

Universidade Estadual Paulista “Júlio de Mesquita Filho”

Instituto de Biociências de Botucatu

Programa de Pós-graduação em Ciências Biológicas (Zoologia)

**Variabilidade em espécies irmãs de caranguejos-violinistas:
uma abordagem genética, comportamental e ecológica**

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Orientadora: Prof.^a Dr.^a Tânia Marcia Costa

Co-orientador: Prof. Dr. Rodrigo Egidio Barreto

Apoio:



Botucatu – SP

-2017-

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*Dedico esta tese aos meus avós
Dico e Dulce, meus grandes
amores, que por toda minha vida
me acompanharam, incentivaram
e, o mais importante, me
ensinaram o significado do amor
incondicional.*

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Introdução geral

Caranguejos-violinistas são pequenos crustáceos pertencentes à família Ocypodidae. Estes são encontrados na zona de entremarés, distribuídos, em sua maioria, em áreas de manguezais e marismas de zonas estuarinas tropicais e subtropicais (Crane 1975, Beinlich & von Hagen 2006, Thurman et al. 2013). Entre outros fatores, a tolerância a um amplo gradiente de salinidade e ao estresse térmico permitiu que estes organismos tivessem uma ampla distribuição (Crane 1975). Atualmente, cento e três espécies são reconhecidas (Rosenberg 2014), sendo dez delas encontradas no Brasil: *Leptuca cumulanta* (Crane 1943), *Leptuca leptodactyla* (Rathbun 1898), *Leptuca thayeri* (Rathbun 1900), *Leptuca uruguayensis* (Nobili 1901), *Minuca burgersi* (Holthuis 1967), *Minuca mordax* (Smith 1870), *Minuca rapax* (Smith 1870), *Minuca victoriana* (von Hagen 1987), *Minuca vocator* (Herbst 1804) e *Uca maracoani* (Latreille 1802-1803) (Thurman et al. 2013).

Por serem ótimos modelos experimentais, este grupo foi alvo de diversos estudos sobre comportamento agonístico (How et al. 2007, Dennenmoser & Christy 2013), seleção sexual (Patricelli et al. 2002, Reading & Backwell 2007), construção de estruturas na entrada da toca (Gusmão-Junior et al. 2012, de O. Rodrigues et al. 2016), competição (Milner et al. 2011, Callander et al. 2011), entre outros. Além disso, esses organismos são considerados engenheiros de ecossistemas (Kristensen 2008), já que durante a construção e manutenção de suas tocas, indivíduos são capazes de transportar sedimento rico em matéria orgânica e outros componentes de dentro da toca para a superfície, modificando a composição da superfície nas áreas próximas a toca, um processo chamado bioturbação (Gribsholt et al. 2003, Cidadin et al. 2016, Natálio et al. 2017).

M. burgersi e *M. mordax* são espécies-irmãs (Thurman et al. 2013, Shih et al. 2016), isto é, espécies muito similares morfologicamente ou quase indistinguíveis, mas que

possuem características biológicas específicas e não se inter cruzam (Ridley 2004). Apesar de serem bastante próximas, as duas espécies apresentam diferentes graus de tolerância em manguezais. *M. burgersi* é frequentemente encontrada em áreas abertas de manguezais próximas a boca do estuário, onde existe uma maior influência da água marinha, enquanto *M. mordax* é frequentemente encontrada em áreas com maior cobertura vegetal próximo à montante, onde a água doce possui maior influência (Crane 1975, Melo 1996, Thurman et al. 2013). Porém, as duas espécies coexistem em áreas com cobertura vegetal e salinidade entre 0,6 e 23,6 (Thurman et al. 2013).

Resultados de análises genéticas obtidos em um projeto Capes/DAAD de cooperação internacional (PROBRAL #D/08/11624 – Programa de Cooperação entre Brasil e Alemanha) mostraram que populações de *M. mordax* da Costa Rica, Pará e São Paulo são geneticamente muito diferentes, enquanto as populações de *M. mordax* e *M. burgersi* encontradas no litoral de São Paulo são bastante semelhantes, tanto em aspectos genéticos quanto nos morfológicos. A partir desses resultados, os autores sugeriram um possível erro de identificação entre essas espécies, sendo as espécies de *M. mordax* do litoral de São Paulo prováveis morfotipos de *M. burgersi*. Com isso, o presente estudo, que integra um projeto sequência (PROBRAL #391/13), teve por objetivo avaliar os aspectos filogenéticos, comportamentais e ecológicos de *Minuca burgersi* e *Minuca mordax*.

A tese foi dividida em três capítulos, cada um contendo um artigo. Os temas de cada capítulo foram escolhidos para testar a hipótese de erro de identificação entre as duas espécies. O primeiro capítulo teve por objetivo apresentar a genética de populações das espécies irmãs. Amostras de DNA das duas espécies foram obtidas em diferentes populações, sequenciadas e, então, utilizadas para avaliar a diversidade haplotípicas e divergência nucleotídica dentro e entre as populações e entre espécies. O comportamento

reprodutivo dos machos, que em muitas espécies é utilizado para identificação interespecífica (How et al. 2007, Perez et al. 2012), foi alvo do estudo no capítulo 2. O aceno, realizado pelos machos com o quelípodo hipertrofiado, foi filmado em campo e então analisado para posterior comparação entre espécies e populações. O terceiro e último capítulo abordou aspectos ecológicos das duas espécies. Para isso, devido ao cenário mundial de aquecimento global, foram realizados experimentos controlados para comparar a tolerância térmica das duas espécies, assim como observações em campo do comportamento de acordo com a temperatura.

Capítulo 1

**Genetic population of two sibling species of fiddler crabs
(Decapoda: Ocypodidae): Brazilian homogeneity and Western Atlantic
heterogeneity**

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ABSTRACT

Fiddler crabs are a widespread group of marine invertebrates. Their large distribution is due to the great capacity of their larvae to disperse among estuary and shelf waters. Therefore, it is expected that all populations along their distribution show molecular similarities due to the extensive gene flow. Thus, we assess the intra and interspecific genetic variation of two sibling species of fiddler crabs, *Minuca burgersi* and *Minuca mordax*, based on COI mtDNA sequences. The molecular data resulted in a high mixed haplotype network for *M. burgersi*, while *M. mordax* sequences were clustered in two groups, one with Brazilian and other with Costa Rica haplotypes. The highest variance in *M. burgersi* was found within population, while in *M. mordax* was found between groups and populations. The analyses of nucleotide divergence showed small genetic variance within populations of both species and between Brazilian populations, and higher variance between Brazilian and Costa Rica populations of *M. mordax*. The highest genetic divergence was found between the two species. Thereby, our results show an intense gene flow along the Brazilian coast, while there is a clear divergence among Brazil and Costa Rica, which may be a result of a physical barrier, as Amazon and Orinoco river flow, and Isthmus of Panama, or the great distance among these populations (stepping-stone model). Lastly, the nucleotide divergence between the species confirms their taxonomic status of distinct, but close related species.

KEY-WORDS: *Minuca burgersi*, *Minuca mordax*, nucleotide divergence, phylogeny.

INTRODUCTION

Marine organisms are typically large populations with large geographical ranges due to the ability of free-swimming adults and/or planktonic larvae to disperse for hundred or thousand kilometers (Scheltema, 1971; Palumbi, 1992; Silva et al., 2010). Estuarine species that have larval phase show two mainly dispersal strategies: to retain the larvae within the estuary, allowing the larvae to stay in an appropriate environment until they turn into adults; and to export them to shelf waters, so they will develop there and then return by a late larval stage or early post-larval stage (Bilton, 2002). The migration to shelf waters is advantageous because the marine environment offers more food supply and stable physico-chemical properties. Besides, the transport to other appropriated areas, ensures an intense gene flow among populations, contributing to species continuity and slowing the species formation (Palumbi, 1992, 1994; Bilton et al., 2002).

Fiddler crabs are a widespread group of small and intertidal brachyuran crabs. They are distributed in estuarine zones of tropical and subtropical areas, mostly in mangrove areas and salt marshes. The occupation of these habitats is partly due to the great tolerance to changes in salinity, from freshwater to sea water conditions (Crane, 1975; Beinlich & von Hagen, 2006; Thurman et al., 2013). The first theory about the dispersion and the diversity of this group was proposed by Crane (1975): primitive narrow-front species arose in Indo-West Pacific, split in other groups, (narrow and broad-front species), which one of the narrow-front lineage dispersed to the New World, originating the narrow and broad-front of America and West Africa; afterwards, these new lineages migrated back to the origin center. Later, Salmon and Zucker (1988) proposed that all lineages arose around Tethys seaway and later they split into Indo-West Pacific group and American group. Independently of where the group started its dispersion, the successful occupation range of

the group is probably due to the evolution of extensive larval phase that allowed them to colonize new areas (Scheltema, 1971; Rieger, 1998; Sanford et al., 2006).

Minuca burgersi (Holthuis, 1967) and *Minuca mordax* (Smith, 1870) are broad-front sibling species of the derived American clade *Minuca* (Rosenberg, 2001). *M. burgersi* is distributed in Western Atlantic, in Caribbean and West Indies, USA (southern Florida), from Mexico to Venezuela and in Brazil (Maranhão State to Santa Catarina State). *M. mordax* distribution ranges from Belize to Brazil (Pará State to Rio Grande do Sul State) (Thurman et al., 2013). *M. burgersi* is usually found in mangrove areas close to the estuary mouth, where the water is brackish due to the great marine influence, while *M. mordax* is found in upstream areas, where there is a great influence of freshwater. As other species of fiddler crab, they can coexist in intermediate areas, where salinity ranges from 0.6 to 23.6 (Crane, 1975; Melo, 1996; Thurman et al., 2013). The dispersion of these larvae depends on the habitat where the adult lives. In species that live far from the hatching areas, as *M. mordax*, the zoea's swimming rhythms match the periods of tidal constituents, while zoea of species that live close to the hatching areas, as *M. burgersi*, showed weak rhythms or arrhythmic patterns (López-Duarte et al., 2011).

As marine invertebrates present an extensive larval phase, high dispersal potential, and large populations' sizes, it is expected low or inexistent genetic divergence between populations (Palumbi, 1992, 1994). Molecular analyses already showed genetic connectivity among distant populations of fiddler crabs: mainland populations of *Minuca rapax* (Smith, 1870) are connected along West Atlantic (Laurenzano et al., 2013) and populations of *Austruca annulipes* (Milne Edwards, 1837) showed genetic homogeneity along East Africa (Silva et al., 2010). For *M. burgersi* and *M. mordax*, as they are close related species, it is expected genetic differences between them, but lower than when

compared to more distant species. Moreover, the presence of a continuous distribution in Western America may favor the genetic homogeneity along the coast. Therefore, the aim of the present study is to evaluate the intraspecific and interspecific genetic distance of populations of two sibling species of fiddler crab, *M. burgersi* and *M. mordax*.

METHODS

Ethics Statement

Our study complies with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation – Brazil (CONCEA). Permission to use the mangroves areas, as well as to manipulate the crabs in the conservation unit, was given by the System of Authorization and Information on Biodiversity (SISBIO) from the Ministry of Environment (MMA), protocol number 38711-1, and the scientific technical committee (COTEC) of the Secretariat for the Environmental (SMA), process 260108-005.660/2013.

Experimental design

We have used for the molecular analysis legs from females and males of each population, which were collected between 2010 and 2014 (Table 1). The DNA was extracted from the pereiopod muscle tissue using Puregene kit (Qiagen). Then, we amplified a fragment of mitochondrial gene cytochrome oxidase subunit I (COI mtDNA) in a polymerase chain reaction (PCR) (40 cycles; 45 sec 94°C/ 1m 48°C/ 90 sec 72°C denaturing/annealing/ elongation temperatures) using the primers COH1b 5'-TGTATARGCRTCTGGRTARTC-3' and COL6a 3'-

TCWACAAATCATAAAGAYATTGG-5' (Schubart, 2009). The PCR products were sending to Macrogen Europe (Amsterdam, The Netherlands).

Obtained sequences were compared to GenBank assemblage using nucleotide Megablast (NCBI). Then, all the sequences were manually aligned using BioEdit 5.0 (Hall, 1999) and eventual mistakes were manually corrected using Chromas Lite 2.1.1 (Technelysium Pty Ltd). Primer regions and non-readable areas at the beginning and at the end were omitted, resulting in fragments of 900 basepair (bp). After alignment and eventual correction, the absence of stop codons was confirmed using the software Artemis 16.0.0 (Rutherford et al., 2000) in order to reduce the possibility of including pseudogenes (Song *et al.*, 2008). A statistical parsimony network was constructed using TCS 1.21 (Clement et al., 2000) with previous data preparation in DnaSP (Librado & Rozas, 2009). Afterwards, analyses of molecular variance (AMOVA) (Excoffier et al., 1992) were performed in Arlequin 3.1 (Excoffier et al., 2006) to determine the genetic distance among haplotypes within the population, among populations of the same groups (FSC), among populations of all groups (FST) and among groups (FCT). In order to estimate intra- and interspecific divergence rates, genetic distances were also calculated by pairwise comparisons using uncorrected p-distances with the program Mega 5.1 (Tamura et al., 2011).

Table 1. Fiddler crabs used in phylogenetic reconstruction (COI mtDNA) and their respective collection sites.

Species	Country	City	Collection sites	Coordinates	N
<i>M. burgersi</i>	Brazil (BRA)	Itacaré (BA)	Contas River	14°16.494'S 38°59.869'W	10
		Bertioga (SP)	Itapanhaú River	23°49.466'S 46°09.466'W	6
		São Vicente (SP)	Mar Pequeno Channel	23°97.511'S 46°39.589'W	9
		Iguape (SP)	Verde River	24°55.341'S 47°22.808'W	9
	Costa Rica (CRC)	Puerto Limon	Tortuguero Channel	10°12.398'N 83°16.348'W	9
<i>M. mordax</i>	Brazil (BRA)	Belém (PA)	Chamá River	1°28'22.9"S 48°26'50.2"W	10
		Bertioga (SP)	Itapanhaú River	23°49.466'S 46°09.466'W	9
		São Vicente (SP)	Mar Pequeno Channel	23°97.511'S 46°39.589'W	8
		Iguape (SP)	Verde River	24°55.341'S 47°22.808'W	8
		Guaratuba (PR)	Guaratuba Bay	25°53'39.7"S 48°37'26.1"W	1

RESULTS

For Brazilian populations of *Minuca burgersi*, the genetics analysis resulted in a highly mixed network (Fig. 1). The 23 haplotypes possibly spanned from an ancestral haplotype h11. Twenty of them (87%) represent individual haplotypes. Considering only the haplotypes shared by more than one individual, the h11 had the highest frequency, and it was shared by six individuals of São Paulo State (four from São Vicente and one from Bertioga and Iguape), followed by h4, with five individuals from all populations (two from Iguape and one from São Vicente, Bertioga and Itacaré), and h2, with three individuals from São Paulo populations (two from São Vicente and one from Bertioga). The maximum distance among the ancestral haplotype was 13 mutational steps away from h3.

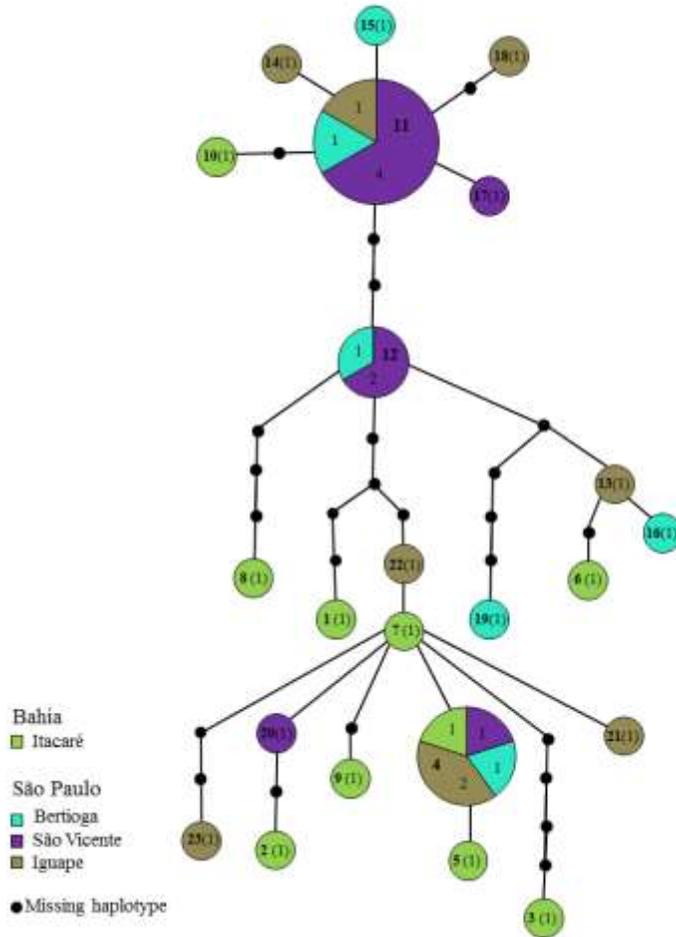


Figure 1. Parsimony haplotype network of Brazilian populations of *Minuca burgersi*. Numbers in bold represent the haplotype identity,

Minuca mordax graphic representation also showed a mixed network, but the 27 haplotypes found for Brazilian and Costa Rica populations were clustered in two regional groups (Fig. 2). The first group, which contains the possible ancestral haplotype h14, was constituted by all the Brazilian populations, and was 28 mutational steps away from the second, constituted by the Costa Rica population. From 27 haplotypes, 22 (81%) of them represented individual haplotypes, being 13 in Brazilian group while 9 in Costa Rica group. All the 5 shared haplotypes occurred in the Brazilian group, and they were as following: the possible ancestral h14, which had the highest frequency and was shared by 11 individuals

(five from Bertioga, two from São Vicente and Iguape and one from Belém and Guaratuba), h19 with five individuals (four from São Vicente and one from Iguape), h10 with three individuals (two from Belém and one from Iguape), h20 with two individuals (one from São Vicente and Iguape) and h26 with two individuals of Bertioga. The maximum distance among the ancestral haplotype was 14 mutational steps away from h15, considering only the Brazilian group and 39 mutational steps away from h2, considering the two groups.

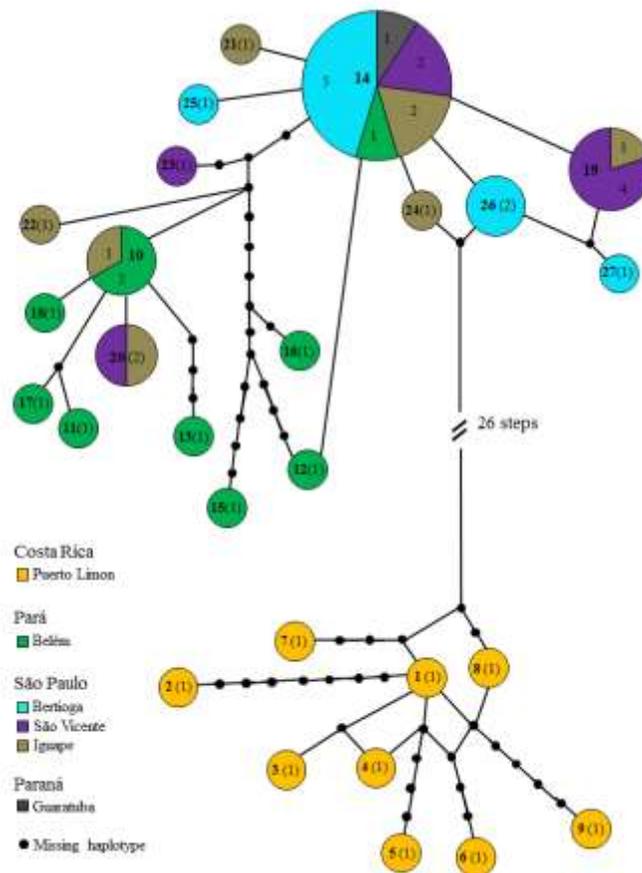


Figure 2. Parsimony haplotype network of Brazilian populations of *Minuca mordax*. Numbers in bold represent the haplotype identity,

The analysis of molecular variance (AMOVA) showed that the highest variation in *M. burgersi* occurred within the populations (93.84%), while the variance among populations was low ($F_{ST} = 0.0616$; $P = 0.11$). For *M. mordax*, the highest variation was among groups ($F_{CT} = 0.8552$; $P < 0.01$) and among all populations ($F_{ST} = 0.8733$; $P < 0.01$), but variation is small among populations within the Brazilian group ($F_{SC} = 0.1248$; $P < 0.01$) and within populations (12.67%). The Paraná population was not included on AMOVA analysis due to its reduced sample size. The variation within the population of both species can also be observed in the haplotype and nucleotide diversity (Tab. 2).

Genetic divergences between specimens of *M. burgersi* and *M. mordax* ranged from 6.1 to 7.7%. Variation within *M. burgersi* ranged from 0 to 1.4%, while within *M. mordax* ranged from 0 to 4.2%, given that the variation in *M. mordax* up to 1.4% occurred between Brazilian and Costa Rica populations (3.2 to 4.2%) (Tab. 3).

Table 2. Estimation of haplotype and nucleotide diversity of *Minuca burgersi* and *Minuca mordax* populations.

		Number of sequences	Number of haplotypes	Haplotype diversity	Nucleotide diversity
<i>M. burgersi</i>	Itacaré	10	10	1.0000	0.007462
	Bertioga	6	6	1.0000	0.005965
	São Vicente	9	5	0.8056	0.003684
	Iguape	9	8	0.9722	0.006082
	Puerto Limon	9	9	1.0000	0.007139
<i>M. mordax</i>	Belém	10	9	0.9778	0.006444
	Bertioga	9	4	0.6944	0.001167
	São Vicente	8	4	0.7500	0.002679
	Iguape	8	7	0.9643	0.003286
	Guaratuba	1	1	0	0

Table 3. Genetic divergence matrix (p-distances) of *Minuca burgersi* and *Minuca mordax* COI mtDNA gene.

	1	2	3	4	5	6	7	8	9	10
1- <i>M. burgersi</i> - Itacaré										
2- <i>M. burgersi</i> - Bertioga	0.007									
3- <i>M. burgersi</i> - São Vicente	0.007	0.005								
4- <i>M. burgersi</i> - Iguape	0.007	0.006	0.005							
5- <i>M. mordax</i> - Puerto Limon	0.067	0.067	0.067	0.067						
6- <i>M. mordax</i> - Belém	0.071	0.070	0.071	0.071	0.037					
7- <i>M. mordax</i> - Bertioga	0.068	0.067	0.068	0.068	0.035	0.006				
8- <i>M. mordax</i> - São Vicente	0.068	0.068	0.068	0.068	0.036	0.006	0.002			
9- <i>M. mordax</i> - Iguape	0.070	0.069	0.070	0.070	0.036	0.005	0.003	0.003		
10- <i>M. mordax</i> - Guaratuba	0.069	0.068	0.068	0.069	0.035	0.005	0.001	0.002	0.002	

DISCUSSION

We evaluated the intra and interspecific genetic distance of two sibling species of fiddler crabs, *Minuca burgersi* and *Minuca mordax*. Molecular data based on COI mtDNA showed that all populations of *M. burgersi* were clustered in only one group, in a high mixed network. The highest genetic variation was within populations. *M. mordax* populations were clustered in two groups, one shared by Brazilian populations and other by Costa Rica population. The highest variance was between groups and populations. Besides that, the analyses of nucleotide divergences showed a small intraspecific variation and a higher interspecific divergence, confirming the species status and the close relationship of the two species.

Marine organisms developed a long duration of larval dispersion that makes possible the larval exchange among different populations (Scheltema, 1971; Silva et al., 2010). Previously, Silva (2010) showed clearly homogeneity among *Austruca annulipes* (Milne Edwards, 1837) populations connected by the shelf, but separated by over three thousand

kilometers. *M. burgersi* showed high level of variation within populations, and low level of variation among populations. Contrarily, *M. mordax* showed small variation within populations, but a high variation between groups and populations. These results suggest a no restrict gene flow among Brazilian estuarine zones, probably due to the larval exchange among sites. This results corroborate with the hypothesis that marine organisms which go through an extensive larval phase show reduced heterogeneity among populations (Scheltema, 1971; Han et al., 2008). *M. burgersi* presents six zoeae stages and one megalopa stage that, together, can spend a hundred forth six days in plankton (Rieger, 1998).

The genetic variance between Costa Rica and Brazil is possible explained by the stepping-stone model (Kimura & Weiss, 1964) and/or the presence of natural barrier. As *M. mordax* has a large distribution, populations among São Paulo State and Puerto Limon (Costa Rica) may present less noticeable differentiation among each other, but more noticeable when we compare populations in the extreme of the distribution. Furthermore, three great barriers in western America are known to produce population genetic structure in sea urchin of genus *Tripneustes*: the Isthmus of Panama, in Central America, the Orinoco River, in Venezuela, and the Amazon River, in Northern Brazil (Lessios et al., 2003). As Laurenzano (2013) showed in *M. rapax* no restrict levels of gene flow between Brazilian (South) and Suriname populations, it is more likely that the stepping-stone model is the main factor that influence the genetic drift among fiddler crab populations.

The genetic divergence between our sibling species was around 7%, while the highest variance within species was 4.2% in *M. mordax*, confirming the distance between Costa Rica and Brazilian population. Even though a high divergence was found between populations of *M. mordax*, we still consider them the same species, once this value is far

below from the interspecific divergence. Compared to other fiddler crabs, our values of intraspecific variation are similar, as *Minuca minax* (LeConte, 1855) populations presented a maximum of 3.9% of nucleotide divergence (Felder & Staton, 1994), and 0.98% within species of *Austruca lactea* complex (Shih et al., 2009). However, the values of nucleotide divergence between species was lower than in species of *A. lactea* complex, as the maximum value here was 7.7%, while the maximum value in *A. lactea* complex was 11.91% (Shih et al., 2009), almost two times higher. Other brachyuran species showed within species variation among 0 and 4.9% (Fuseya & Watanabe, 1996; Schubart & Koller, 2005) and interspecific divergence up to 10% (Fuseya & Watanabe, 1996).

Our findings showed a clearly genetic homogeneity among Brazilian populations of *M. burgersi* and *M. mordax*, while the widespread *M. mordax* populations (Brazilian and Costa Rica) showed considerable genetic differences. The intraspecific Brazilian homogeneity may occur due to the extended larval phase, as showed in other species. However, the Costa Rica and Brazilian heterogeneity may be consequence of the Amazon and Orinoco river flows, which are natural barriers to aquatic organisms, or because the great distance among sites (stepping-stone model). Further, the low nucleotide divergence within species showed a small variability among specimens, and a high interspecific value between *M. burgersi* and *M. mordax*.

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Capítulo 2

Waving display similarities in sibling species of fiddler crabs

(*Minuca burgersi* and *Minuca mordax*)

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HIGHLIGHTS

- *Minuca mordax* and *Minuca burgersi* presented a similar waving display
- Waving components did not differ between populations
- Claw length did not influence waving duration
- Waving duration is not used in interspecific recognition

Male fiddler crabs are known to produce a claw-waving display, which consist in vertical and/or lateral enlarged claw movement. It is used in agonistic interactions and in courtship. Here, we investigate whether temporal traits of waving display in two sibling species, *Minuca mordax* and *M. burgersi*, change between species and populations when sympatric species were present or not. We measure the time spent by an individual in the complete waving, as in each component of the waving (upward and downward movement) and in interwave interval. We also investigated if the claw length affects the display speed. Both species showed the same lateral waving behavior, and the duration of the complete waving and its components were also similar. Considering populations of the same species, only *M. mordax* populations of Belém, which do not share its habitat and Iguape, which lives in sympatry, showed complete waving duration differences. Last, the claw length was similar in both species and did not influence the waving duration. These results suggest that the waving rate is not a trait used in interspecific recognition, since even sympatric populations produced a similar waving. As there are no differences between the species in the same habitat, differences between Belém and Iguape may not be a result of sympatric population's influences, but maybe a result of environmental differences.

In nature, where there are all several environmental stimulus, the communication system evolved due to the decisions that an individual had to make based on other animals signals (Alcock, 2009; Endler, 1993; Green & Marler, 1979). The signals are changes in behavior, morphology and/or physiology of an animal, the emitter, which may produce information to another animal, the receiver (Alcock, 2009; Endler, 1993). The benefits of a signal for either emitter and receiver depends on receiver decision about what to do whether there was no signal (Bradbury & Vehrencamp, 2000). Therefore, signals and receiving mechanisms coevolved to increase the efficiency and reliability of communication and information reception, respectively, benefiting both emitter and receiver (Endler, 1993).

The display behavior is one type of animal communication in which an individual provides specific information to conspecifics and/or individuals of other species (Alcock, 2009; Ridley, 1995). It usually provides an honest information (honest signals) about the quality of the signaler or about the environment (Fitch, 2008). The information can be sent through the following components: auditory, as monkey and suricate alarm vocalization (Cheney & Seyfarth, 1996; Manser et al., 2002); olfactory, as illness odor signals in rats (Arakawa et al., 2010; Maex et al., 2016); and, the most common, visual, as birds plumage and crabs enlarged claw (Järvi et al., 1987; von Hagen, 1982). Yet, all these displays play a role in survivorship and/or sexual selection, since their function is linked to resources holding and defense (e.g., territory and food) (Morales et al., 2014; Peek, 1972), anti-predatory behavior (Brodie III, 1993; Olofsson et al., 2013; Sánchez-García et al., 2016), parent-offspring communication (Cocroft, 1999; Lucass et al., 2016), courtship and mating (Cornuau et al., 2012; Perez et al., 2012; Prum, 1990).

Fiddler crabs are known to produce a visual display called waving, which consist in a vertical and/or horizontal movement of the major claw, followed by the minor claw in some

species (Crane, 1975; Perez et al., 2012; von Hagen, 1982). It is a flexible behavior, so it can change according to environmental conditions and community traits (How et al., 2008; Koga et al., 2001; Murai & Backwell, 2006; Pope & Haney, 2008; Sakagami et al., 2015). Moreover, the waving can be used in interspecific recognition, as many species live in mixed areas (How et al., 2007; Perez et al., 2012), and, as displays are honest signaling of males' health and condition (Andersson, 1994), to access male's quality, given that females access during the reproductive season and males in resource competition (Christy et al., 2002; How et al., 2007; Muramatsu, 2011).

Previous studies used both phylogenetical analyses and courtship waving to analyze the relationship between the group evolution and its behavior. Some of them showed that closely related species, as sibling species, produce more similar displays than more distantly related species (Crane, 1975; How et al., 2009; Perez et al., 2012). Nevertheless, species of fiddler crabs that perform similar waving display and share their habitats tend to perform a display with more species-specific components than conspecific populations which do not share their habitats (How et al., 2009). So, according to these previous studies, it is expected that two sibling species of fiddler crabs, *Minuca mordax* (Smith, 1870) and *Minuca burgersi* (Holthuis, 1967), which often share the same habitats (Thurman et al., 2013), perform a similar behavior, since they are close related. Besides that, it is also expected that sympatric populations have more species-specific wave components than their conspecifics populations which do not share their habitats. Thus, the aim of this work is to investigate the waving display traits in sibling species of fiddler crabs, considering the presence/absence of sympatric species in their habitats and whether claw length affects the waving speed.

METHODS

Study crabs

Fiddler crabs live in estuarine zones, mostly in mixed populations. In reproductive season, when males of burrow mate species start displaying to attract females (Crane, 1975), two types of waving are produced, and they can be classified into ‘vertical’, which involve a simple movement up and down of the major claw, and ‘lateral’, which consist in a more complex horizontal and vertical movement (How et al., 2009). *Minuca mordax* and *Minuca burgersi*, sibling species of the derived American clade *Minuca*, are found in Brazil from Pará to Rio Grande do Sul and Maranhão to Santa Catarina, respectively (Rosenberg, 2001; Thurman et al., 2013). *Minuca mordax* lives in mangrove areas close to the upstream, where there is a great supply of fresh water, while *M. burgersi* lives in downstream areas, where the water is brackish due to the great sea water supply, but they can coexist in intermediate areas where salinity ranges from 0.6 to 23.6 (Crane, 1975; Melo, 1996; Thurman et al., 2013).

We chose three different sites in Brazil where at least one population is present: two sites in São Paulo state, Verde river (24°33'03.3"S, 47°13'43.5"W), Iguape, and Itapanhaú river (23°49'45.6"S, 46°09'48.7"W), Bertioga, and one site in Pará state, Chamá river (1°28'22.9"S, 48°26'50.2"W), Belém. The two species share the same habitats in São Paulo (sympatric), while in Pará area only *M. mordax* is found. The density in Belém, Bertioga and Iguape were 52.64 ± 17.82 crabs/m², 42.31 ± 15.56 crabs/m², 21.08 ± 12.67 crabs/m², respectively. We took only mature males to behavioral analyses, which carapace width ranged from 11.8 to 22.7 mm, and 10.9 to 19.3 mm for *M. mordax* and *M. burgersi*, respectively.

Experimental design

Our strategy was to study the waving display of the two sibling species to know if populations of the same species may present waving variations due to the presence of heterospecifics in the same area in order to avoid possible interspecific inbreeding. In reproductive season, during the spring tide, in low tide, when the crabs started the surface activity, we searched for displaying males, and then, we arranged a video camera Handycam DCR – SR68 (Sony) in front of their burrow entrance, pointed onto a mirror angled 50° in the opposite side of the burrow, so we could use the mirror reflection to measure the waving parameters. When males leave the burrow, we let the video camera shooting for 15 minutes to get at least 10 consecutives waves. The time spent recording was longer than we needed because we wanted to disregard the researcher effect in the beginning and in the end of the film. After all, we took the animal to measure the major claw propodus length (cm), here referred as “claw length”. Claw length was used in analyses to investigate if this trait may influences the movement speed, since size-dependence was already found in *Leptuca pugilator* (Aspey, 1802) (Hyatt, 1977).

All records were analyzed frame-by-frame (0.017-s intervals) using Kinovea 0.8.15 software. As the interspecific variation in waving is mostly a response of changes in display velocity (Perez et al., 2012), we decided to use in our analyses only the vertical movement (up and down) of the lateral display. We made four measurements, based on Perez and coworkers (2012): the first one was the duration of the upward movement, which consisted in the claw movement from the resting position until the maximum height position, followed by the second, the downward movement, which was the opposite movement, from the height position to the resting position; the third was the duration of the complete wave, which was the sum of the upward and downward movement; and the last, the interwave

interval, that was the interval between the end of a precursor wave and the beginning of the next wave. Then, considering the possible variations in display of an individual, we calculated the average of ten consecutive wave components, so we decrease the possibility of use an unusual movement in our analyses, making our data more reliable.

Statistical analyses

We measure the duration of each compound (upward, downward, complete wave and interwave interval) as the time spent in each movement. The normality and homoscedasticity were evaluated using the Kolmogorov and Smirnov test and Levene test, respectively. The Levene test showed that the data was heteroscedastic, so a transformation to log 10 was performed. Then, we applied a General Linear Model (GLM) to compare the time spent in each component between populations and species, considering the claw length as a covariate and a one-way ANOVA complemented by Tukey test to compare the complete wave duration of each population and species. Significant differences were considered when $P < 0.05$.

Ethics Statement

Our study complies with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation – Brazil (CONCEA). Permission to use the mangroves areas, as well as to manipulate the crabs in the conservation unit, was given by the System of authorization and information on biodiversity (SISBIO) from the Ministry of Environment (MMA), protocol number 38711-1, and the scientific technical committee (COTEC) of the Secretariat for the Environmental (SMA), process 260108-005.660/2013.

RESULTS

For all populations of both species, the waving was a circular path of the major claw, which moved obliquely up and down. The minor claw also performed the oblique movement at the same time. During the movement, the second or the third pair of walking-legs may have accompanied the wave, moving only up and down (straight movement). The waving duration was significantly different between populations (one-way ANOVA: $F_{4,46} = 2.959$, $P = 0.029$), but only between Belém and Iguape populations of *M. mordax* (Fig.1).

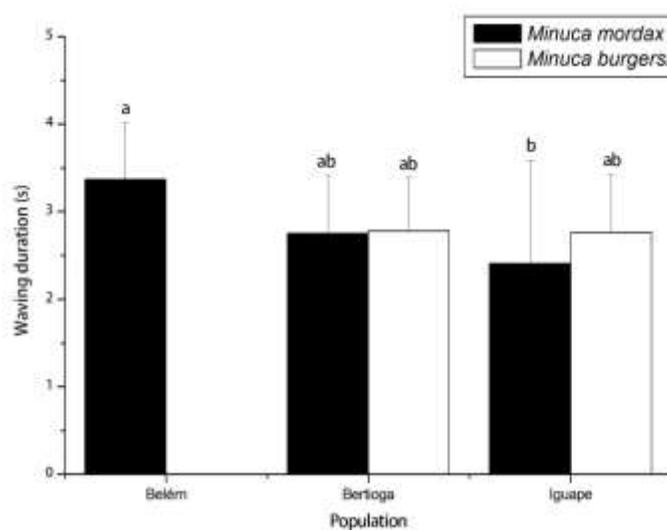


Figure 1. Duration of waving display (mean \pm SD) in *Minuca mordax* and *Minuca burgersi*. The comparison was made between populations of both species. Same letters above bars indicate no statistical differences.

The minimum and maximum duration of the upward movement, downward movement and interwave interval were 0.71 to 2.39s, 0.56 to 2.71s and 0.13 to 12.79s, respectively. Claw length was similar between species (General Linear Model: $F_{4,82} = 1.245$, $P = 0.307$, Fig. 2). The duration of the waving compounds was similar between them (General Linear Model: $F_{2,82} = 0.300$, $P = 0.742$, Fig. 3), between populations of both species (General Linear Model: $F_{8,82} = 1.629$, $P = 0.130$, Fig. 3) and according to claw

length, regardless the species (General Linear Model: $F_{2,82} = 0.501$, $P = 0.608$, Fig. 4). Moreover, the waving behavior compounds were similar between populations regardless the claw length of each population (General Linear Model: $F_{8,82} = 1.538$, $P = 0.157$).

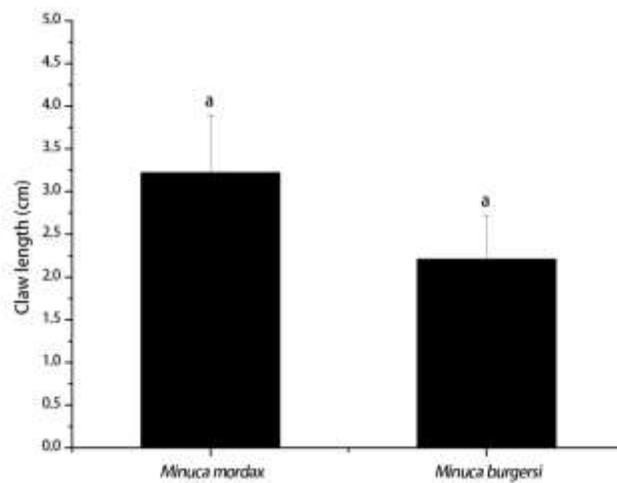


Figure 2. Claw length (mean ± SD) of *Minuca mordax* and *Minuca burgersi*. Same letters above bars show no statistical differences.

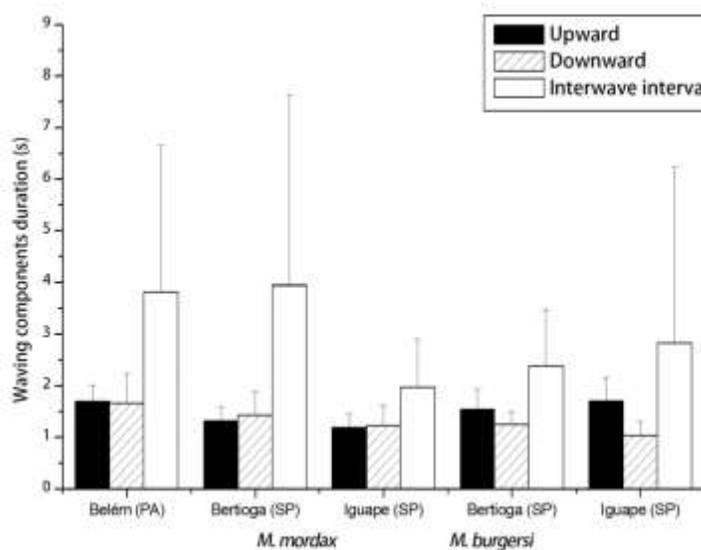


Figure 3. Duration of each waving compound (mean ± SD) in all population of *Minuca mordax* and *Minuca burgersi*.

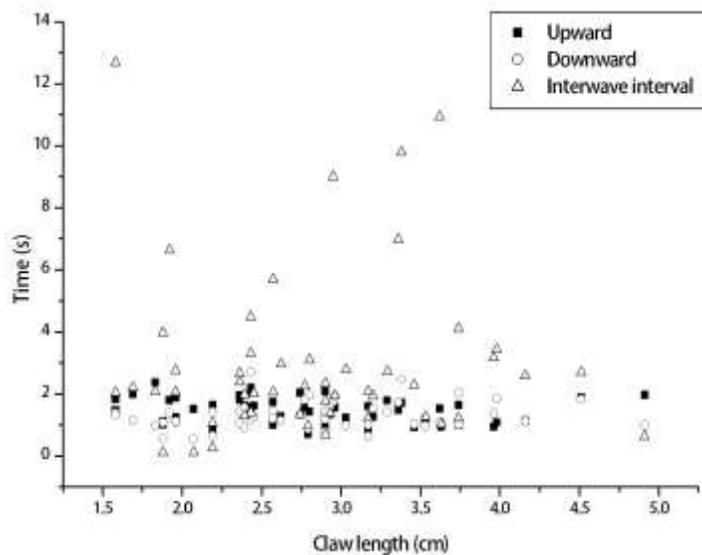


Figure 4. Movement duration against major claw length of *Minuca mordax* and *Minuca burgersi*.

DISCUSSION

In this work, we compared the waving behavior of Brazilian populations of two sibling species of fiddler crab, *Minuca mordax* and *Minuca burgersi*. The waving duration was similar between all populations, regardless the species, except for two populations of *M. mordax*: individuals of Belém waved faster than Iguape individuals. When we analyzed each compound of the waving behavior (upward and downward movement and interwave interval), all populations showed the same pattern of movement, regardless of claw length, which was similar in both species. Although the waving behavior is an important factor for the mate choice in reproductive season, the display similarities between the sympatric species suggest that the waving is not the main factor used by females to recognize their own species males to mate.

Fiddler crabs are known to produce two types of waving movement, a vertical wave, which is fast, simple and produced in high rates, and a lateral wave, which is more elaborated than the first, as it is larger and has an effective range (How et al. 2007, 2009,

Zeil & Hemmi 2006). When courting females, males of both species presented the same pattern of wave, producing the lateral wave. Before starts the display, the major and the minor claw were close to the crab's body. Then, the individual raised obliquely both claws in an opposite direction, and when the claw tip was at an angle of 90° to the carapace, it started the opposite movement (downward) until the claw reaches the body again. von Hagen (1982) showed the same pattern of waving display, but, differently of our work, he also observed a precursory downstroke before the upward movement in 60 per cent of the waves of *M. mordax*, whilst only less than 10 per cent of waves of *M. burgersi* started with it. Besides that, the waving movement found was also according to other broad-fronted species, as, e. g., *Austruca mjoebergi* (Rathbun, 1924) and *Austruca perplexa* (Milne-Edwards, 1837) (How et al. 2009).

In populations of fiddler crabs that live in sympatry with others, females' choice should favor traits that are species specific to avoid interspecific breeding, otherwise the correct breeding would be lost, as well as hybrids and/or inviable offspring would be produced (Callander et al., 2013; Salmon et al., 1978). Despite this, we showed that waving duration was similar, considering only sympatric populations of *M. mordax* and *M. burgersi*. Furthermore, when we compared separately the upward and downward movement and interwave interval between species, both species spent time in each compound equally. Thus, although the waving behavior is used to attract females and/or male-male competition (Izumi et al., 2016; Muramatsu, 2011; Ohata & Wada, 2009) the behavioral similarities between these sympatric populations suggest that the waving is not a trait used for them to interspecific recognition. Instead, this information may be accessed by other morphological and behavioral traits, as claw shape and color (Callander et al., 2011; Detto et al., 2006) and acoustic display (von Hagen, 1984).

Comparing within species, the only difference found was between the waving duration of two *M. mordax* populations, Belém and Iguape, which the first was higher than the other. The presence of sympatric species in Iguape area cannot explain this difference, since, as showed previously, there were no differences between sympatric populations. A possible explanation is that the distance between Belém and Iguape is longer than the others, which may favor the isolation between these two populations. Besides that, some environmental traits may influence the display variability, as the presence of vegetation, which may block the crabs' view, depressing the waving activity (Sakagami et al., 2015), and the presence of predators, which also has a negative influence on waving activity (Daleo et al., 2003). Thus, the geographic isolation, combined to distinct selection pressure, may result in changes in the waving pattern or in receivers preferences (How et al., 2009; Mark Ridley, 2004).

The inter and intraspecific variation of waving behavior can also be influenced by crabs' morphology and/or major claw muscular traits (Callander et al., 2011; Detto et al., 2006; Takeda & Murai, 1993). For example, crabs which perform a lateral waving have the first and the second ambulatory legs on major claw side longer than the other side (Miller, 1973; Takeda & Murai, 1993). In our work, we did not find a relation between the claw length and the waving duration, showing that the waving duration and interwave interval do not depend on this trait. Thus, considering the individuals of a given population, the duration of the waving and the interwave interval may be linked to other factors such as male maturity, since, in some species, males increase the frequency of waving until they reach the fully grown size (Hyatt, 1977), and the female distance, since males increases the signal intensity when females approach (How et al., 2008).

Here, we found a clear behavioral similarity between the sibling species *M. mordax* and *M. burgersi* considering the waving display, even when we considered the claw length in the analyses. This suggests that the waving rate is not the main trait in interspecific recognition. The only difference was found between the wave duration of two populations of *M. mordax*, which may indicate that waving behavior is flexible and can be modified when there are some possible differences in habitats. Future studies should compare habitat traits to investigate if environmental differences can influence the waving behavior.

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Capítulo 3

Desiccation depends on claw length in two sibling species of fiddler crab (*Minuca burgersi* and *Minuca mordax*)

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Running page head: Desiccation in fiddler crabs

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ABSTRACT

Fiddler crabs are exposed to a large variety of physical and chemical processes of intertidal zones. Elevated air temperatures can affect negatively exposed crabs, once water loss will be necessary to decrease body temperature. Some behavioral and morphological traits help to decrease body temperature, but water lost in desiccation process will only be restored by contact with water or substratum. Thus, the goal of this work was to evaluate the intra and interspecific temporal effect of temperature in water loss and water gain in sibling species of fiddler crab (*Minuca burgersi* and *Minuca mordax*), according to males' major claw length (propodus length). Afterwards, we evaluated interspecific differences in male retreating to their burrow from environmental observations. There were no differences in water loss, rehydration and claw length within species in controlled conditions. Interspecific differences were found in water loss, as *M. burgersi* lost more water in each moment and in the end of experiment, and claw length, as *M. mordax* presented larger claws. Both species gained water equally after desiccation. Claw length was negatively correlated to water loss, although no correlation was found with water gain. Lastly, mortality was higher in *M. burgersi*. In nature, males of both species retreated to burrow equally, spending a similar time into the burrow. These results suggest interspecific differences in thermal tolerance, but it may be a result of interspecific differences of claw size.

KEY WORD: mangrove, rehydration, thermoregulation, water loss.

INTRODUCTION

Intertidal zone are characterized by gradients and high variety of physical and chemical process over small spatial scales (Allen et al. 2012). During low tide, the aerial exposure can often leads intertidal organisms to higher physiological stress (Helmuth et al. 2002, Somero 2002). The air temperature, for example, can directly affect body temperature and water loss of intertidal organisms (Helmuth et al. 2002, 2005). Thence, organisms may respond to environmental stress differently over different time scales: some organisms will not survive, as the increasing stress becomes lethal; they can rapidly adjust their physiological function to habitat change; and, in a large scale, environmental changes can favors genetic adaptation via natural selection (Helmuth et al. 2005). All of these responses will affect directly individual performance (Deutsch et al. 2008), as well as affect indirectly intra and interspecific relationships (Mitchell & Angilletta 2009).

Fiddler crabs are a small and intertidal group of widespread brachyurans, distributed mostly in mangrove areas and salt marshes in estuarine zones of tropical and subtropical areas. They were successfully in occupying these habitats partly due to the great tolerance to a salinity gradient and thermal stress (Crane, 1975; Beinlich & von Hagen, 2006; Thurman et al., 2013). In reproductive season, males spend almost all low tide in the surface performing courtship display (Crane 1975). When displaying, crabs decrease their foraging rates that, associated to thermal stress, may lead to an energy intake reduction (Allen & Levinton 2014). Then, fiddler crabs present three mainly process to minimize the thermal effect: (1) increase transpiration, once water loss transfer heat to the air, decreasing body temperature (Edney 1961, Wilkens & Fingerman 1965); (2) high growing rates of major claw, which has a thermoregulatory function, since it movement may expels part of the heat from the body to the surrounding air (Darnell & Munguia 2011); and (3)

behavioral process as herding, once crabs that forage in large herds showed a water-conservation group effect (Yoder et al. 2005), and retreat to the burrow, since temperatures within the burrows are considerably cooler than surface temperatures (Wilkins & Fingerman 1965, Powers & Cole 1976). As compensatory mechanisms to water loss, crabs can only obtain water by direct contact to substratum or water, since crabs cannot take water from atmosphere (Thompson et al. 1989, Yoder et al. 2005). Fiddler crabs has pairs of hydrophilic setal tufts between walking legs that, in contact to soil, can obtain sand capillary water by suction to replace lost hemolymph volume when desiccating (Thompson et al. 1989).

This study focus on the temporal effect of temperature in desiccation and rehydration in fiddler crabs. *Minuca burgersi* (Holthuis, 1967) and *Minuca mordax* (Smith, 1870), sibling species of West Atlantic (Beinlich & von Hagen 2006, Thurman et al. 2013, Shih et al. 2016), are good models to study thermal stress once, although the close relationship and the behavioral similarity (Rodrigues et al 2017a, 2017b), they tolerate different environmental traits. *Minuca burgersi* is mostly found in mangrove open areas close to the estuary mounth, whilst *M. mordax* is mostly found in shaded areas close to upstream (Crane 1975, Melo 1996, Thurman et al. 2013). Therefore, the aim of this study was to evaluate the temporal effect of temperature in water loss and water gain in controlled conditions, considering the influence of claw length in these processes. We also evaluated how many times male retreated to its burrow (compensatory behavior to thermal stress) in field to compare to laboratorial analyses.

METHODS

Study crabs

Fiddler crabs (genus *Minuca*) are small and intertidal brachyurans. They live in mixed-sex populations in estuarine zones, mostly in mangrove areas and salt marsh (Crane 1975). *Minuca burgersi* and *Minuca mordax* are sibling species of the Atlantic-East Pacific clade *Minuca* (Shih et al. 2016). They are distributed along the Brazilian coast, from Pará to Rio Grande do Sul and Maranhão to Santa Catarina, respectively (Rosenberg, 2001; Thurman et al., 2013). They coexist in areas where salinity ranges from 0.6 to 23.6, and the soil has mud substrate with great sand components (Crane 1975, Melo 1996, Thurman et al. 2013). From this information, we chose two sampling sites where their populations are mixed: one site in Verde river (24°33'03.3"S, 47°13'43.5"W), Iguape, and other in Itapanhaú river (23°49'45.6"S, 46°09'48.7"W), Bertioga, both in São Paulo State, Brazil. The density of crabs in both areas was 21.08 ± 12.67 crabs/m² and 42.31 ± 15.56 crabs/m², respectively. were 52.64 ± 17.82 crabs/m², 42.31 ± 15.56 crabs/m², 21.08 ± 12.67 crabs/m², respectively. We used in our work only adult males, which carapace width ranged from 1.14 to 2.1mm, and 0.86 to 1.5 mm for *M. mordax* and *M. burgersi*, respectively.

Desiccation experimental design

Water loss and water gain experiments were performed in a temperature-regulated drying oven, at 30°C. Water loss and water gain were estimated as weight loss/gain under controlled conditions (Thurman 1998). Methodology was adapted from preview study (Levinton et al. 2015). In laboratory, crabs were placed in plastic containers with moistened soil removed from their living area. The species and specimens were shared into containers with few individuals to avoid major claw autotomy. Before the experiment, we took 10

mixed-species individuals of the containers, cleaned using freshwater and put them in a 40x20x20 glass aquaria containing brackish water and let them from 48 to 72 hours, until the experiment beginning. Water layer height varied according to the small individual height, once we wanted to let the crabs' body exposed.

After 48 – 72 hours period, each individual was dried using towels, weighed in a Sartorius analytical balance (accuracy of 0.0001g) and then taken directly into a pre-weighed dry plastic vessel. When all crabs were weighed, the vessels were taken to a drying oven with circulation/air exchange (MA035/1 - Marconi Laboratory Equipment) and kept there for 2 hours at 30°C. Each individual was weight again 15 minutes and 30 minutes after the beginning, and then every 30 minutes until 120 minutes. We always took only 5 vessels per time, so the crabs did not spend too much time outside the oven (less than 30 seconds), and the oven did not increased or decreased the temperature. Right after the last weighing, we put a brackish water layer into each vessel, as well as in acclimation period, and replace vessels into the oven for more two hours. After two hours, we dried and weighed the crabs, in order to estimate the rehydration. The mortality was also obtained in each period, as the claw length (propodus length) and carapace width in the end of the experiment.

Field behavioral observations

As burrow temperature decrease with deep increase (Powers & Cole 1976), crabs would benefit from the balance between stay in a exposed area and to retreat to the burrow to decrease its temperature (Levinton et al. 2015). Thus, we performed field observations to investigate whether there are interspecific differences in how many times each crab retreated to their own burrow. During spring tide, in low tide, crabs start living the burrow

and perform surface activity. All low tide selected start about the same time of the day and have similar patterns of humidity (~60%), atmospheric pressure (1010 to 1014 hPa), and no cloud cover. When apparently all crabs are in the surface, we arranged a video camera Handycam DCR – SR68 (Sony) directed to a group of males. For our analyses, we estimated 50 minutes of video, as Thurman (1998) found weigh changes between 30 to 45 minutes in other fiddler crabs. The video camera filmed 70 minutes, 20 minutes more than would be used to disregard the researcher effect in the beginning and in the end of the film. All records were analyzed frame-by-frame (0.017-s intervals) using Kinovea 0.8.15 software. In a 50 minutes period, we measured how many times each crab entered in their burrow and the time spent there. Whether the controlled experiments show no significant differences between populations, the behavior will be compared only between species.

Statistical analyses

In desiccation experiment, we calculated the percent of water loss of each individual through the time (moment weight - initial weight)/ initial weight). These values were used in the statistical analyses. To examine the effect of the temperature in desiccation across the time in terms of populations, species and claw length, we performed a General Linear Model. After GLM, whether claw length is linked to water loss, a Spearman correlation test will be performed to examine the correlation between water loss and claw length. The same analyses were made to compare the initial, 120 minutes and rehydration weight in terms of population, species and claw size. Goodman's proportion test (Goodman 1964, 1965) was performed in pairwise comparisons to evaluate differences between multinomials (mortality between species). For the field observations, we also performed a Goodman test to investigate the proportion of crabs that entered into the burrow, performing it in pairwise

comparisons to evaluate differences between multinomials (*M. burgesi* vs. *M. mordax*), and within the same multinomials (males that entered into the burrow vs. males that spent all time outside the burrow). Significant differences were considered when $P < 0.05$.

Ethics Statement

This study complies with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation – Brazil (CONCEA). Permission to use the mangroves areas, as well as to manipulate the crabs in the conservation unit, was given by the System of authorization and information on biodiversity (SISBIO) from the Ministry of Environment (MMA), protocol number 38711-1, and the scientific technical committee (COTEC) of the Secretariat for the Environmental (SMA), process 260108-005.660/2013.

RESULTS

According to our data, *Minuca burgersi* and *Minuca mordax* showed no intraspecific differences in water loss (General Linear Model, $p = 0.471$, Fig. 1), rehydration (General Linear Model, $p = 0.576$, Fig. 2), and claw length (General Linear Model, $p = 0.330$). However, water loss was significantly higher in *M. burgersi* (General Linear Model, $p = 0.000$, Fig. 1). There was significant different within temporal data (General Linear Model, $p = 0.000$, Fig. 1), as well as temporal data among species (General Linear Model, $p = 0.000$, Fig. 1). Rehydration data showed no differences between species (General Linear Model, $p > 0.05$, Fig. 2).

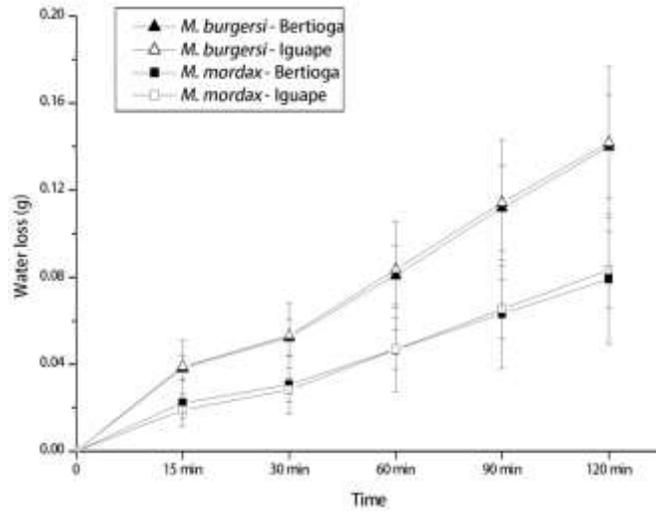


Figure 1. Percent of water loss (\pm SD) across time in populations of *Minuca burgersi* and *Minuca mordax*.

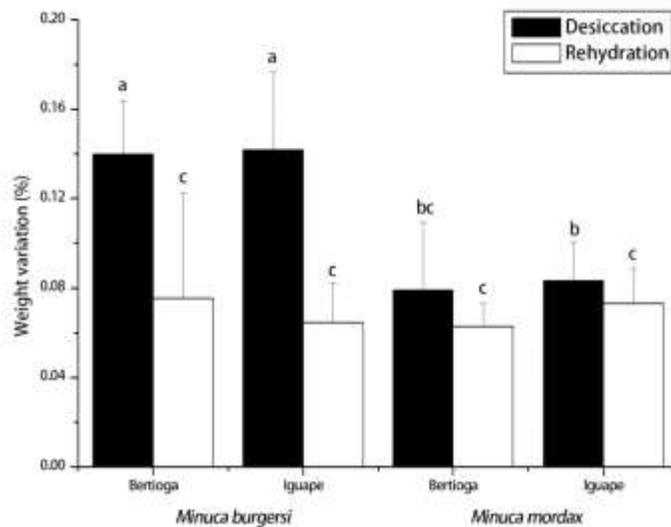


Figure 2. Percent of weight variation (mean \pm SD) between populations and species (*Minuca burgersi* and *Minuca mordax*) in the end of desiccation and rehydration experiments. Same letters above bars indicate no significant differences.

Claw length was greater in *M. mordax* (General Linear Model, $p = 0.000$, Fig. 3). The data presented a strong negative correlation between claw length and desiccation (Spearman correlation test, $r = -0.908$, $p = 0.000$, Fig. 4a), but no correlation between claw

length and rehydration (Spearman correlation test, $r = -0.184$, $p = 0.109$, Fig. 4b). The proportion of dead males was higher (Goodman test, $G_{\text{observed}} = 4.688 > G_{\text{expected}} = 1.96$, $p < 0.05$) in *M. burgersi* (16/38, 42.10%) than *M. mordax* (1/39, 2.56%).

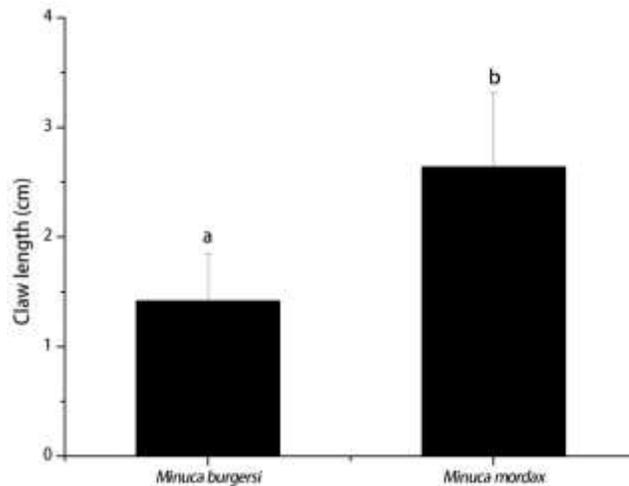


Figure 3: Claw length (\pm SD) of *Minuca burgersi* and *Minuca mordax* specimens.

Same letters above bars indicate no statistical differences.

Field data showed that, in a 50 minutes period, males of *M. burgersi* retreated to burrow more than twice (once in 25 minutes), while males of *M. mordax* retreated almost twice (once in 25 minutes). The proportion of males who entered into their burrow during the observation was similar between species (Goodman test, $G_{\text{observed}} = 0 < G_{\text{expected}} = 1.96$, $p > 0.05$). The proportion of males that entered or not into the burrow within the species was not different (6/9, 67%), assuming a critical level of significance $\alpha = 0.05$ and $A = 3.84$ (2 classes of multinomial), and calculated lower and upper limits of -0.28 and 0.94 , respectively. Males of *M. burgersi* remained inside the burrow 1.17 ± 0.71 seconds every time they entered into the burrow, whilst males of *M. mordax* remained 1.09 ± 0.49 seconds.

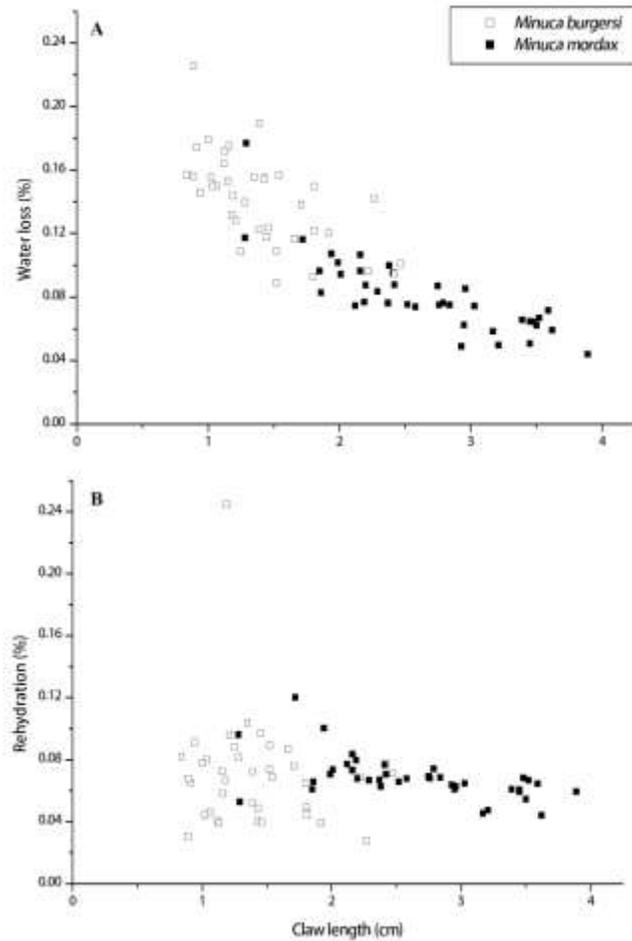


Figure 4. Correlation between claw length and percent of water loss (A) and rehydration (B) of *Minuca burgersi* (empty square) and *Minuca mordax* (black square).

DISCUSSION

Our study examined the desiccation and rehydration traits, besides the field behavior in two sibling species of fiddler crab, *Minuca burgersi* and *Minuca mordax*. We found that under laboratorial conditions, there are no intraspecific differences in water loss, water gain and claw length, but an interspecific significant difference in desiccation. The water loss within temporal sampling was also different, as well as the water loss in each time for each

species. Rehydration was similar in both species. Claw length was different between species. Although claw length was related to water loss, rehydration pattern was not linked to it. Mortality was also higher in *M. burgersi*. Field observations revealed that both species spent the same time inside the burrow. These evidences suggests that although both species respond differently to high temperature, the claw length is the mainly trait that affect water loss.

Physiological and morphological divergences are not directly influenced by geographic distance. However, the geographic distance can generate genetic divergences, and this new haplotypes, then, may produce different physiological and morphological traits (Grant et al. 2000). Here, we found no differences within species in water loss, rehydration and claw size. The intraspecific similarities were already expected, since Brazilian populations of both species share the same haplotypes (Rodrigues et al 2017a, unpublished). Furthermore, besides the genetic similarities, the same populations also showed behavioral similarities, as the individuals of both populations spent a similar time performing vertical components of the lateral waving display (Rodrigues et al 2017b, unpublished).

Water loss is a physiological process that occurs naturally in organisms. Independently of the acting environmental process, crabs may lose water in many ways, as by evaporation from the gills and its chamber during respiration, discharging water from gill chamber, urination, fecal content and by integumentary losses (Herreid II 1969). Species that live in higher and drier sites evolved the capacity to prevent water loss, while species that live in lower and wetter areas still show a considerable variation in water loss, which may be balanced by behavior (Levinton et al. 2015). Although *M. burgersi* can occurs in higher and drier areas, *M. mordax* lost less water in the end of the controlled

experiments, as well as in each evaluated time, suggesting that physiological or morphological traits of this species are more efficient in prevent water loss. Moreover, water loss increased along the time in both species, which may be a mechanism to decrease the body temperature, as water loss can transfer heat to the air (Wilkins & Fingerman 1965, Smith & Miller 1973). Afterwards, rehydration was similar between species, indicating that crabs will gain water independently of how much they previously lost.

It was already showed that males major claw of fiddler crabs are not only used to attract females, but also has a thermoregulatory function. The major claw loss may increase rapidly the body temperature, while no effect occurs when the minor claw is removed (Darnell & Munguia 2011). Claw length was higher in *M. mordax*. Furthermore, claw length was negative correlated to water loss, while it did not have correlation to rehydration traits. As major claw increases isometrically with body increasing (Darnell & Munguia 2011), our findings suggest that the thermoregulatory function of the major claw linked to the body size influenced the water loss differences in *M. burgersi* and *M. mordax*. As examples, small males of *Leptuca pugilator* (Bosc 1802) lost more water than large males (Yoder et al. 2007, Allen et al. 2012) and *Leptuca panacea* (Novak & Salmon 1974) lost considerably more water when its major claw was removed (Darnell & Munguia 2011).

Birgus latro (Lineaus 1767), *Cardisoma carnifex* (Herbst 1796), and *Gecarcoidea lalandii* (Milne Edwards, 1837), terrestrial crabs form Pacific, tolerate losing about 15 to 22% of body mass in high temperature environments, until they loss the locomotor function (desiccation stress) (Harris & Kormanik 1981). All specimens lost around 10 to 18% of body mass before die, and *M. burgersi* specimens died significantly more than *M. mordax* specimens. It is possible that the great mortality in *M. burgersi* occurred due to the small claw size that may decrease the water loss tolerance. During desiccation experiments,

respiration might be slowed until the individual die. After death, organism keep losing approximately 75-95% water of its living rate, but then due to integumentary water loss (Herreid II 1969).

In an environment under summer conditions as high temperatures and radiation, waving males in the surface can lose approximately 10% of body mass in few minutes, which may indicate a significant risk to wandering males, as they do not have access to a burrow for ride (Allen et al. 2012). Although *M. burgersi* and *M. mordax* present differences in water loss traits, males of both species retreated to their burrow equally, as well as the number of males that retreated was similar to males that did not enter in their burrow. These findings suggest that burrow retreat is not the mainly behavior to control temperature and, consequentially decrease water loss in these populations. Additionally, water uptake by setal tufts contact to substratum capillary may be able to replace most part of the water lost during the expose (Thompson et al. 1989).

Our results demonstrate a behavioral homogeneity among populations and a variation in water loss, followed by a similar water gain in controlled experiments using *M. burgersi* and *M. mordax* as models. Besides that, claw length, which was different between species, showed a strong correlation with water loss, but exhibits no correlation to rehydration. Mortality was varied between species. Lastly, field observations showed no differences in burrow utilization. These results suggest that the major claw is an essential trait to control water loss, but water gain after thermal stress is controlled by other morphological and behavioral traits.

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Considerações finais

A partir dos resultados obtidos, chegamos às principais conclusões:

1- Populações brasileiras de *M. burgersi* e *M. mordax* estão geneticamente conectadas devido ao fluxo genético entre elas. Costa Rica e Brasil apresentam divergência genética, provavelmente explicada pelo modelo “stepping stone” e pela presença de barreiras físicas (Ístmo do Panamá, Rio Orinoco e Amazonas);

2- *M. burgersi* e *M. mordax* apresentam o mesmo padrão de movimento no aceno. Além disso, o tempo gasto em cada componente do aceno foi igual entre populações e entre espécies, indicando que o aceno não deve ser utilizado no reconhecimento interespecífico. A diferença encontrada no tempo total do aceno entre duas populações de *M. mordax* pode ser consequência de diferenças ambientais;

3- *M. burgersi* possui menor tolerância ao estresse térmico. Independente da espécie, a perda d'água aumenta consideravelmente ao longo do tempo. O comprimento do quelípedo hipertrofiado variou entre as espécies, além de estar fortemente relacionado a perda d'água. A reidratação, pelo contrário, mostrou-se igual entre as espécies e independente do comprimento do quelípedo. Então, acredita-se que a perda d'água varie entre as espécies devido a diferença de comprimento do quelípedo.

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