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ECOSYSTEMS

Phylogenomic analyses reveals gene flow between populations of the freshwater shrimp *Potimirim brasiliana* (Caridea, Atyidae) along its wide distribution

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Abstract: *Potimirim* is one of the 40 genera of Atyidae restricted to America, which occurs in coastal freshwater habitats and questions about population status and variability have been emerging. *Potimirim brasiliana* occurs in Brazil from the northeastern to southeastern region. In order to evaluate the hypothesis of genetic structure among populations, we performed molecular analyses with specimens from all known limit of distribution of the species. The molecular markers used were COI and 28S. Phylogenetic trees were obtained by maximum likelihood and Bayesian analyses, and a haplotype network was obtained based only on COI. We found clear separation between *P. brasiliana*, *P. potimirim*, *P. glabra* and *Potimirim* sp 2. No pattern of structuration was found among *P. brasiliana*, but the haplotype network showed geographic pattern of structuration for the congener *P. potimirim*. The lack of genetic structuration among *P. brasiliana* can be explained by its life cycle that requires brackish water to complete their larval development. The larvae and juvenile in contact with these habitats can spread through oceanic currents, especially in higher rainfall seasons, maintaining the gene flow. The explanation for the geographical pattern found among *P. potimirim* is still missing and aspects about its lifecycle and larval development should be investigated.

Key words: Brazilian drainages, COI, connectivity, Decapoda, 28S.

INTRODUCTION

The freshwater shrimp's diversity found in the tropical and subtropical regions of South America is represented by 5 families, 11 genera, 88 species (Magalhães et al. 2016). Among them, *Potimirim* Holthuis, 1954, is one of the 40 genera of the speciose family Atyidae De Haan, 1849 (De Grave & Fransen 2011) restricted to the American continent. Members of the genus show short rostrum, with teeth only on the ventral edge, antennal and pterigostomial spines, no supraorbital spines, cornea not wider than the ocular peduncles, basis of the pereiopods without exopod, basis of the first three or four pairs of pereiopods with epipod, slender chelae and endopod of the first pair of pereiopods of males different from the females (Holthuis 1954).

The controversial taxonomic background of the genus *Potimirim* received an important contribution by Torati & Mantelatto (2012) that used combination of alpha morphology and molecular analyses to elucidate some aspects of its evolution and help the identification of species. Many of these previous difficulties on species identification arise from the wide distribution in similar habitats and high intraspecific variability (Müller 1892, Villalobos 1959, Smalley 1963, Page et al. 2008).

According to the most current literature (Torati & Mantelatto 2012, WoRMS 2020), there are five recognized freshwater species in the genus *Potimirim*: *Potimirim americana* Guérin-Méneville, 1855, *Potimirim mexicana* De Saussure, 1857, *Potimirim potimirim* (Müller, 1881), *Potimirim glabra* Kingsley, 1878 and *Potimirim brasiliana* Villalobos, 1959. Other than these, there are two new species pending description (Torati & Mantelatto 2012): *Potimirim* sp 1 and *Potimirim* sp 2. All these species are restricted to America and occur along both Pacific and Atlantic coast in Neotropical region (Torati & Mantelatto 2012).

Potimirim brasiliana is a tiny freshwater shrimp, endemic of coastal rivers and streams of Brazil, distributed from the states of Bahia (northeastern region), Espírito Santo, Rio de Janeiro, São Paulo (southeastern region), to Paraná and Santa Catarina in southern region (Villalobos 1959, Barros & Fontoura 1996a, Torati 2009, Torati & Mantelatto 2012). It is found beneath submerged marginal vegetation, hidden under rocks and gravel (Chace & Hobbs 1969, Ramos-Porto & Palácios 1981, Lima et al. 2006, Torati 2009), or in fast waters (Chace & Hobbs 1969, Ramos-Porto & Palácios 1981). This shrimp genus depends on brackish water to complete its lifecycle (Molina 1987) and some abiotic factors can influence the population structure, such as temperature affecting the time of larval development (Hoffmann & Negreiros-Fransozo 2010), and the reproductive patterns (Barros & Fontoura 1996b, Rocha et al. 2013).

Potimirim brasiliana is a gonochoric species (Grilli et al. 2014), and shows sexual dimorphism, in which the females are commonly larger than males, besides the presence of *appendix masculina* in males (Parker 1992). The seasons of year and geographical location affect the reproductive activity and fertility of this species (*e.g.* populations of southern Brazil show lower reproductive activity) (Rocha et al. 2013). According to the IUCN (International Union for Conservation of Nature and Natural Resources) criteria, the conservation status of this species has previously been suggested as Least Concern (LC), but additional studies in different areas are necessary to make inferences more precise on this status (Mantelatto et al. 2016).

Despite of the knowledge accumulated during the past years, and the recent molecular phylogenetic picture between species of *Potimirim*, there is no conclusive study about the genetic relationships between populations of *P. brasiliana*. Variable molecular markers can provide substantial background on the genetic structure of populations (Palsbøll et al. 2007). COI is a mitochondrial marker with high mutation rate compared with nuclear genes, and it is used to determine intra and interspecific phylogenetic relationships. The gene 28S is a nuclear marker and was chosen for its low mutation rate, being efficient for the study of interspecific relationships (see Timm & Bracken-Grissom 2015 for review on genetic markers).

Due its wide geographical distribution along the Brazilian drainages, we performed more robust genetic analyses in order to elucidate the relationships and to evaluate the hypothesis of genetic structure among the populations of *P. brasiliana*. In addition to the comparative analysis, genetic information on the congener *P. potimirim* was furnished.

MATERIALS AND METHODS

Sampling

We analyzed specimens of the genus *Potimirim* obtained from field collections in Brazil and from the Crustacean Collection of the Department of Biology (CCDB) at the Faculty of Philosophy, Science and Letters at Ribeirão Preto (FFCLRP), University of São Paulo (USP). Specimens of *P.*

brasiliana were obtained from all known limit of its distribution. The collections complied with current applicable state and federal Brazilian laws (SISBIO license to FLM for the collection and genetic analysis of decapods No. 11777-1, 16/09/2007).

We checked the identification of the material based on diagnostic morphological characteristics of the species (Holthuis 1954, Villalobos 1959, Chace & Hobbs 1969, Chace 1972, Melo 2003, Torati & Mantelatto 2012), especially the shape and size of the *appendix masculina*, rostrum and dactyl of the third and fourth pereiopods.

Molecular Data

Nucleotide sequences were obtained based on protocols described by Schubart et al. (2000), modified by Pileggi & Mantelatto (2010) and Torati & Mantelatto (2012).

We obtained new sequences of *Potimirim* for two molecular markers: COI and 28S; complementary sequences were retrieved from GenBank [COI: 7 nominated in GenBank as *Potimirim glabra* but identified as *Potimitim* sp 2 by Torati & Mantelatto (2012), and 1 of *P. glabra*. 28S: 1 of *P. potimirim* and 1 of *Potimirim glabra*, identified as *Potimirim* sp 2 by Torati & Mantelatto (2012)]. COI sequences of *Atya scabra* Leach, 1815 and *Micratya poeyi* Guérin-Méneville, 1855 and 28S sequences of *Jonga serrei* Bouvier, 1909, *Micratya poeyi*, *Atya ortmannioides* Villalobos, 1956, *Atya scabra* and *Atyoida bisulcata* Randall, 1840 (all from GenBank) were used as outgroups (Table I).

DNA Extraction

Adult males were selected whenever possible, which helps the identification of the species. Genetic vouchers were deposited at CCDB.

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 with 200 µL of proteinase K (500 µg/mL) at 55°C, followed by addition of 200 µL of ammonium acetate (7.5M). The sample was centrifugated 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 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).

DNA Amplification

The regions of interest [fragments of the mitochondrial gene cytochrome c oxidase subunit I (COI) – primers LCO1490 (5´ - GGT CAA CAA ATC ATA AAG ATA TTG G - 3´) and HCO2198 (5´ - TAA ACT TCA GGG TGA CCA AAA AAT CA - 3´ (Folmer et al. 1994) – and nuclear gene 28S – primes 28S Rd1a (5' - CCC SCG TAA YTT AAG CAT AT - 3') and 28S Rd4b (5' - CCT TGG TCC GTG TTT CAA GAC - 3') (Edgecombe & Giribet 2006)] – 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 MgCl2, 4 µL of 0.25 µM each DNTP, 2 µL of primer (1 µL of forward and 1 µL of reverse), 0.5 µL of Taq DNA recombinant polymerase (5 U/µL), and 1 µL of DNA at 10 ng/µL. The amplification was performed in an Applied Biosystems Veriti 96 Well Thermal Cycler® (thermal cycles: initial denaturing for 2 min at 94°C; pairing for 40 cycles: 30 s at 94°C, 30 s at 48°C, 1 min at 72°C; final extension 10 min at 72°C for COI; and initial denaturing for 5 min at 95°C; pairing for 40 cycles: 45 s at 95°C, 45 s at 52°C, 1 min

Table I. Data of the sequences obtained from GenBank. *Species named as *P. glabra* in GenBank, but posteriorly identified as *Potimirim* sp 2.

Species	Locality	GenBank	Gene	Reference
P. glabra	Panama	JF811005.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811012.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811011.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811010.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811009.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811008.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811007.1	COI	Page et al. 2011
*P. glabra**	Trinidad and Tobago	JF811006.1	COI	Page et al. 2011
P. potimirim	Panama	FN995614.1	28S	Von Rintelen et al. 2012
*P. glabra**	Trinidad and Tobago	FN995613.1	28S	Von Rintelen et al. 2012
Atya scabra	Trinidad and Tobago	JF810990.1	COI	Page et al. 2013
Micratya poeyi	Caribbean	FJ348827.1	COI	Cook et al. 2010
Atya scabra	----	FN995550.1	28S	Von Rintelen et al. 2012
Micratya poeyi	----	FN995595.1	28S	Von Rintelen et al. 2012
Jonga serrei	----	FN995587.1	28S	Von Rintelen et al. 2012
Atya ortmannioides	----	FN995549.1	28S	Von Rintelen et al. 2012
Atyoida bisulcata	----	FN995553.1	28S	Von Rintelen et al. 2012

at 72°C; final extension 3 min at 72°C for 28S). Electrophoresis was performed using 1% agarose gel with PCR product. We photographed the results with a digital camera C-7070 Olympus® in a transilluminator UV M20 UVP®.

Purification and Sequencing

We purified the PCR products using the SureClean Plus® kit, following the manufacturer's instructions. The sequencing of the samples was performed in automated sequencer (ABI 3730 XL DNA Analyzer®, Applied Biosystems) at Department of Technology of the Faculty of Agrarian and Veterinary Sciences at Jaboticabal, Paulista State University "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. Sequences were 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 & Standley 2013), using the default setting. Identical

sequences of the same locality were excluded from analyses.

Phylogenetic Analysis

Statistical selection of nucleotide substitution models was conducted in jModelTest v2.1.4 (Darriba et al. 2012). Bayesian analyses were conducted in MrBayes v3.2.2 program (Ronquist et al. 2012), using parameters (frequency of nucleotides and transition/transversion ratio, gamma distribution and variable sites proportion, when it was present in the model) chosen from among the models available in jModelTest. Analyses were performed with 10^9 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 COI and 28S 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 model that best applied to each gene. Maximum likelihood analyses were conducted in the RAxML program (7.6.3) (Stamatakis 2006), 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. The semi-strict consensus tree between Bayesian inference method and maximum likelihood was obtained using the software Mesquite v3.01 (Maddison 2003).

Haplotype Network

The number of haplotypes was obtained in DnaSP v.5.10.1 (Rozas & Rozas 1999). The haplotype network was obtained using the method median joining network in the software PopArt (Population Analysis with Reticulate Trees) (Leigh & Bryant 2015). A GeoTags block was used to establish the geographical coordinates were each specimen was collected and to color the network according to them. Only COI sequences were used in this analysis.

RESULTS

We analyzed the greatest possible diversity of specimens throughout their distribution, as detailed below. In the list of analyzed material there are the specimens that served as genetic vouchers, of which sequences were generated by us, deposited in GenBank and used in the phylogenetic analyzes. Cols. = collectors; ♂ = male; ♀ = female; ♀♀ = ovigerous female.

Potimirim brasiliana

Analyzed material. Uruçuca, Bahia, Brazil, 08/xi/2011, Cols. Carvalho, F.L. et al., 8 ♀, 1 ♀♀, COI – GenBank Access KP202789-93, 28S – KP202727-31, KP202753-56 (CCDB 1585); Ilhéus, Bahia, Brazil, 31/iii/2009, Cols. Mantelatto, F.L. and Almeida, A.O., 7 ♀, COI – KP202794-97, KP202821, 28S – KP202732, KP202757-58 (CCDB 2633); Mangaratiba, Rio de Janeiro, Brazil, 28/ii/2008, Col. Lima, G.V., 6 ♂, 2 ♀, 1 ♀♀, COI – KP202811-14, KP202819-20, 28S – KP202736, KP202743-45, KP202759-61 (CCDB 2142); Paraty, Rio de Janeiro, Brazil, 15/viii/2007, Cols. Mantelatto, F.L. et al., 3 ♂, 3 ♀, COI – KP202786, KP202802-05, 28S – KP202725, KP202737-39, KP202746 (CCDB 2005); Paraty, Rio de Janeiro, Brazil, 12/xii/1996, Cols. unknown, 3 ♂, 1 ♀, 2 ♀♀ (CCDB 2693); Ubatuba, São Paulo, Brazil, 16/viii/2007, Cols. Mantelatto, F.L. et al., 3 ♂, 5 ♀, COI

– KP202781, KP202787, KP202806, KP202815-17, 28S – KP202724, KP202740-41, KP202747, KP202749-51, KP202784 (CCDB 2006); Caraguatatuba, São Paulo, Brazil, 09/xi/2007, Cols. Mantelatto, F.L., 2 ♀ (CCDB 0049); São Sebastião, São Paulo, Brazil, 11/vii/2006, Cols. Mantelatto, F.L. et al., 3 ♂, 7 ♀ (CCDB 3105); Ilhabela, São Paulo, Brazil, 13/vii/2006, Col. Mossolin, E.C., 2 ♂, COI – KP202785, 28S – KP202724 (CCDB 1991); Ilhabela, São Paulo, Brazil, 14/vii/2006, Col. Mossolin, E.C., 5 ♂, 2 ♀ (CCDB 6404); Iguape, São Paulo, Brazil, 13/v/2006, Cols. Mantelatto, F.L. et al., 1 ♂, 1 ♀, COI – KP202808, 28S – KP202752 (CCDB 2386); Cananéia, São Paulo, Brazil, 18/iv/2011, Col. Mantelatto, F.L., 4 ♀, 2 ♀♀ (CCDB 3213); Matinhos, Paraná, Brazil, 20/02/2008, Cols. Mantelatto, F.L. & Mossolin, E.C., 3 ♂, COI – KP202788, KP202807, KP202818 (CCDB 2115); BR376, Santa Catarina, Brazil, 19/ii/2008, Col. Mantelatto, F.L. and Mossolin, E.C., 1 ♂, 3 ♀, COI – KP202798-99, KP202809-10, 28S – KP202733-35, KP202742 (CCDB 2116).

Potimirim potimirim

Analyzed material. Isla Colon, Bocas del Toro, Panama, 13/viii/2008, Cols. Torati, L.S. and Page, T., 3 ♂, 3 ♀, COI – KP202783, KP202827-29, 28S – KP202764, KP202777-80 (CCDB 2399); Iconha, Espírito Santo, Brazil, 19/vi/2012, Cols. Carvalho, F.L. et al., 5 ♂, 3 ♀, 1 3 ♀♀, COI – KP202801, KP202822-24, 28S – KP202766-71 (CCDB 4066); Piúma, Espírito Santo, Brazil, 15/vi/2012, Col. Carvalho, F.L. et al., 2 ♀♀, COI – KP202825-26 (CCDB 4069); Ilhéus, Bahia, Brazil, 31/iii/2009, Cols. Mantelatto, F.L. and Almeida, A.O., 1 ♂, 28S – KP202765 (CCDB 2634); Canavieiras, Bahia, Brazil, 17/viii/2010, Col. Carvalho, F.L., 10 ♂, 3 ♀, 28S – KP202772 (CCDB 3037); Ilhabela, São Paulo, Brazil, 07/vii/2011, Cols. Mantelatto, F.L. et al., 5 ♀, 28S – KP202775 (CCDB 3942); Cananéia, São Paulo, Brazil, 29/viii/2011, Cols. Mantelatto, F.L. et al., 15 ♂, 35 ♀, 28S – KP202773 (CCDB 3720); Cananéia, São Paulo, Brazil, 29/viii/2011, Cols. Mantelatto, F.L. et al., 1 ♀, 28S – KP202763 (CCDB 3721); Cananéia, São Paulo, Brazil, 09/xi/2011, Cols. Carvalho, F.L. et al., 1 ♀, COI – KP202784, 28S – KP202762 (CCDB 3743); Ariri, São Paulo, Brazil, 10/xi/2011, Cols. Carvalho, F.L. et al., 15 ♂, 6 ♀, COI – KP202800, 28S – KP202774 (CCDB 3741); Caieira do Norte, Santa Catarina, Brazil, 18/iv/2007, Cols. Mantelatto, F.L. et al., 3 ♂, 3 ♀, 28S – KP202776 (CCDB 1893).

Potimirim glabra

Analyzed material. Pacific Ocean, Costa Rica, 10/ii/2009, Cols. Mantelatto, F.L. et al., 7 ♂, 1 ♀, COI – KP202782 (CCDB 3341).

We obtained 56 sequences of ~750 base pairs (bp) of *Potimirim* for 28S (37 of *P. brasiliana* and 19 of *P. potimirim*). For COI, we obtained 49 sequences of ~530 bp (36 of *P. brasiliana*, 1 of *P. glabra* and 12 of *P. potimirim*). The number of haplotypes of each species was: 13 of *P. brasiliana*, 1 of *P. glabra*, 5 of *Potimirim* sp 2 and 7 of *P. potimirim*, including the new sequences and the ones retrieved from GenBank.

The analysis conducted in the software jModelTest showed that the substitution models that best fit our data were HKY+I for 28S and HKY+Γ for COI, considering both Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

The consensus tree between the Bayesian inference and maximum likelihood, based on the concatenate matrix of both genes (fig. 1), showed a clear separation between *Potimirim potimirim*, *Potimirim glabra* and the other species. *Potimirim* sp 2 is also a well-supported clade, but among a polytomy of *Potimirim brasiliana*.

Separately, the nuclear gene 28S was inefficient for the study of phylogenetic relationships among *Potimirim*. The trees

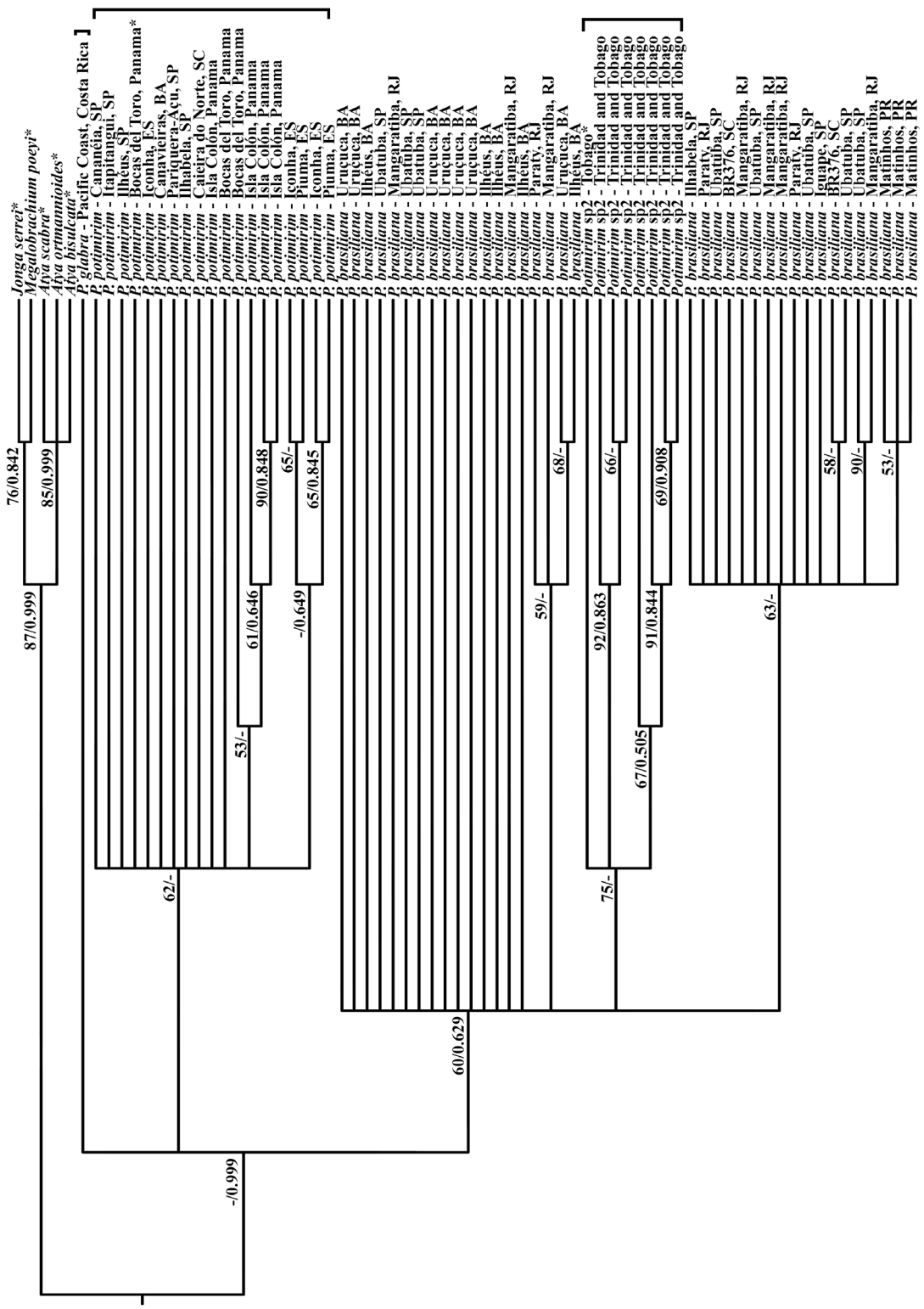

Figure 1. Consensus tree between the maximum likelihood and Bayesian inference based on the concatenate matrix of COI and 28S. Node numbers represent the bootstrap values and a posteriori probability. *Sequence from GenBank. Brackets show *Potimirim* species that were retrieved as monophyletic groups by the analysis. BA – Bahia, ES – Espírito Santo, PR – Paraná, RJ – Rio de Janeiro, SC – Santa Catarina, SP – São Paulo.

obtained based on this molecular marker showed very poor resolution of the clades and only showed the separation of *P. potimirim* from other species. On the other hand, the mitochondrial gene COI showed clear delimitations between the studied species. The consensus tree between the Bayesian inference and maximum likelihood, based only on COI (fig. 2), showed that each one of the studied species form a distinct clade, including *Potimirim brasiliana*.

Although *Potimirim brasiliana* has a wide distribution along the Brazilian drainage, there is no geographical pattern of structuration among the populations, what is shown by the tree and the haplotype network (figs. 2 and 3) based on COI. Nevertheless, the haplotype network showed that there is a geographical pattern of distribution of the haplotypes of *Potimirim potimirim* (i.e. Panama – São Paulo/Parana – Espirito Santo). *P. brasiliana*'s COI intraspecific distances ranged from 0.0 to 8.6%. Interspecific distance between *Potimirim* species ranged from 12.8 to 16.9%.

DISCUSSION

Potimirim brasiliana's COI intraspecific distances (0.0 to 8.6%) were lower than the mean interspecific gap of Atyidae shrimps (13.2 to 13.8%) calculated by Silva et al. (2011), and lower than the interspecific distance between *Potimirim* species (12.8 to 16.9%) calculated in the present study. In addition, we found 13 haplotypes of COI among *P. brasiliana*, in which the topology showed no geographical pattern, which means that the populations present gene flow between them. The explanation about this intriguing genetic connection even within smaller scales is not totally conclusive since it was expected that species with wide geographic distribution in association with the differences in the environmental conditions, presents expressive genetic divergence. In this sense and no exclusive factor, we found some characteristics of larval cycle of atyids to support our hypothesis. Although *P. brasiliana* is an amphidromic shrimp, in which adult individuals live and reproduce in freshwater, the larvae of *Potimirim* species require brackish water to successfully complete their larval development and lifecycle (Villalobos 1959, Molina 1987, Hoffmann & Negreiros-Fransozo 2010). In most its area of occurrence, *P. brasiliana* shows reproductive activity throughout the whole year, with more intensity during the summer, which is coincident with the higher rainfall period in southern Atlantic region and that are favorable to the passive transport of larvae to the estuary and their development in different salinity (see Molina 1987, Hoffmann & Negreiros-Fransozo 2010, Rocha et al. 2013 for review). Therefore, it is likely that the gene flow occurs when these potential colonizers (larvae) are in contact with these habitats in conditions which the dispersion of larvae or juvenile by ocean currents in facilitated. This is considered the main explanation of the widespread distribution and genetic connectivity seen in amphidromous species (Page et al. 2008, Fujita et al. 2016).

As observed in some other members of Atyidae (Molina 1987, Hoffmann & Negreiros-Fransozo 2010, Rocha et al. 2013), *Potimirim brasiliana* presents many favorable and convergent characteristics that fits with this model of dispersion: 1) produces many small elliptic eggs per brood (mean fecundity = 515 eggs/female; mean diameter = 0.57 mm x 0.37 mm; Molina 1987); 2) high reproductive activity throughout the year (Hoffmann & Negreiros-Fransozo 2010, Rocha et al. 2013); 3) a moderate incubation period (extends from 14 to 27 days according to the temperature) (Molina 1987),

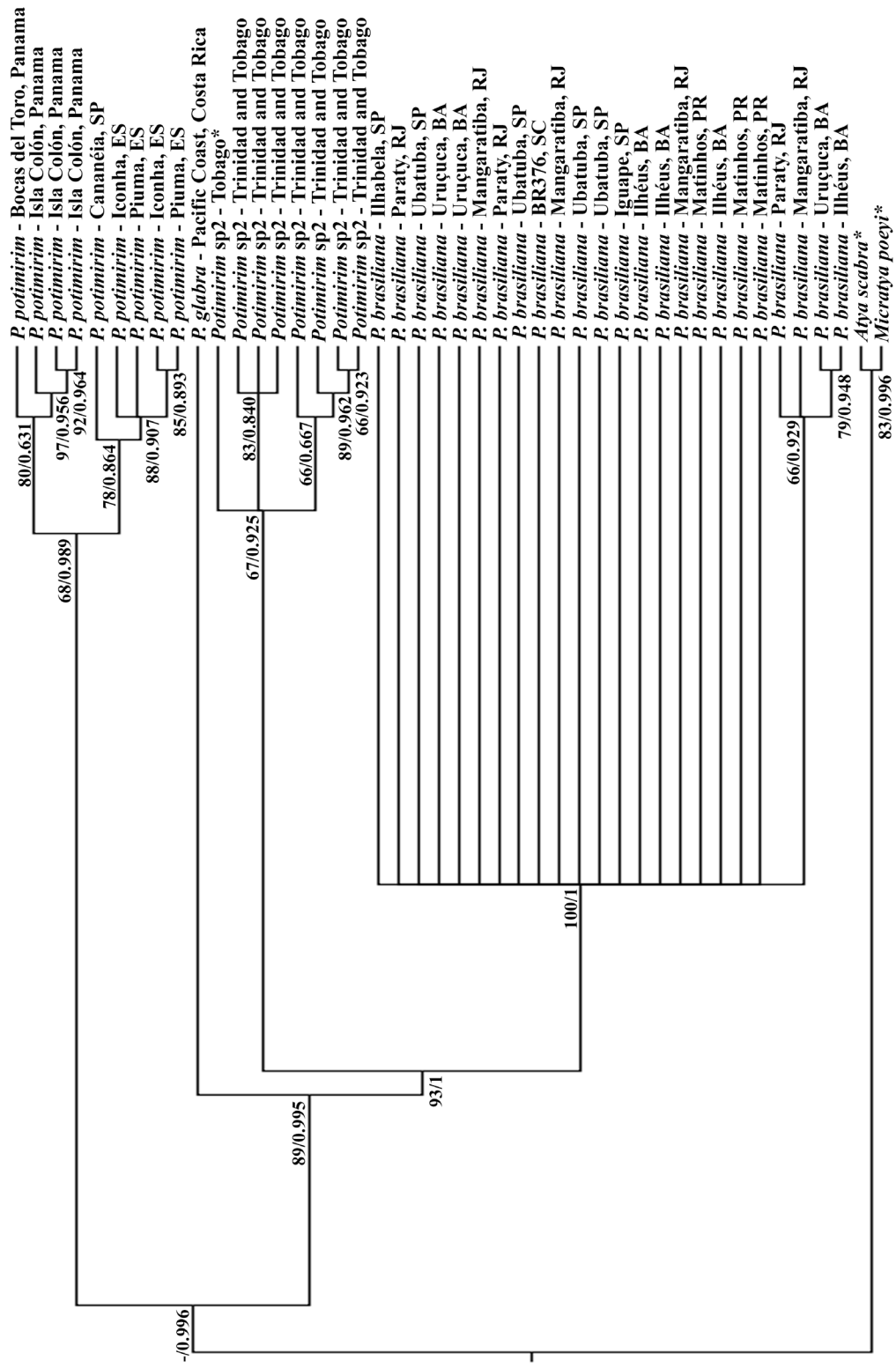

Figure 2. Consensus tree between the maximum likelihood and Bayesian inference based on COI. Node numbers represent the bootstrap values and a posteriori probability. *Sequence from GenBank. BA – Bahia, ES – Espírito Santo, PR – Paraná, RJ – Rio de Janeiro, SC – Santa Catarina, SP – São Paulo.

and 4) extended larval developmental period (11 zoea and 1 decapodid stages reached in about 45 days (14-16 salinities range) under laboratory conditions (Molina 1987). These population and larval cycle profiles are key features to the success of this dispersion in low scales and gene flow.

In contrast, the haplotype network and the tree based only on COI showed geographical pattern of separation for populations of *Potimirim potimirim*, divided into populations from Panama, São Paulo and Espírito Santo. Unfortunately, as far as we know, nothing is known about larval cycle of *P. potimirim* which do not permit us make a conclusive inference regarding the capacity of dispersion and the absence of connection among the studied populations. Considering the phylogenetic relationship between both species (see below more details), studies on larval development and experiments on survival in different salinities of *P. potimirim* could help to explain why there is gene flow between populations of *P. brasiliana*. They would also explain why this does not occur in *P. potimirim* and allow to test the idea that those populations might be experiencing different selective pressures during the evolution in America. In addition, due the phylogenetic separation in two different clades (see Torati & Mantelatto 2012 and discussion below), we can hypothesize that *P. potimirim* much probably present a different pattern of larval development that should be investigated.

The mitochondrial marker COI showed a clear separation of the four studied species of *Potimirim*, corroborated Torati & Mantelatto (2012) and reinforced its utility, as a highly variable mitochondrial molecular marker, to delimit species and even populations (Harrison 2004, Toon et al. 2009). On the other hand, the nuclear gene 28S was not efficient to show the relationships between populations of

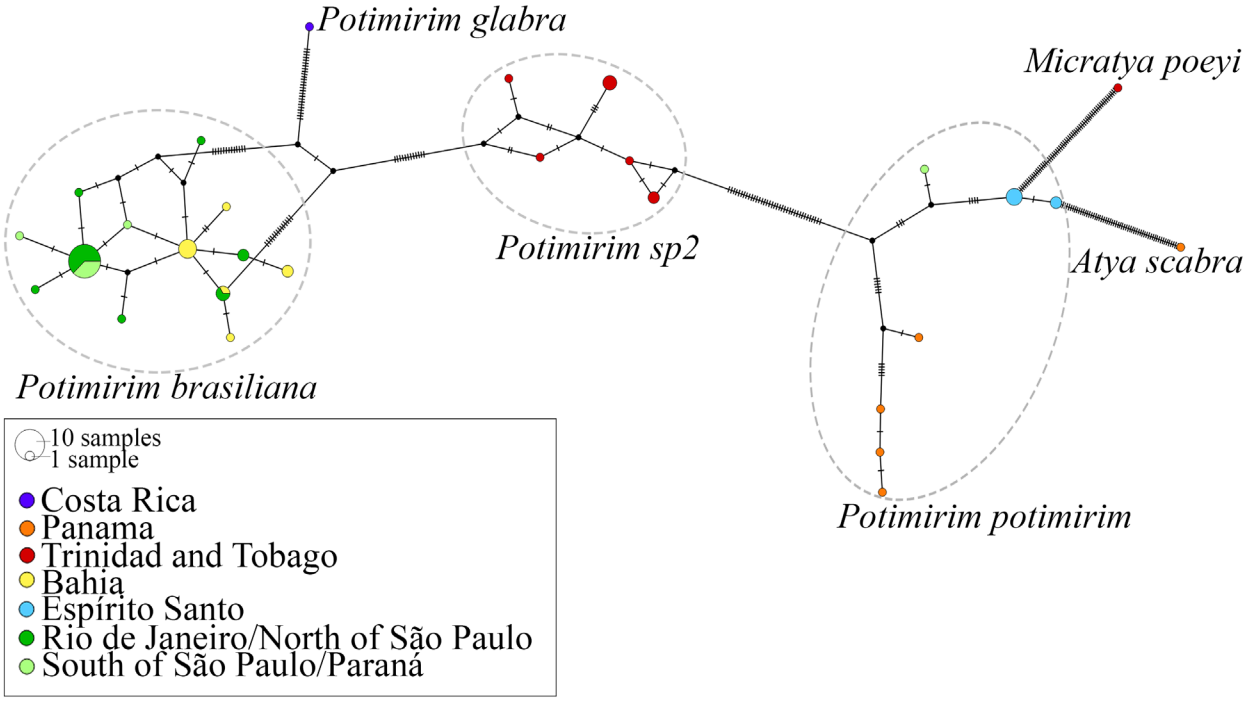

Figure 3. Haplotype network based on COI (530 bp) for populations of Potimirim and two outgroups (*Atya scabra* and *Micratya poeyi*).

P. brasiliana but showed a clear separation between this species and *P. potimirim*. In general, nuclear markers are well conserved and are normally used to delimit genera (Toon et al. 2009).

The evolution of the genus *Potimirim* is strongly related to the closure of the Isthmus of Panama (Torati 2009). This biogeographic barrier was responsible for separating *Potimirim* populations from the Pacific Ocean from those in the Atlantic Ocean, which made possible the speciation of these populations. According to Torati (2009), the cladogenesis of *P. brasiliana* and *Potimirim* sp 2 occurred more recently than the cladogenesis of *P. glabra* and the ancestral population of *P. brasiliana* and *Potimirim* sp 2, and our results corroborate this once the trees shows that *Potimirim* sp 2 is more closely related to *P. brasiliana* than to *P. glabra*.

Although *P. glabra* and *Potimirim* sp 2 are morphologically similar (Torati & Mantelatto 2012), our molecular analyses showed that *Potimirim* sp 2 is more closely related to *P. brasiliana*. These three species share an important morphological character: the shape of the *appendix masculina*, which distances them from *P. potimirim*. Torati & Mantelatto (2012; Fig. 3, page 635) obtained two groups of *Potimirim* supported by both the 16Smt topology and morphology of the *appendix masculina*: one formed by *P. potimirim*, *P. mexicana* and *Potimirim* sp 1, and the other containing *P. brasiliana*, *P. glabra* and *Potimirim* sp 2. This separation, however, was not supported by Grilli et al. (2014), considering the sexual system of the species, once *P. mexicana* is protrandric hermaphrodite and *P. potimirim* is gonochoric, a difference that should be investigated.

Our findings provide a new perspective about the evolution of the genus *Potimirim*, especially regarding the life cycle and gene flow particularities observed in the studied species. This scenario serve as appropriate theoretical basis for the development of management strategies for conservation proposes since *P. brasiliana* is endemic to Brazilian drainages and the genetic connectivity found within the geographical regions is important to maintain the genetic variability and prevent local extinction, as observed in some invertebrates (Bohonak & Jenkins 2003), including mangrove crab (Buranelli et al. 2019). Additionally, our findings highlighted the need of studies on larval development of *Potimirim potimirim* to fully understand its genetic structuration and the taxonomic status.

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