aiuaba, Ceará	CCDB 4569	KP054350	KP054323
	Açude Sabiá, Juazeiro do Norte, Ceará	CCDB 4712	KP054345	KP054327
	Rio Piranhas, São Bento, Paraíba	CCDB 4568	KP054343	KP054321
	Rio Itamirim, Sergipe	CCDB 4567	KP054361	KP054332
	Rio Inhambupe, Bahia	CCDB 4566	KP054344	KP054322
	Ilha de Itaparica, Vera Cruz, Bahia	CCDB 2625	KP054364	KP054328
	Rio Vermelho, Elísio Medrado, Bahia	CCDB 3794	KP054339	KP054335
	Mutuípe, Bahia	CCDB 3796	KP054347	KP054319
	Rio de Contas, Bahia	CCDB 2788	KP054356	KP054317
	Rio Tijuípe, Itacaré, Bahia	CCDB 1566	–	KP054326
	Rio Pancadinha, Uruçuca, Bahia	CCDB 1254	KP054352	KP054320
	Rio Jacão, Una, Bahia	CCDB 3087-1	KP054360	KP054331
	Rio Jacão, Una, Bahia (II)	CCDB 3087-2	–	KP054329
	Estação Veracel, Porto Seguro, Bahia	CCDB 1657	KP054351	–
	Córrego da Lagoa Grande, Prado, Bahia	CCDB 4203	KP054342	KP054324
	Rio Jucuruçu afluente, Prado, Bahia (II)	CCDB 4201	KP054354	KP054316
	Rio São Francisco, Januária, Minas Gerais	CCDB 549	KP054346	–
	Pirapitinga, Três Marias, Minas Gerais	CCDB 2952	KP054341	–
	Lagoa Grande, Vila Velha, Espirito Santo	CCDB 2627-1	KP054358	–
	Lagoa Grande, Vila Velha, Espirito Santo (IV)	CCDB 2627-2	KP054365	–
	Córrego Sete, Vila Velha, Espirito Santo (II)	CCDB 2628-1	KP054359	KP054334
	Córrego Sete, Vila Velha, Espirito Santo (III)	CCDB 2628-2	KP054362	KP054333
	Lagoa de Maeaípe, Guarapari, Espirito Santo	CCDB 2842	KP054363	KP054330
	Córrego Caranguejo, Cardoso Moreira, Rio de Janeiro	CCDB 4991	KP054348	KP054325
	Pureza, Cambuci, Rio de Janeiro	CCDB 4650	KP054353	KP054318
	Pereira Barreto, São Paulo	CCDB 2129	KP054357	–
	Clube Náutico de Araraquara, Araraquara, São Paulo	CCDB 3520	KP054338	KP054336
Outgroups				
M. amazonicum	Aquidauana, Mato Grosso do Sul	CCDB 1970	HM352442*	–
M. brasiliense	Rio Claro, Serra Azul, São Paulo	CCDB 2135	HM352429*	KP054314
M. potiuna	Iguape, São Paulo	CCDB 2496	JX466934**	KP054315

Purification and Sequencing

We purified the PCR products using the SureClean Plus® kit (Bioline, Taunton, MA, USA), following the manufacturer's instructions.

The sequencing of the samples was performed in automated sequencer (ABI 3730 XL DNA Analyzer®, Applied Biosystems, Foster City, CA, USA) at Departamento de Tecnologia da Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Universidade Estadual Paulista "Júlio de Mesquita Filho," with the reaction kit ABI Big Dye® Terminator Mix (Applied Biosystems).

Editing Sequences

Both DNA strands (sense and antisense) were sequenced for higher reliability in the obtained sequences. The sequence of each strand was edited to generate a consensus sequence using the computational program BioEdit v7.0.5 (Hall, 2005). The sequence alignment was conducted in the software MAFFT v7.158 (Katoh and Standley, 2013), using the default setting.

Phylogenetic Analysis

Uncorrected genetic divergences (p-distance) were calculated using the MEGA 6 program (Tamura et al., 2011). Statistical selection of nucleotide substitution models were conducted in jModelTest v2.1.4 (Darriba et al., 2012).

Bayesian analyses were conducted in MrBayes v3.2.2 program (Ronquist et al., 2012). The nucleotide substitution model initially used was GTR + Γ + I. We also performed analyses with simpler models: TPM2u + I for 16S and HKY + Γ for COI. These models were selected using Bayesian Information Criterion (BIC), chosen from among jModelTest available models. BIC provided the values of the parameters for analysis (frequency of nucleotides and transition/transversion ratio, gamma distribution and variable sites proportion, when it was present in the model). These values were used to set *a priori* model parameters in MrBayes. Analyses were performed with 10^6 generations in two independent parallel simulations with four chains each and were stopped when they reached stationarity (average standard deviation between two simulations less than 0.01). The parameter values were saved once every 1000 rounds. The first quarter of the parameters and trees were discarded. Only nodes with *a posteriori* probability higher or equal to 50% were shown (see Ronquist et al., 2012).

Initial analyses were performed using 16S and COI matrices separately. Analysis of the concatenate matrix of both genes of interest was also conducted in MrBayes program. Data were divided into two independent partitions, each following the simplest model that best applied to each gene (TPM2u + I for 16S and HKY + Γ for COI), with parameters provided by jModelTest. This analysis was initially performed with all sequences obtained in this study, but it was not possible to obtain sequences of both genes for all individuals. Initial analysis thus included 16S sequences without 100% of the corresponding COI and vice versa, i.e., COI sequences without 100% of the corresponding 16S. Analysis was also performed only with sequences from individuals who had sequences for both genes.

Maximum likelihood analyses were conducted in the RAxML program (7.6.3) (Stamatakis, 2006) and implemented in Cyberinfrastructure for Phylogenetic Research (CIPRES; available on line at http://www.phylo.org). The assumed nucleotide substitution model was GTR + Γ + I. The consistency of the topologies was measured by bootstrap method (1000 pseudoreplicates), and only confidence values higher than 50% were reported. This analysis was performed for the genes 16S and COI separately and for a concatenate matrix of both.

The majority rule consensus tree between Bayesian inference method and maximum likelihood was obtained using the software Mesquite v3.01 (Maddison, 2003).

Molecular Dating

Likelihood ratio test (LRT) was performed in order to test the null hypothesis that data evolved under a molecular clock, which was accepted ($p > 0.05$). Likelihood values for the molecular trees obtained by means of Bayesian inference with and without an imposed molecular clock (both using the models TPM2u + I and HKY + Γ) were compared using a LRT calculator in jModelTest. We therefore used a strict molecular clock in order to estimate the approximate divergence time between *M. jelskii* clades. We assumed mutation rates per site per million years for corrected values of divergence of 0.007 (± 0.00133) for 16S and 0.0120 (± 0.00169) for COI. To obtain these rates, the molecular clock was calibrated with two groups of palaemonid species with known divergence time: *Palaemon carteri* (Gordon, 1935), *Palaemon ivonicus* (Holthuis, 1950), and *Palaemon yuna* Carvalho, Magalhães and Mantelatto, 2014, from 9.5 Ma, and *Palaemon paivai* Fausto Filho, 1967 and *Palaemon ritteri* Holmes, 1895, from 3 Ma (see Carvalho et al., 2014). The calibration points were then removed and several mutation rates were tested. We selected the rates that resulted in divergence times that were closest to those obtained with calibration points.

RESULTS

We obtained approximately 1130 bp of sequence, i.e., 16S partial sequence (about 550 bp) of 28 individuals of *M. jelskii* from 26 locations as well as specimens of three congeners (*M. amazonicum*, *M. brasiliense* (Heller, 1862) (from Pileggi and Mantelatto, 2010), and *M. potiuna* (from Carvalho et al., 2013)), and the partial sequences of COI (about 580 bp) of 22 individuals of *M. jelskii* from 20 locations and two other species of *Macrobrachium* (*M. potiuna* and *M. brasiliense*).

The topology showed the presence of two groups within the species, here named "Amazonian clade" (consisting of specimens from all sampled regions) and "coastal clade" (consisting of some specimens from eastern Brazilian basins in the states of Sergipe, Bahia, and Espírito Santo). These names were chosen because they refer to the likely natural distribution of each clade. Both Bayesian inference analysis and maximum likelihood showed the monophyly of these two groups, based on 16S rRNA, COI and on the concatenated sequences of the two genes (Fig. 2).

None of the substitution models used (GTR + Γ + I and TPM2u + I for 16S and GTR + Γ + I and HKY + Γ for COI) resulted in significant differences in the tree topologies obtained by Bayesian inference. Only a slight variation in the clades support values was shown.

The concatenated analysis that includes 16S sequences without a COI correspondent and COI sequences without a 16S correspondent did not present differences in tree topologies compared to the analysis done only with sequences from individuals who had sequences for both genes.

The intraspecific genetic divergence of *M. jelskii* ranged from 0.0 to 3.6% for 16S and from 0.0 to 10.0% for COI. Genetic divergence between the two groups ranged from 2.3 to 3.6% for 16S and from 6.0 to 10.0% for COI. Genetic divergence among specimens of the Amazonian clade varied from 0.0 to 1.6% for 16S and from 0.0 to 3.2% for COI. In the coastal clade, this variation was 0.0% for 16S and from 0.0 to 0.5% for COI (Figs. 3 and 4).

The time of divergence between the two clades (based on 16S rRNA and COI genes) was estimated to be approximately 3 million years. This average ranged from approximately 2.5 to approximately 3.5 Ma and was lower in 16S analysis than in COI analysis. The 95% credibility confidence interval (HPD, or highest posterior density) ranged from 1.7 to 3.7 Ma for 16S, 2.4 to 4.4 Ma for COI, and from 2.3 to 4.3 for concatenate analysis, using a strict molecular clock (Fig. 5). These data indicate that the divergence between the Amazonian and coastal clades occurred in the Pliocene.

DISCUSSION

Molecular analysis showed the existence of two clades, Amazonian and coastal, of *M. jelskii*. The Amazonian clade

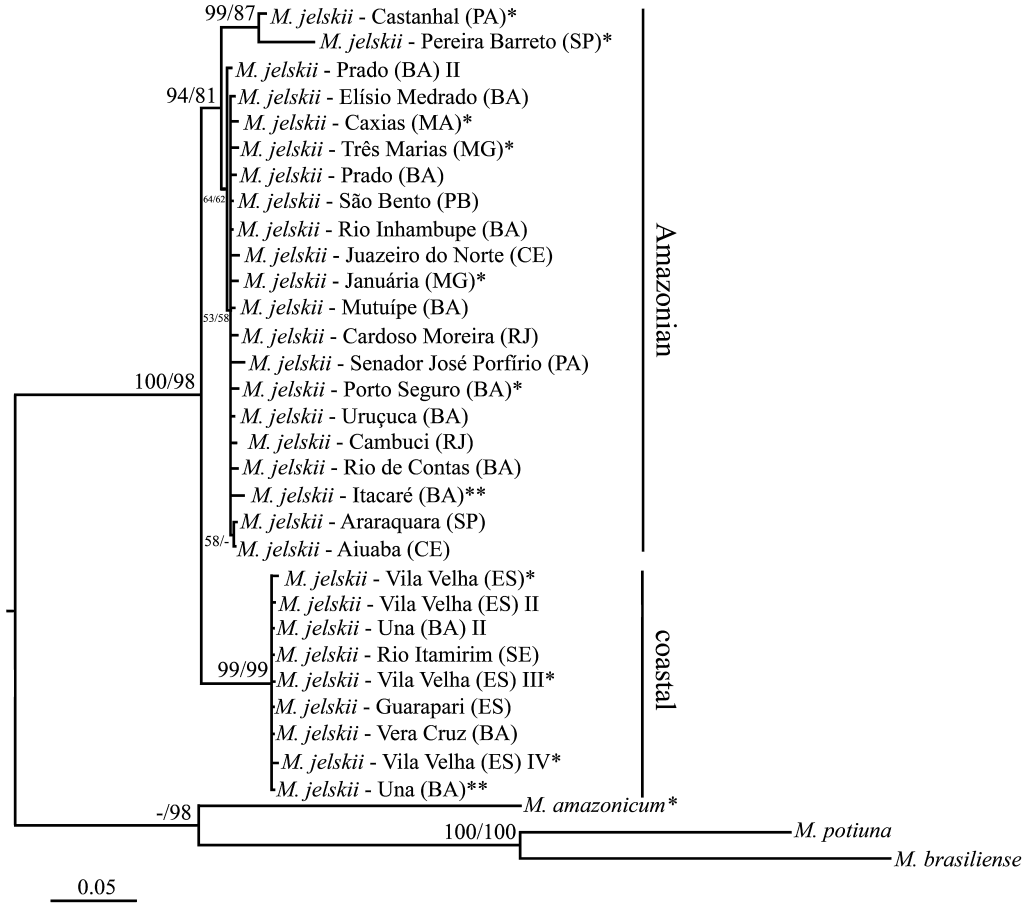

Fig. 2. Consensus tree between Bayesian inference (TPM2u + I and HKY + Γ models) and maximum likelihood for the genes 16S and COI concatenated. Node numbers represent the *a posteriori* probability for Bayesian inference and bootstrap values for maximum likelihood, respectively; * individuals that only had 16S sequences, ** individuals that only had COI sequences. Abbreviations for Brazilian states: PA, Pará; SP, São Paulo; BA, Bahia; MA, Maranhão; MG, Minas Gerais; PB, Paraíba; CE, Ceará; RJ, Rio de Janeiro; ES, Espírito Santo.

is distributed in both coastal and interior areas, including the states of Pará, Maranhão, Ceará, Paraíba, Minas Gerais, and São Paulo, and areas closer to the coast of the states of Bahia and Rio de Janeiro. The coastal clade is exclusively distributed in coastal areas of Sergipe, Bahia and Espirito Santo states (Fig. 6). Although the two clades occur sympatrically today, they underwent an allopatric speciation process during the Pliocene. We propose two sets of events to explain the current distribution of *M. jelskii*: ancient and recent events. The ancient events clarify the divergence between the coastal and Amazonian clades and recent events explain the occurrence of the Amazonian clade in several areas.

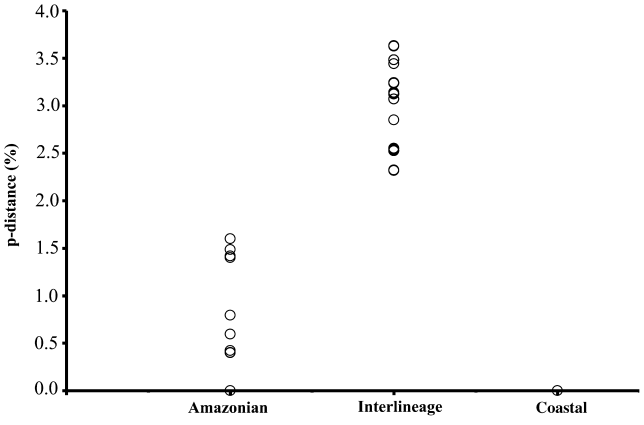

Fig. 3. Uncorrected genetic divergence values (p-distance) interlineage and intralineage for Amazonian and coastal clades of *Macrobrachium jelskii* based on gene 16S.

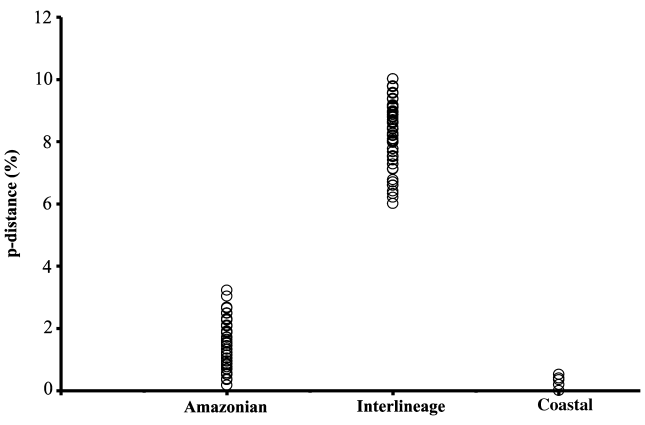

Fig. 4. Uncorrected genetic divergence values (p-distance) interlineage and intralineage for Amazonian and coastal clades of *Macrobrachium jelskii* based on gene COI.

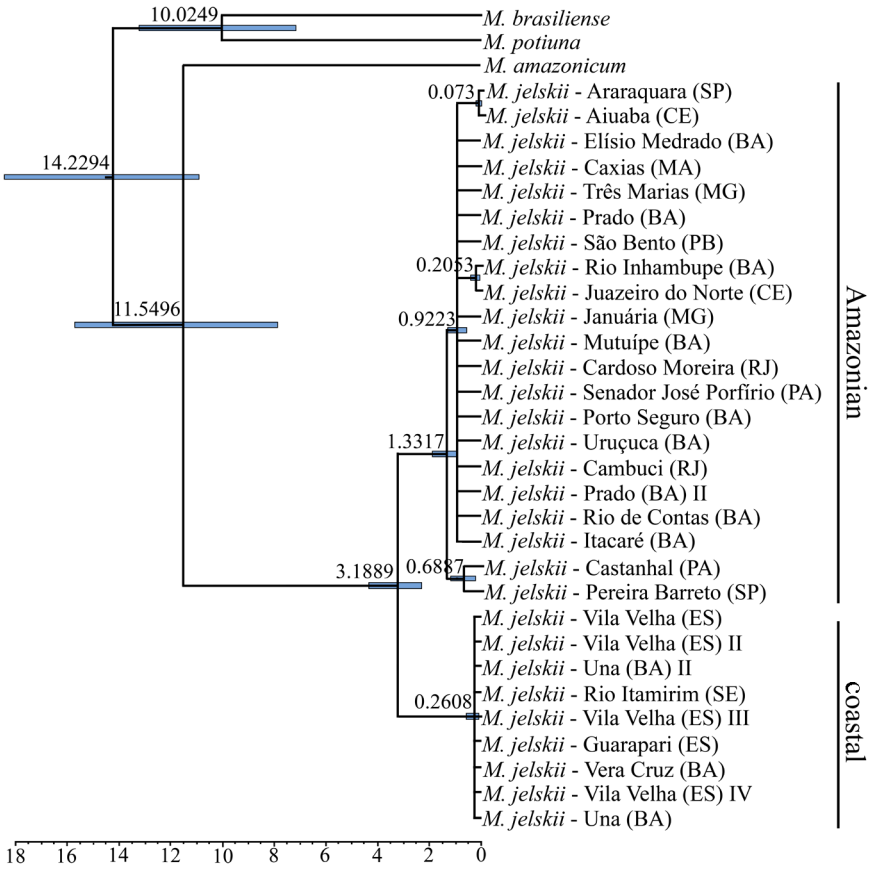

Fig. 5. Tree obtained by Bayesian inference for the genes 16S and COI concatenated (TMP2u + I and HKY + Γ models, respectively). Node numbers represent the divergence time in million years, and the bars represent the highest posterior density – HPD. Abbreviations for Brazilian states: PA, Pará; SP, São Paulo; BA, Bahia; MA, Maranhão; MG, Minas Gerais; PB, Paraíba; CE, Ceará; RJ, Rio de Janeiro; ES, Espírito Santo. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

Ancient Events

We consider the Amazonian region as the likely center of origin of the *M. jelskii* lineage. Marine incursions of the Miocene onto northern South America enabled the invasion of marine organisms in this area, the Pebas system. Hololimnetic lineages of *Macrobrachium* arose from lineages of those immigrants that were trapped in inland waters during the later regressions and could survive to the gradually changing environment (see Anger, 2013). The proto-Amazon-Orinoco basin and adjacent basins (such as modern upper Paraguay and Paraná basins) can therefore be considered the dispersion center of some marine derived lineages, such as the species of *Macrobrachium*. This species is known from several Brazilian river basins, but Magalhães et al. (2005) proposed that the natural distribution includes the Orinoco, Amazon, Paraguay, and low Paraná basins.

The divergence of the two *M. jelskii* lineages occurred during the Pliocene, as indicated from our molecular clock analysis. This result is due to the low genetic distances verified between the two groups, which indicate recent divergence of the lineages. The same divergence pattern was suggested for the congeners *M. amazonicum* and *M. potiuna*, which show genetic structuration similar to *M. jelskii* (Vergamini et al., 2011; Carvalho et al., 2013).

Based on the above evidence, and considering both the Amazonian region as the likely dispersion center of *M. jelskii* and the estimated time of divergence, we suggest two evolutionary scenarios for the divergence into the Amazonian and coastal clades. Both scenarios demand the movement of *M. jelskii* from the Amazon basin to the northeastern coast of Brazil. The first one takes into account the capacity, although limited, of *M. jelskii* to tolerate seawater (Holthuis, 1980). From the Amazon basin, *M. jelskii* would have colonized the eastern Brazilian basins through coastal waters, creating the coastal clade. This hypothesis is based on the relative resistance that freshwater shrimps have to saline environments reported for some species of *Macrobrachium* (Moreira et al., 1983; Freire et al., 2003). Although a strictly freshwater, or hololimnetic, species, its abundance in coastal basins and its relative resistance to saline environments (Holthuis, 1980) are indicative that *M. jelskii* might have dispersed throughout coastal basins and then colonized continental waters near the eastern Brazilian coast (Sergipe, Bahia and Espirito Santo states). A similar situation was observed for *M. potiuna*, whose juveniles show some resistance to temperature and salinity changes, so it is dispersed among coastal basins in periods of high rainfall (Moreira et al., 1983; Carvalho et al., 2013).

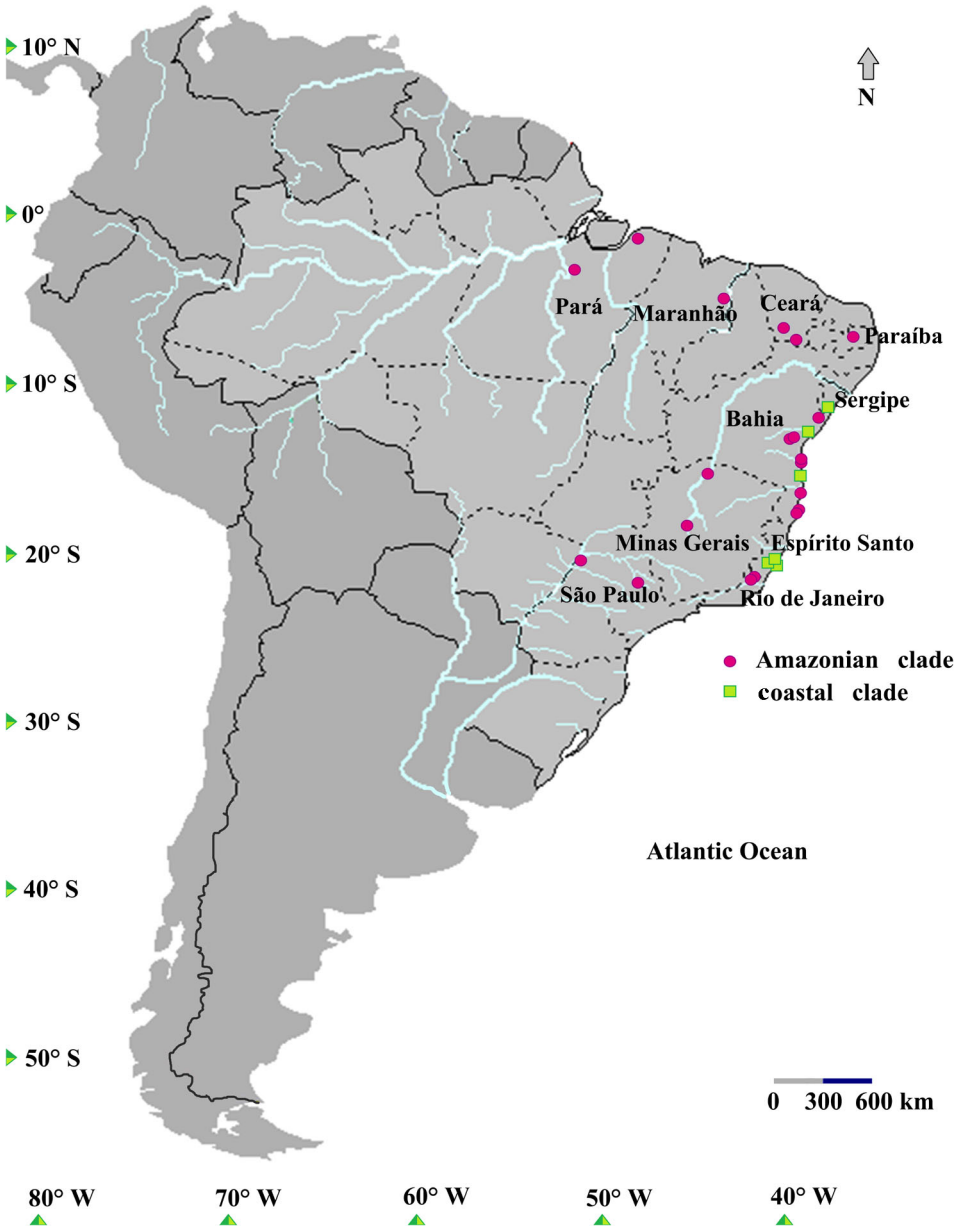

Fig. 6. Geographical distribution of *Macrobrachium jelskii* populations, showing the Amazonian and coastal clades. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

The second scenario takes into account climate change during the Miocene/Pliocene. By that time the climate had become warmer and drier and the sea level had dropped (Pellegrino et al., 2005; Dwyer and Chandler, 2008). This marine regression event probably led to river confluence at their lower courses throughout the Brazilian coast, and it is considered to be one of the ways freshwater fishes spread through South American basins (Costa, 2010). The dispersion of *M. jelskii* from the Amazon basin to the northeastern coast would have therefore been facilitated, later moving from north to south along the eastern coast and occupied coastal basins. The subsequent reestablishment of the sea level would have prevented the continuation of this flow by river confluence, isolating *M. jelskii* populations in distinct basins. The water salinity in these habitats would have exceed the tolerance of *M. jelskii*, constituting a barrier to the species dispersal.

Recent Events

Macrobrachium jelskii populations of the Amazonian clade from distinct drainage basins show low genetic divergence. Considering the time of isolation in the basins where the Amazonian clade is found, this pattern of distribution would hardly be found if the occurrence of *M. jelskii* in those basins were natural. For example, the São Francisco basin was consolidated during the Neoproterozoic without evidence of communication with the Amazon basin since this period (Mabesoone and Neumann, 2005; Buckup, 2011). The Amazonian clade of *M. jelskii* is nevertheless found in both basins. The possibility of dispersion by human activi-

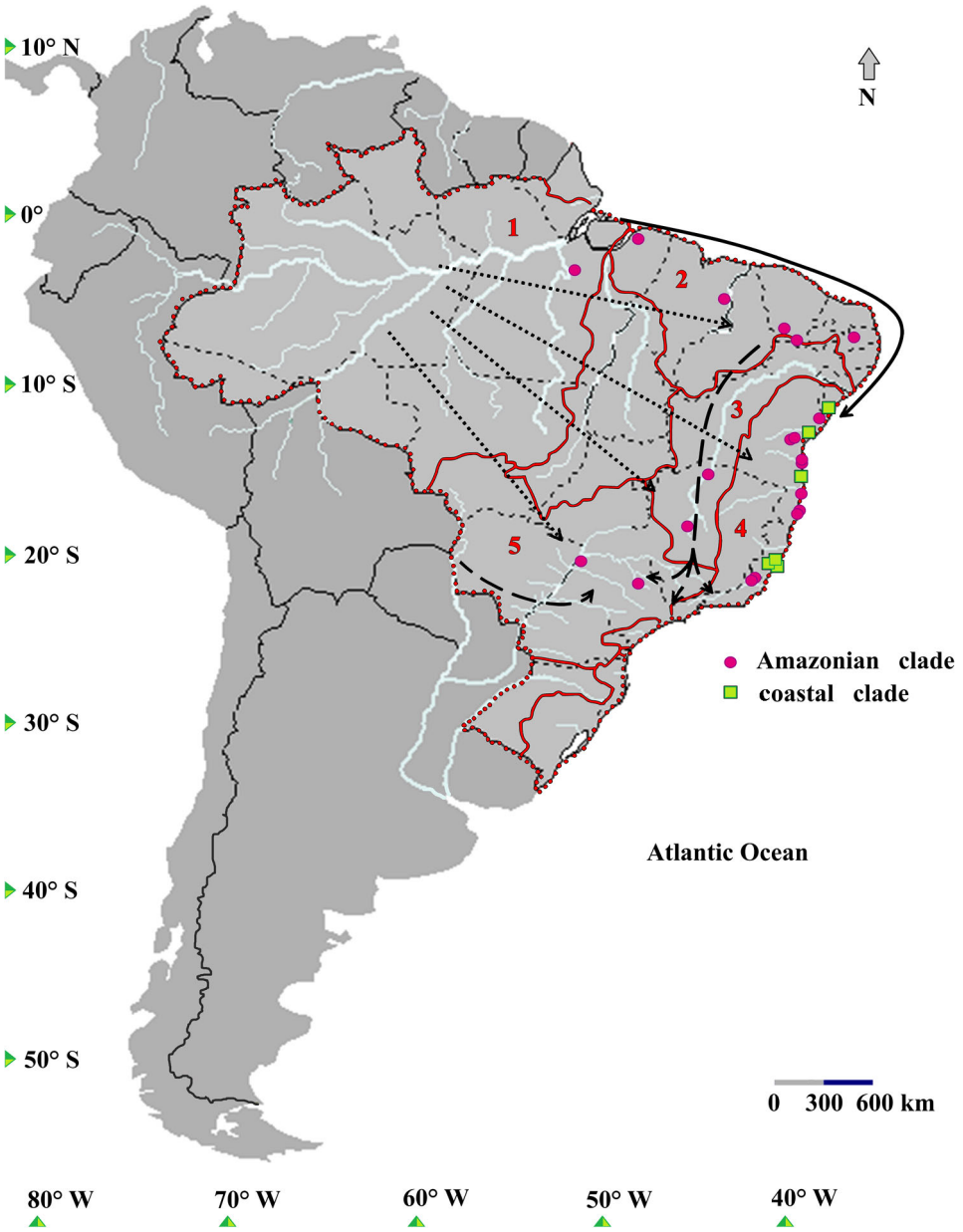

Fig. 7. Hypothetical dispersion events of *Macrobrachium jelskii*. The solid arrow indicates the likely dispersion that occurred before the Pliocene divergence from the center of origin in the Amazon basin. Dashed arrows represent the dispersion events cited in the literature: transplantation of *Plagioscion squamosissimus* from Brazilian northeastern reservoirs to southeast (longer arrow) and dispersion by commercial fishing preserves (shorter arrow). Dotted arrows illustrate dispersion from the Amazon basin to several areas by human activities. Regions indicated by outline: (1) Amazon, (2) northeastern Atlantic, (3) São Francisco, (4) east Atlantic, and (5) Paraná/Paraguay river basins. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

ties therefore must be considered because this species is used for fishing, the aquarium trade, and as food (Magalhães et al., 2005; Pileggi et al., 2013). The introduction of *M. jelskii* in aquaculture stations from CESP (Companhia Energética de São Paulo) between 1966 and 1973 were confirmed by Torloni et al. (1993). The introduction occurred as part of the transplantation process of a fish, the South American silver croaker *Plagioscion squamosissimus* (Heckel, 1840), from reservoirs from northeastern Brazil. By 1973, small individuals of the fish had reached reservoirs from Ilha Solteira and Jupiá, upper Paraná River, dispersing to Grande River after they had escaped from their previous environments in Pardo River in 1970. It is therefore possible that freshwater shrimps followed the same dispersion pattern (Magalhães et al., 2005). CESP also has dams at the upper Paraíba do Sul River basin, which could explain the occurrence of individuals from the Amazonian clade in the state of Rio de Janeiro.

Magalhães et al. (2005) mentioned that *M. jelskii* could have also been brought to certain São Paulo localities by commercial (pay for, or "fish and pay") fishing preserves from Pantanal populations because fishes from this region are used to populate pounds and reservoirs used in

these commercial activities. Larvae, juveniles, and immature forms of *M. jelskii* could also have been transported inadvertently with macrophyte roots, which are used as shelter for fishes during transportation in containers (see Montoya, 2003; Paschoal et al., 2013).

The introduction and transference of fishes into inland waters has been taking place, whether intentionally or accidentally. The peacock bass, *Cichla* sp. (Bloch and Schneider, 1801), a carnivorous fish from the Amazon basin, is used in sport fishing and for food (Nascimento et al., 2001) and is currently widely distributed throughout Brazil. Native to the same basin, *M. jelskii* could have been transported along with this fish and other Amazonian species, either inadvertently as larvae or juveniles, or to be used as food or bait for fishes. Crustaceans, mainly species of *Macrobrachium*, are part of diet of carnivorous fishes (Santos et al., 2001). These likely events affecting the dispersal of *M. jelskii* are shown in Fig. 7.

Considering human action as an important factor in the current distribution of *M. jelskii*, another possibility for the existence of a coastal clade, although unlikely, is that these populations could have been introduced from regions other than the Amazon basin. Vergamini et al. (2011) suggests the introduction of *M. amazonicum* from the Paraná and Paraguay hydrographic regions, which has a natural distribution and genetic structure similar to those of *M. jelskii* and is divided into three intraspecific clades.

Magalhães et al. (2005) claimed that no negative ecological effects were reported within 60 years after *M. jelskii* introduction in northeastern Brazil. Despite the possibility of not causing environmental problems, however, there is the risk of "anthropogenic homogenization" (Souza et al., 2009) once the hybridization of populations is favored by the introduction (McNeely and Schutyser, 2003). The genetic diversity detected here in *M. jelskii* populations can be lost through time if continuous introduction by human activity (leading to homogenization) persists.

Taxonomic Implications

Even though it is possible to propose hypotheses to explain the current distribution of *M. jelskii* and suggest distinct evolutionary scenarios that would have led to the formation of two clades as shown by molecular analysis, the absence of consistent morphological characters (synapomorphies) precludes the description of a new taxon for the coastal clade of *M. jelskii*. It is possible, however, that such morphological differences do exist but were not detected. The two clades were the result of recent divergence, hence they might not yet been expressed by conspicuous morphological differences. The existence of cryptic species with likely differences in other biological characters is still possible. Ecological speciation has been already observed in other groups (Schluter, 2009), including a species of *Macrobrachium* (Carvalho et al., 2013). Studies on the habitat and lifestyle demonstrating ecological barriers between the two clades could help clarify the causes of this divergence, contributing to delimit a cryptic taxonomic entity and suggesting speciation into distinct ecological niches.

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