Marine Pollution Bulletin 116 (2017) 440–447

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Can environmental pollution by metals change genetic diversity? *Ucides cordatus* (Linnaeus, 1763) as a study case in Southeastern Brazilian mangroves

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ARTICLE INFO

Article history:
Received 9 July 2016
Received in revised form 13 January 2017
Accepted 19 January 2017
Available online 25 January 2017

Keywords:
Biomonitoring
Conservation
Crab
Estuary
Metals
Pollution

ABSTRACT

Industrial areas on estuarine systems are commonly affected by heavy metals, affecting all local biota. Random Amplified Polymorphic DNA (RAPD) was used to evaluate genetic diversity of *Ucides cordatus* at mangroves in southeastern Brazil (Juréia, J; São Vicente, SV; and Cubatão, C), with distinct pollution levels by metals. The genetic diversity of this species was compared with concentrations of metals (Cd, Pb, Cu, Cr and Hg) in the environment. A pollution gradient was confirmed (SV > C > J), with low levels detected in water, except for mercury in SV. All metals in the sediment samples were below Threshold Effect Level (TEL), without an apparent biological risk to the biota. Genetic distance was very similar between J and C, with SV occurring as an out-group. RAPD was a powerful tool to investigate the effect of metal pollution on genetic diversity of this mangrove crab, and to evaluate the conservation status of the mangrove ecosystem.

1. Introduction

Mangroves are an ecosystem with intense recycling of nutrients, providing natural nursery grounds for many animal species (e.g. fish, crustaceans and mollusks) (Schaeffer-Novelli, 1995; Spalding, 2012; Lee et al., 2014), and an ideal site for feeding and nesting of birds (Vannucci, 2001; Luther and Greenberg, 2009; Huertas et al., 2016). Biodiversity is relatively low in mangroves, due to physiological adaptations required to support the wide variation of salinity and due to its anoxic/unstable/contaminated sediment (Pinheiro et al., 2008a; Thilagavathi et al., 2013; Remaili et al., 2016).

Recently, mangroves has been purposed to monitor climatic changes (Schaeffer-Novelli et al., 2016), mainly using some species of decapod crustacean that inhabitant this environment (Gilman et al., 2008; Pinheiro & Almeida, 2015; Siddig et al., 2016). Among these crustaceans *Ucides cordatus* (Linnaeus, 1763) is an endemic brachyuran that is widely distributed in tropical mangroves of the western Atlantic (Melo, 1996). This species builds its galleries in the sediment and feeds on senescent leaves and propagules present on the substrate (Koch and Nordhaus, 2010; Christofoletti et al., 2013), processing 84% of the mangrove litter (Koch & Wolff, 2002). Besides playing a relevant role in carbon cycle by litter processing (Begon et al., 1996; Hogarth, 1999), this crab species is also an important food and subsistence source to coastal communities (Alves & Nishida, 2003), and has been recently used as bioindicator species for evaluating the conservation status of mangrove areas (Pinheiro et al., 2013). *U. cordatus* has also been successfully used as a tool to diagnose and classify the human impacts on environmental quality of mangrove ecosystem based on a multi-level analysis (Duarte et al., 2016).

In Brazil, the southeastern coast is the most disturbed, mainly the central coast of the São Paulo State. From this, the Metropolitan Region of 'Baixada Santista' (MRBS) including nine municipalities with around 1.7 million inhabitants (Pinheiro et al., 2008b) stands out. The ecosystems in this coastal region are under significant pressure due to industrial and port activities, with a historic use that dates back >500 years (Oliveira et al., 2008). Some estuarine areas (e.g. São Vicente municipality) are characterized by the presence of many stilt houses on the river banks and estuaries (Azevedo et al., 2012), without sanitary condition and correct destination of solid wastes (Cordeiro & Costa, 2010). While in Brazilian mangroves the main threats are the harvesting of mangrove wood, deforestation for aquaculture ponds, and intense

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http://dx.doi.org/10.1016/j.marpolbul.2017.01.039
0025-326X/

property speculation (Macintosh & Ashton, 2002; Ellison, 2008), at RMBS industrial activities, such as a port complex, prevails, posing an imminent risk, with toxic waste, which may affect the biota (De Wolf et al., 2004). Duarte et al. (2016) quantified and qualified the main pollutants in São Paulo coast areas, with a high concentration of metals and organic contaminants in Central area (where São Vicente and Cubatão are placed) when compared to those registered in South area (where there are conservation units as Juréia).

Several studies have described the effects of pollution on mangrove vegetation (Silva et al., 1990; Arrivabene et al., 2015; Souza et al., 2015) and crustaceans (Harris & Santos, 2000; Valdovinos & Zúñiga, 2002; Álvaro et al., 2015), but the use of molecular tools for detecting pollution impacts on the genetic level, particularly in estuarine ecosystem, are scarce. The Random Amplified Polymorphic DNA (RAPD), i.e., is one technique applied in ecotoxicology studies (De Wolf et al., 2004; Giantsis et al., 2012; Liu et al., 2012; Salem et al., 2014; Zhang et al., 2016) for quantification of alleles (bands) and analyses of their addition/loss due to mutation, inversion, deletion or chromosomal rearrangement (De Wolf et al., 2004). This genetic fingerprinting tool is a relatively inexpensive and fast method for evaluating pollutants effects on a broad range of DNA damages, thus, improving environmental risk assessment, mainly in developing countries as Brazil, where this study was carried out.

Our main objective was to evaluate if the presence of heavy metals in environmental matrices (water and sediment) could affect the genetic diversity and structure of the mangrove crab *U. cordatus*. To this aim, we used Random Amplified Polymorphic DNA (RAPD) technique to assess the genetic variation of *U. cordatus* from three mangrove areas from Southeastern Brazil (Juréia, São Vicente and Cubatão), characterized by distinct pollution levels by metals (Cd, Pb, Cu, Cr and Hg). A positive association between genetic parameters and concentration of pollutants will allow the use of this tool in studies about environmental conservation, helping government management plans related to the mangrove ecosystem and the target species.

2. Materials and methods

2.1. Study area

Three mangrove areas, which presumably differ in their degree of environmental impacts, were investigated (Fig. 1) from January to March 2009. Juréia is a legally protected area that composes a great 'Mosaic of Conservation Units' near the mouth of the Una River (24°26′0″ S–47°04′5″ W), with presence of a traditional community comprising a few people (São Paulo, 2006). São Vicente was represented by a mangrove area near the Branco River (23°56′2″ S–46°28′1″ W) in São Vicente Municipality, with an impact of 316,324 inhabitants (IBGE, 2010), 11 sources of industrial pollution (Pinheiro et al., 2013), and also impacted. In turn, Cubatão was comprised by a mangrove close to USIMINAS (an important Brazilian steel Company), and adjacent to the Morrões River (23°52′5″ S–46°22′2″ W), in Cubatão Municipality, with 116,010 inhabitants (IBGE, 2010). This is one of the largest industrial centers in Brazil (23 industrial complexes, 111 factories, and >300

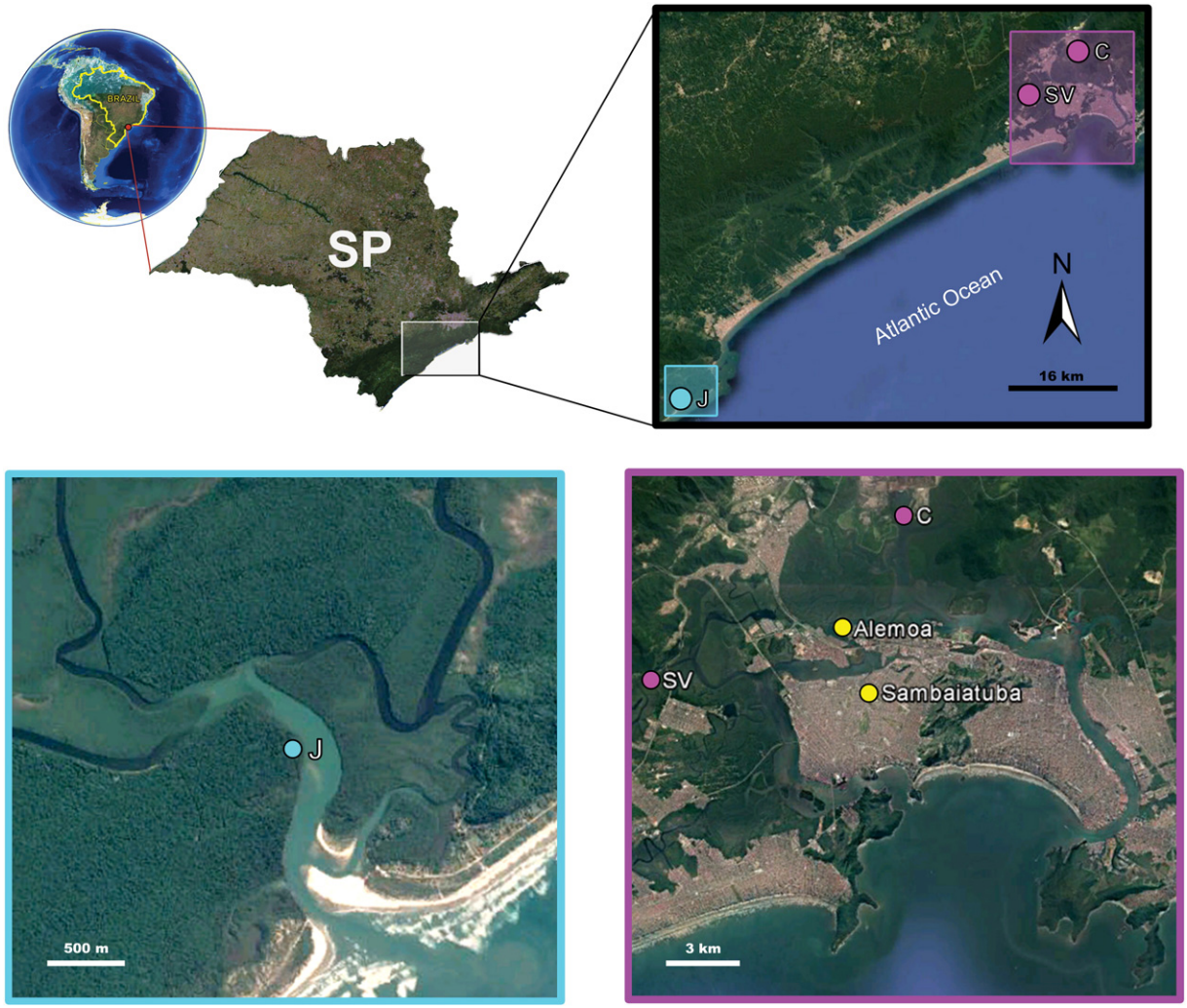

Fig. 1. Map of São Paulo state coast (Brazil), showing the three mangrove areas studied (C, Cubatão; J, Juréia; and SV, São Vicente), and the locations of Sambaiatuba and Alemoa dumps.