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**Conectividade do caranguejo *Grapsus grapsus* (Linnaeus, 1758) em  
ilhas oceânicas brasileiras**

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**CONECTIVIDADE DO CARANGUEJO *GRAPSUS*  
*GRAPSUS* (LINNAEUS, 1758) EM ILHAS OCEÂNICAS  
BRASILEIRAS**

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## **RESUMO**

O ambiente marinho favorece a troca de indivíduos entre as populações promovendo a conectividade entre elas, o que pode contribuir para esforços na conservação. No Brasil, as ilhas oceânicas (Atol das Rocas (RA), Fernando de Noronha (FN), Arquipélago de São Pedro e São Paulo (SPSPA) e Ilha da Trindade (TR)) têm diferentes estratégias de conservação. O caranguejo *Grapsus grapsus* (Linnaeus, 1758), no Brasil, ocorre somente nestas ilhas oceânicas, além disso, ele dispersa apenas no estágio larval e depois passa a fase adulta nos costões rochosos. Portanto, essa espécie não se utiliza de trampolins ecológicos submersos e precisa das correntes marinhas superficiais para dispersão. Para entendermos o papel do oceano para cada espécie é importante estudarmos as diferenças genéticas e morfológicas entre as populações. O objetivo deste estudo foi verificar o nível de diferenças genéticas e morfométricas entre as populações do caranguejo *G. grapsus* em todas as ilhas oceânicas brasileiras e esclarecer o “status” de identificação da população na Ilha da Trindade. Além disso, nós também avaliamos esta espécie como modelo de conectividade para o planejamento de áreas marinhas protegidas. Assim, um total de 564 indivíduos de *G. grapsus* foi utilizado nas análises morfométricas e 84 nas análises genéticas. O DNA genômico utilizado foi um fragmento de 570bp da região controle do mtDNA. As análises morfométricas mostraram similaridade entre as populações do SPSPA e TR, tanto para os machos quanto para as fêmeas. Considerando os aspectos genéticos, SPSPA foi o local com maior diversidade haplotípica, enquanto TR foi o menos diverso. Além disso, este último local apresentou haplótipos exclusivos, enquanto as outras ilhas compartilharam alguns. Diferentes padrões encontrados na genética e na morfometria são comuns em caranguejos decápodos. Consequentemente, é importante combinarmos análises morfométricas e genéticas quando comparamos populações, para uma visão mais completa sobre sua diferenciação. *G. grapsus* aparenta ser capaz de dispersar livremente entre as ilhas brasileiras equatoriais, onde a distância é relativamente curta, mas incapaz de alcançar ilhas muito distantes. Portanto, TR é um local importante para a manutenção de *G. grapsus*, pois representa um estoque genético com haplótipos diferenciados o que é essencial para a manutenção da diversidade genética da espécie e, portanto, ela deveria ser objeto de maior atenção.

**Palavras-chave:** morfometria, genética, dispersão, áreas marinhas protegidas



## ABSTRACT

The marine environment favors the exchange of individuals among population promoting the connectivity among them which contribute to conservation efforts. In Brazil, the oceanic islands (Rocas Atoll (RA), Fernando de Noronha (FN), St. Peter and St. Paul Archipelago (SPSPA) and Trindade Island (TR)) have different conservation strategies. The crab species *Grapsus grapsus* (Linnaeus, 1758) occurs in Brazil only in these islands, it disperses just as larvae, and then it spends the adult life on the rocky shores. Therefore it does not use seamounts as stepping stone and needs the superficial currents to disperse. In order to understand which role the ocean plays for each species it is important to study genetic and morphological differentiation among populations. The aim of this study was to verify the level of genetic and morphometric differences among *G. grapsus* populations in all Brazilian oceanic islands and confirm the population status in TR. In addition, we also evaluated this species as a model of connectivity for designing marine reserve area. A total of 564 individuals of *G. grapsus* were used for morphometric analyses and 84 for genetic analyses. All measured characters were analysed as proportions. The genomic DNA used was a 570bp fragment of the control region of the mtDNA. Morphometrics analyses showed morphology similarity between SPSPA and TR *G. grapsus* populations, both for male and female, and FN and RA presented a different population pattern. Considering genetics aspects SPSPA was the most haplotype diverse site and TR the less diverse. Interestingly, the latter had exclusives haplotypes while the other islands shared a few. Mismatched patterns of genetic and morphometric variation are common in decapods crabs. Consequently, it is important to combine morphometric and genetic analyses when comparing populations to obtain a more correct and complete view about the basis of their differentiation. *G. grapsus* seems to be able to freely disperse through Equatorial islands but incapable to reach long distances according to Brazilian superficial currents. Therefore, TR is an important maintenance site for *G. grapsus* because it represents a gene reserve of different genotypes that are essential to keep the species genetic diversity and thus, it should be safeguarding with more attention.

**Keywords:** Morphometric, genetic, dispersal, Marine Protected Areas



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## **LISTA DE ABREVIATURAS E SIGLAS**

- AEU - Atlantic Equatorial Undercurrent  
BC - Brazilian Current  
CCL – Chelipod carpus length  
CL - Carapace length  
CML - Chelipod merus length  
CPL - Chelipod propodus length  
CPH - Chelipod propodus height  
CW - Carapace width  
FAW - Female abdominal width  
FMG - First male gonopod  
FN - Fernando de Noronha  
MAW - Male abdominal width  
NBC - North Brazilian Current  
RA - Atol das Rocas  
RW - Rostrum width  
SEC - South Equatorial Current  
SECC - South Equatorial Countercurrent  
SMG - Second male gonopod  
SPSPA - Arquipélago de São Pedro e São Paulo  
TPL – Total pereiopod lenght  
TR – Trindade Island



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## **1 INTRODUÇÃO**

Conectividade marinha – a troca de indivíduos entre as populações marinhas – tem implicações relevantes à evolução e ecologia das espécies (Becker et al. 2007). É importante diferenciarmos a conectividade ecológica da genética, não obstante, a comparação entre as duas deve ser feita com cuidado, pois a escala temporal delas é distinta. A proporção de migrantes necessários para conectar ecológicamente as populações precisa ser bem maior do que aquela para conectar populações no sentido evolutivo. Isto significa que mesmo uma pequena quantidade de indivíduos pode ser suficiente para conectar populações geneticamente, mas não o bastante para ser notado ecológicamente (Sale et al. 2010). Portanto, de acordo com estes autores, conectividade genética é a quantidade de fluxo gênico entre as populações em uma escala de tempo de várias gerações e determina a extensão das diferenças genéticas entre as populações. Estas diferenças podem incluir tamanho populacional efetivo, diversidade genética, adaptação local e em alguns casos, especiação (Freeland 2005), enquanto a conectividade ecológica é a troca de indivíduos entre as populações que podem influenciar a demografia e dinâmica populacional. A troca de indivíduos entre as populações pode ser por dispersão larval, recrutamento de juvenis em idade reprodutiva e movimento de grande escala de juvenis e adultos entre os locais (Sale et al. 2010). Além disso, a evolução molecular é um processo contínuo e populações isoladas provavelmente divergirão ao longo do tempo. Por outro lado, a morfologia não necessariamente muda com o passar dos anos se os fatores ecológicos permanecerem constantes, como foi demonstrado pela comparação de várias espécies de invertebrados marinhos presentes nos dois lados do Istmo do Panamá (Schubart et al. 2000). Ainda, mudanças moleculares podem levar a mudanças fisiológicas, ecológicas ou comportamentais, que não são rapidamente evidentes na morfologia (Schubart et al. 2000).

A vida no oceano favorece a dispersão larval dentro e entre as populações. Indivíduos dispersores podem se beneficiar por evitar o endocruzamento, encontrar um novo lugar com menos competidores ou escapar de patógenos, parasitas e predadores (Freeland 2005). No entanto, eles podem não encontrar em lugar favorável ou parceiros, e há o risco de serem predados no caminho (Freeland 2005). Para muitas espécies, o primeiro estágio de dispersão é tipicamente associado com os primeiros estágios de vida (p. ex., esporos, ovos ou larvas). No entanto, em espécies móveis os juvenis e adultos podem também

dispersar (Cowen and Sponaugle 2009). A extensão espacial da dispersão larval em sistemas marinhos tem sido tradicionalmente inferida através de estimativas da duração pelágica larval, pelos modelos de movimentos de partículas passivas pelas correntes oceânicas ou pela análise de variação na freqüência alélica mitocondrial ou nuclear dos genes (Scheltema 1986; Hellberg et al. 2002; Treml et al. 2008). A dispersão entre as populações pode recuperar populações em declínio, prevenir metapopulações de extinções locais e manter a variação genética dentro e entre as populações (Waser and Strobeck 1998). Portanto, taxas de dispersão ou fluxo gênico são importantes para predizer o tamanho efetivo da população, sua persistência e sua estrutura, os quais são parâmetros importantes para esforços para conservação.

Há uma grande preocupação em conservar a biodiversidade marinha, maximizar os rendimentos pesqueiros e um apelo à base da gestão de ecossistemas devido ao declínio excessivo dos estoques pesqueiros e a rápida degradação do habitat costeiro (Cowen et al. 2006). Ultimamente, a maioria dos esforços teóricos para o planejamento de áreas marinhas protegidas considera o papel da conectividade pela troca de larvas, e, em alguns casos, o movimento de adultos (Guichard et al. 2004; Gerber et al. 2005; Gaines et al. 2007). Além disso, dados empíricos disponíveis sobre o potencial de distância da dispersão larval tem sido usado para fazer recomendações referentes ao tamanho da reserva e local em que esta deve ser inserida (Kinlan and Gaines 2003; Palumbi 2003; Shanks et al. 2003). Os dados genéticos também contribuem para esforços para a conservação e para o planejamento do manejo (Moritz 1994; Gompert et al. 2006; Schwartz et al. 2007). A distribuição geográfica da variação genética dentro e entre os táxons providencia informações sobre a demografia histórica e contemporânea e processos evolutivos (Sunnucks 2000; Costello et al. 2003; Aspi et al. 2006). Segundo Palumbi (2003), por exemplo, ilhas separadas por centenas de quilômetros talvez troquem somente uma pequena quantidade de recrutas ou não haja nenhum recrutamento, o que significa um baixo fluxo gênico entre as populações e, portanto, elas deveriam ser manejadas como populações ecologicamente separadas.

A função e a efetividade das reservas marinhas dependem dos seus objetivos, mas são inquestionáveis os benefícios ecossistêmicos nestas áreas, mesmo assim somente uma pequena parte (menos de 1%) dos oceanos é mundialmente protegida (Wood et al. 2008). No caso da costa brasileira, as ilhas oceânicas (Atol das Rocas, RA; Fernando de Noronha, FN; Arquipélago de São Pedro e São Paulo, SPSPA; e Ilha da

Trindade, TR) são áreas biologicamente importantes para o país. No entanto, somente uma delas (RA) é uma Reserva Marinha. FN tem parte de sua ilha principal como Parque Nacional, onde o acesso de turistas é permitido com restrições, e a outra parte como Área de Proteção Ambiental, onde se encontram as habitações, o comércio e o acesso livre pelo turista. SPSPA é também uma Área de Proteção Ambiental, mas a grande distância da costa ( $> 1.000$  km) faz com que o acesso e o controle sejam naturalmente difíceis. TR é administrada pela Marinha do Brasil e localiza-se ainda mais distante da costa do que SPSPA, o único meio de transporte para chegar à ilha é através da marinha ou outra, desde que com sua autorização.

As ilhas oceânicas brasileiras compartilham algumas espécies, como por exemplo, a lagosta *Panulirus equinatus* (Melo, 1999), peixes (Floeter et al. 2008), esponjas (como *Plakinastrella microspiculifera* Moraes and Muricy, 2003 e *Erylus latens* Moraes and Muricy, 2007), algumas espécies de moluscos (Gomes et al. 2006), o polvo *Octopus insularis* (Leite et al., 2008) e o caranguejo de costão rochoso *Grapsus grapsus* (Linnaeus, 1758). No Brasil, *G. grapsus* é encontrado nas ilhas oceânicas (Freire et al. 2011), mas sua distribuição se estende pelo Pacífico Oriental, desde os Estados Unidos (Califórnia) até o Chile incluindo as ilhas, como também no Atlântico Ocidental, desde os Estados Unidos (Carolina do Norte) até as ilhas brasileiras e Atlântico Oriental, desde Portugal até as ilhas espanholas e Camarões, Congo e Guiné Equatorial (Ratti 2004). É curiosa a distribuição desta espécie somente em ilhas no Atlântico Sul, atribuímos isto a características ambientais e ecológicas, como talvez a interação com outras espécies. Freire et al. (2009a,b; 2011) estudou o comportamento, a fecundidade e a maturidade da população desta espécie no SPSPA e Koettker et al. (2010) e Brandão et al. (in press) estudaram sua ecologia larval. Uma particularidade desta espécie comparada a peixes, lagostas e esponjas, é o seu modo de dispersão. *Grapsus grapsus* dispersa através de sua larva planctônica mas se estabelece na zona supralitoral e, portanto, não pode utilizar os montes submarinos como trampolins ecológicos, o que a faz um bom modelo para estudos de dispersão larval por correntes marinhas superficiais e não corresponde ao mesmo modo de dispersão das outras espécies. Segundo dados disponíveis, Palumbi (2003) sugere uma média de dispersão entre 25 – 150 km de distância para larvas de peixes e invertebrados. No entanto, as distâncias entre as ilhas oceânicas brasileiras variam de 145 km a mais de 2.000 km, o que possivelmente permite a conectividade das espécies entre algumas ilhas, mas não entre outras. Apesar de o oceano favorecer a dispersão por ser um ambiente

fluido, ele pode também atuar como uma barreira e separar populações distantes (Palumbi 1994). Assim, para entendermos o papel do oceano para cada espécie é importante estudarmos as diferenças genéticas e morfológicas entre as populações.

Com relação à morfologia de *Grapsus grapsus* alguns estudos apresentam dúvidas quanto ao “status” das populações das ilhas oceânicas do Atlântico Sul. Manning and Chace (1990), Guerao et al. (2001), Ratti (2004) e Hartnoll (2009) consideram que *G. grapsus* das Ilhas de Trindade e Ascensão são espécies diferentes. Hartnoll (2009), por exemplo, considerou *Grapsus* da Ilha da Ascensão como *Grapsus adscensionis* (Osbeck 1765) e ele recomenda confirmar a espécie da Ilha da Trindade. O objetivo deste estudo foi verificar as diferenças morfológicas e genéticas entre as populações de *G. grapsus* em todas as ilhas oceânicas brasileiras, esclarecer o “status” de identificação da população da Ilha da Trindade, além de avaliar a espécie como modelo de conectividade para o planejamento das áreas marinhas protegidas.

## **2 CAPÍTULO ÚNICO**

**Conectividade marinha de *Grapsus grapsus* (Linnaeus, 1758) nas ilhas oceânicas brasileiras, Atlântico Sul**

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Marine connectivity of *Grapsus grapsus* (Linnaeus, 1758) in the Brazilian oceanic islands, South Atlantic

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## 2.1 ABSTRACT

In this study, the level of genetic and morphometric differences among all Brazilian populations of *Grapsus grapsus* (Linnaeus, 1758) were studied. Specimens were collected in Saint Peter and Saint Paul Archipelago ( $0^{\circ}55'N$ ,  $29^{\circ}20'W$ ), Fernando de Noronha ( $3^{\circ}50'S$ ,  $32^{\circ}24'W$ ), Rocas Atoll ( $3^{\circ}50'S$ ;  $33^{\circ}49'W$ ) and Trindade Island ( $20^{\circ}30'S$ ;  $29^{\circ}20'W$ ) from 2003 to 2011. A total of 564 individuals were used for morphometric analyses and 84 for molecular ones. The gene used was a 570bp fragment of the control region of the mtDNA. Morphometric results demonstrated a similarity between Trindade Island and Saint Peter and Saint Paul Archipelago populations while genetic results showed a similarity among all islands, except Trindade. The major differences in male morphologies were detected in carapace and chelipods while in females the same occurs, in addition to the abdomen. A total of 31 haplotypes were found in all islands. Saint Peter and Saint Paul Archipelago was the most diverse site (haplotype diversity = 0.873 and nucleotide diversity = 0.004) and Trindade Island was the less (haplotype diversity = 0.462 and nucleotide diversity = 0.001). These results clarify the status of the population in Trindade Island, help to understand connectivity through superficial ocean currents among Brazilian oceanic islands, and give basement for a best performance of Brazilian marine reserve areas.

## 2.2 INTRODUCTION

Marine connectivity – the exchange of individuals among marine populations – has important implications to evolution and ecology of species (Becker et al. 2007). It is important to differentiate between ecological and genetic connectivity, however it should be in mind their different time scale. The rates of migrant exchange necessary to connect populations ecologically need to be much higher than those which connect populations in an evolutionary sense. This means that even a small number of individuals can be sufficient for evolutionary connectivity, despite not being noticeable in an ecological sense (Sale et al. 2010). Therefore, according to that author, evolutionary connectivity is the amount of gene flow occurring among populations over a timescale of several generations and it determines the extent of genetic differences among populations. These differences include population size, genetic diversity, local adaptation and in some cases, speciation (Freeland 2005), while ecological connectivity is an exchange of individuals among local populations that can influence population demography and dynamics. It can include exchange of offspring between populations through larval dispersal, recruitment of juveniles with their survival to reproductive age, and large-scale movement of adults between locations (Sale et al. 2010). Besides, molecular evolution is a continuous process and isolated populations will probably diverge over time. On the other hand, morphology does not necessarily change over extended time periods if ecological factors remain constant, as shown by comparison of several trans-isthmian marine invertebrate species (Schubart et al. 2000). In addition, molecular changes might lead to physiological, ecological or behavioral changes that are not readily evident in morphology (Schubart et al. 2000).

The life in the ocean favors the dispersal within and among populations. Dispersing individuals may benefit by avoiding inbreeding, finding a new place with few competitors, or escaping from pathogens, parasites or predators (Freeland 2005). However, they may be unable to locate a suitable new site or mate, and they also are in risk of being preyed during dispersal (Freeland 2005). For many species, the primary dispersal phase is typically associated with the earliest life history stage (i.e. spore, egg, or larva). However, for more mobile species, the juveniles and adults may also disperse (Cowen and Sponaugle 2009). The spatial extent of larval dispersal in marine systems has been inferred from estimates of pelagic larval durations, from the modeled movements of passive particles by ocean currents, or from analyses of variation in

allele frequencies of mitochondrial or nuclear genes (Scheltema 1986; Hellberg et al. 2002, Treml et al. 2008). Dispersal between populations can rescue declining populations, prevent metapopulations from local extinction, and maintain genetic variation within and between populations (Waser and Strobeck 1998). Therefore, rates of dispersal or gene flow are important to predict population size, its persistence and its structure which are important parameters for conservation efforts.

There is a big concern about conserving marine biodiversity, maximizing fishery yields and calls for ecosystem-based management due to major declines in fishery stocks and rapid degradation of natural coastal habitat (Cowen et al. 2006). Most theoretical efforts to design effective reserves now consider the role of connectivity via larval exchange and, in some cases, adult movement (Guichard et al. 2004; Gerber et al. 2005; Gaines et al. 2007). Besides, empirical available data on potential larval dispersal distances has been used to make recommendations regarding reserve size and spacing (Kinlan and Gaines 2003; Palumbi 2003; Shanks et al. 2003). Genetic data also contributes to conservation efforts and designing management (Moritz 1994; Gompert et al. 2006; Schwartz et al. 2007). The geographical distribution of genetic variation within and among taxa provides information on historical and contemporary demographic and evolutionary processes (Sunnucks 2000; Costello et al. 2003; Aspi et al. 2006). According to Palumbi (2003), for example, islands separated by hundreds of kilometers may exchange just a small number of recruits or no recruits at all, which means low gene flow between populations, therefore they should be managed as ecologically separate populations.

The operation and effectiveness of marine reserves depends on their goals, but it is undoubted the benefits to the ecosystem in these areas, even though just a small part of the ocean (less than 1%) is protected (Wood et al. 2008). In the case of the Brazilian coast, the oceanic islands (Rocas Atoll, RA; Fernando de Noronha, FN; St. Peter and St. Paul Archipelago, SPSPA; and Trindade Island, TR) represent important biological areas to the country. However, only one of them (RA) is a Marine Reserve, no-take area. FN has part of its main island as National Park where tourists are allowed with restriction and the other part as Area of Environmental Protection where habitations, trade markets and tourism area allowed. SPSPA is also an Area of Environmental Protection but it is distant more than 1,000 km from the coast, making the access and control naturally difficult. TR is under Brazilian Navy administration and it is even farther from the coast, the

only approach to this island is made by the Navy otherwise with its authorization.

The Brazilian oceanic islands share some species as *Panulirus equinatus* lobster (Melo, 1999), *Plakinastrella microspiculifera* Moraes and Muricy, 2003 and *Erylus lateens* Moraes and Muricy, 2007 sponges, some mollusks (Gomes et al. 2006), *Octopus insularis* Leite et al., 2008, some fishes (Floeter et al. 2008) and the rocky crab *Grapsus grapsus*. In Brazil, *G. grapsus* is only found in oceanic islands (Freire et al. 2011) but its distribution in the Pacific Ocean extends from the United States (California) to Chile including islands, in the West Atlantic from the United States (North Carolina) to Brazilian islands and in the East Atlantic in Portugal and Spain Islands, Cameroon, Congo and Equatorial Guinea (Ratti 2004). It is interesting to notice the occurrence of this species only in islands in the South Atlantic, we considered this distribution related to the habitat and the species ecology, for example, its interaction with other species. Freire et al. (2009a,b; 2011) have studied behavior, fecundity and maturity within the SPSPA population and Koettker et al. (2010) and Brandão et al. (in press) focused on larval ecology. One particular difference among fishes, lobsters, sponges and *G. grapsus* is the latter species way of dispersion. *Grapsus grapsus* disperses through planktonic larvae but settle in supralitorial zones, and therefore it is unable to use seamounts as stepping stones, which makes it a good model for larval dispersal through superficial currents and not corresponding to the same dispersal as the other species. Palumbi (2003) suggested a mean dispersal distances of 25–150 km for fish and invertebrates larvae based on available data. The distances among the Brazilian oceanic islands range from 145 km to more than 2,000 km, which also means that it may be possible to have connectivity between some islands but not between others. However ocean favors species dispersal due to its fluid environment, it can also act as a barrier and separate distant populations (Palumbi 1994). In order to understand which role the ocean plays for each species it is important to study genetic and morphological differentiation among populations.

Based on morphological studies, there is a dubious status about *Grapsus grapsus* populations in the South Atlantic oceanic islands. Manning and Chace (1990); Guerao et al. (2001); Ratti (2004) and Hartnoll (2009) are inclined to consider *G. grapsus* from Trindade and Ascension Islands as different species. Hartnoll (2009), for instance, considered *Grapsus* from Ascension Island as *Grapsus adscensionis* (Osbeck, 1765) and he recommends confirming the species from Trindade Island. The aim of this study was to verify the level of genetic

and morphometric differences among *G. grapsus* populations in all Brazilian oceanic islands and clarify the status of the population in Trindade Island. In addition, we also evaluated this species as a model of connectivity for designing marine reserve area.

## 2.3 MATERIALS AND METHODS

### 2.3.1 Study area

Specimens of *Grapsus grapsus* (Linnaeus, 1758) were collected from all Brazilian oceanic islands: Saint Peter and Saint Paul Archipelago (SPSPA, 0°55'N, 29°20'W), Fernando de Noronha (FN, 3°50'S, 32°24'W), Rocas Atoll (RA, 3°50'S; 33°49'W) and Trindade Island (TR, 20°30'S; 29°20'W) (Fig. 1) between 2003 and 2011. SPSPA is approximately 600 km from FN; FN and RA are 145 km apart; and TR is almost 2,000 km distant from FN. These islands are subject to a set of ocean currents. SPSPA is influenced by the South Equatorial Current from East-West direction and the South Equatorial Countercurrent in the opposite direction. FN and RA are also influenced by these two currents but, due to their proximity to the coast, they are also under the North Brazilian Current effect which connected these two islands. These three locations are also connected by the Atlantic Equatorial Undercurrent. TR is in the middle of the South Atlantic Subtropical Gyre originated by the Brazilian Current and the South Equatorial Current (Silveira et al. 2000) (Fig. 1).

### 2.3.2 Sampling collection and laboratory procedures

A total of 564 individuals of *Grapsus grapsus* were used for morphometric analyses (359 females and 205 males (Table 1). Measurements included a variety of variables in the carapace, rostrum, major chelipod, major pereiopod (forth), fifth segment of abdomen and gonopods; for a total of 10 to 12 measurements per individual, depending on its sex (Table 2, Fig. 2). Only sexually mature specimens were included (carapace width larger than 51 mm for males and 35 mm for females, according to Freire et al. 2011). Individuals from SPSPA and TR were manually captured, immediately frozen and further measured with a digital vernier caliper (0.01 mm). Individuals from FN and RA were manually captured, and using a milimetric scale over the crab surface, at least 3 pictures of each dimension were taken and then crabs were released to the rocks. Afterwards, the measurements were obtained with the free software AxionVision 4.8 (0.01 mm). These methods were carried out due to differences in logistics during field

work on each island. Destruction of crabs was not allowed in RA and FN by the environmental laws and sampling time in the other islands was insufficient to take pictures. A *t* test was made between all measures ( $\log x+1$ ) used and the result were significant ( $n = 30$ ,  $p < 0,001$ ) only for two measures in a total of 15, therefore we accepted the two methods as similar. Pereiopod muscle samples were obtained immediately after capture from 11 to 34 individuals (Table 1) from each island, preserved in absolute ethanol and maintained at -20°C for genetic analyses.

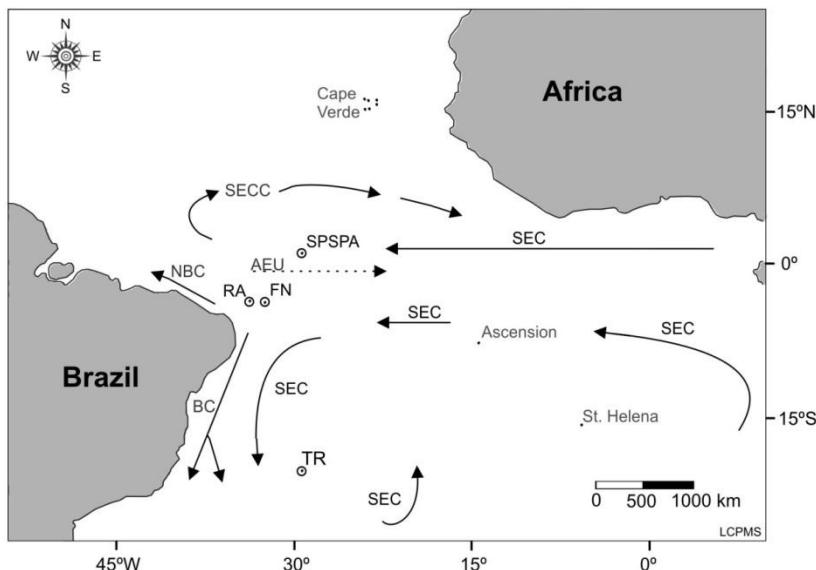


Fig. 1 Map of oceanic islands and main superficial ocean currents. Circles with a dot represent the sampling sites. SPSPA = St. Peter and St. Paul's Archipelago; RA = Rocas Atoll; FN = Fernando de Noronha; TR = Trindade Island. SEC = South Equatorial Current; SECC = South Equatorial Countercurrent; BC = Brazilian Current, NBC = North Brazilian Current, AEU = Atlantic Equatorial Undercurrent. Modified from Macedo-Soares et al. (2012) and Silveira et al. (2000)

Table 1. Number of *Grapsus grapsus* individuals used for morphometrics analyses per site, number of pereiopods used for genetic analyses and sampling year. SPSPA = St. Peter and St. Paul's Archipelago; RA = Rocas Atoll; FN = Fernando de Noronha; TR = Trindade Island

| Location     | Female     | Male       | Genetic   | Year            |
|--------------|------------|------------|-----------|-----------------|
| SPSPA        | 57         | 41         | 11        | 2003-2005, 2011 |
| RA           | 105        | 56         | 20        | 2011            |
| FN           | 95         | 42         | 34        | 2010            |
| TR           | 102        | 66         | 19        | 2010, 2011      |
| <b>TOTAL</b> | <b>359</b> | <b>205</b> | <b>84</b> |                 |

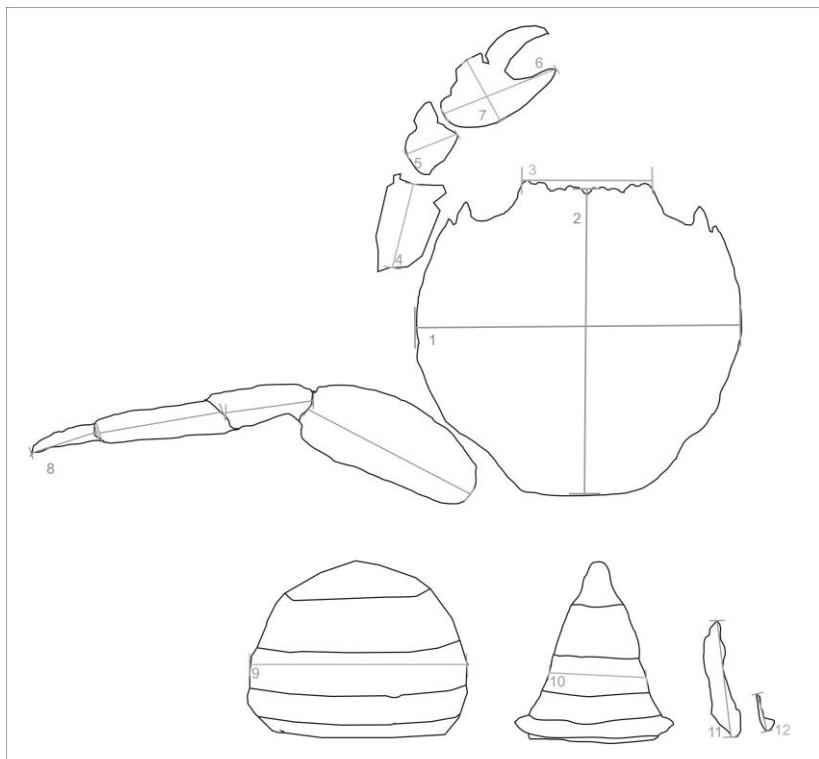


Fig. 2 *Grapsus grapsus* morphometric measurements used in this study. See Table 2 for details

Table 2. Brachyura morphological measurements used in this study. See Fig. 2 for visual description

| Code    | Character                  |
|---------|----------------------------|
| 1. CW   | Carapace width             |
| 2. CL   | Carapace length            |
| 3. RW   | Rostrum width              |
| 4. CML  | Chelipod merus length      |
| 5. CCL  | Chelipod carpus length     |
| 6. CPL  | Chelipod propodus length   |
| 7. CPH  | Chelipod propodus height   |
| 8. TPL  | Total pereiopod length     |
| 9. FAW  | Female abdominal width     |
| 10. MAW | Male abdominal width       |
| 11. FMG | First male gonopod length  |
| 12. SMG | Second male gonopod length |

### 2.3.3 DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA was extracted using the DNeasy kit (QIAGEN®) and used in the amplification of a 570bp fragment of the control region of the mtDNA (DLoop) of *Grapsus grapsus* using primers based on the flanking 12S rDNA and ILE-tRNA regions of the brachyuran mitochondrial genome (DL.USSA.R1: 5'-GGTTAGAGAGAAGGTTAGAGGAC-3', and DL.USSA.F1: 5'-GTATAACCGCGAATGCTGGCAC-3') (Oliveira-Neto et al. 2007). Amplification was carried out in 25 µL reactions with 1.25 units of AmpliTaq DNA polymerase, 1X PCR buffer, 2 mM of MgCl<sub>2</sub>, 0.4 mM of dNTPs and 0.5-1 µM of each primer.

Thermocycler settings included an initial denaturation period of 2 min at 95 °C, followed by 35 cycles of 30 s at 92 °C, 30 s at 54 °C, and 60 s at 68 °C, and by a final extension period of 10 min at 72 °C. Results were visualized using 2% agarose electrophoresis followed by ethidium bromide staining. A negative control (no genomic DNA) was included in each PCR set to check for reagent contamination. Successfully amplified PCR products were sequenced using BigDye v.3.1 terminator sequencing (GeneCodes, Ann Arbor, MI, USA) and run on an ABI3500 automated sequencer. Contigs were assembled and overlapping chromatograms edited with the STADEN 1.6.0 package (Staden 1996). Sequence alignments were adjusted manually using BioEdit program (Hall 1999), no indel (insertion and deletion) was observed. However

heteroplasmy occurred and comparisons of heteroplasmy sequences were performed with DnaSP5.10 (Librado and Rozas 2009).

### 2.3.4 Data analysis

#### 2.3.4.1 Morphometrics

In this study morphometrics (Table 2) were made in order to differentiate *Grapsus grapsus* populations and they were based on Ratti (2004), Pinheiro and Fransozo (1998) and Freire et al. (2011), the two latter studies are related specially to sexual morphologies. An analysis of variance ANOVA was performed with carapace width measures to describe general size of populations.

Afterwards, all morphometric values were log-transformed to achieve normality (Legendre and Legendre 1998). A multivariate analysis of variance (MANOVA) with a canonical variate analysis (CVA) was performed using PAST free software (Hammer et al. 2001) and Statistica 7 to describe patterns of differentiation among populations. When statistically significant variation was recorded among groups, multiple comparisons between each pair of sites were made using the Hotelling  $T^2$  (Legendre and Legendre 1998). Only one measure (CPL) for females was not significant and it was removed from the analysis. Males and females were analyzed separated (Table 1).

#### 2.3.4.2 Genetic

Nucleotide and haplotype diversity estimated for *Grapsus grapsus* populations from each island and for all islands together were calculated using the program DnaSP 5.10 (Librado and Rozas 2009). Haplotype diversity represents the probability that two alleles randomly chosen from the population will be different from one another and the closer this value is from 1.0, more diverse is the population. On the other hand, nucleotide diversity quantifies the mean divergence between haplotypes sequences which might be more informative in some cases (e.g. in relatively rapidly evolving genomes such as animal mtDNA) (Freeland 2005). To quantify the spatial distribution of genetic variation, an analysis of Molecular variance (AMOVA) was conducted in Arlequin 3.5. (Excoffier and Lischer 2010). For the AMOVA, F-statistics ( $F_{ST}$ , Wright, 1969) were used to estimate the relative contribution of molecular variance at three levels: (i) among populations; (ii) within populations and; (iii) pairwise divergence between populations.  $F_{ST}$  values of 0 - 0.05 are generally considered to indicate little genetic differentiation; values of 0.05 - 0.25 indicate moderate genetic

differentiation; and values of  $> 0.25$  represent pronounced levels of genetic differentiation.

Interpopulation evolutionary relationships were estimated by constructing unrooted parsimony haplotype networks using the Templeton et al. (1992) method in the software package TCS 1.21 (Clement et al. 2000). MrBayes 3.1.2 program (Huelsenbeck and Ronquist 2001) was used to produce an unrooted haplotype tree with all populations which was generated using FigTree 1.3.1 (Rambaut 2009). The values used to carry out the computations at MrBayes were: chain length = 10,000,000; sample frequency = 10000; number of chains = 4; burn-in = 200 and the model used was HKY (Hasegawa et al. 1985), according to Posada *in press*; Guindon and Gascuel (2003). Bayesian skyline reconstruction of *Grapsus grapsus* populations was estimated using the BEAST program which employs a Bayesian MCMC approach (5,000,000 permutation burn-in followed by 50,000,000 permutations). For each analysis, the result was generated using Tracer 1.5 software (<http://beast.bio.ed.ac.uk>).

## 2.4 RESULTS

Although only mature crabs were collected, during field work it was possible to notice a slightly tendency of RA crabs to be larger than other populations. This can also be observed by the significant maximum size difference in carapace width measurements (Fig. 3), both for male and female, where maximum sizes were found in this site. This pattern also occurred for most part of the measures, females from SPSPA were the smallest in most cases and FN and TR crabs had intermediate sizes. Another observation presented in all population was the presence of a bifid spine in carpus chelipods.

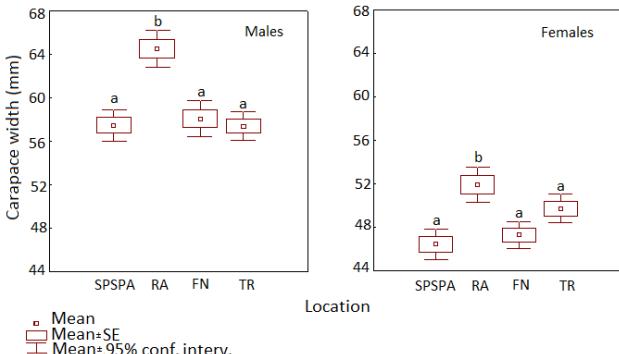


Fig. 3 Box plot with mean, mean  $\pm$  SE and mean  $\pm$  95% confidence interval representing ANOVA of *Grapsus grapsus* carapace width for all collected sites. Males: F (3; 201) = 22,57; p = 0.000. Females: F (3; 355) = 11,03; p = 0.000. SPSPA = St. Peter and St. Paul's Archipelago; RA = Rocas Atoll; FN = Fernando de Noronha; TR = Trindade Island

The MANOVA/CVA analysis showed a significant difference in the morphology among *G. grapsus* populations (males: Wilk's  $\Lambda$  = 0.14; Hotelling's T<sub>2</sub> p < 0.0001; females: Wilk's  $\Lambda$  = 0.16; Hotelling's T<sub>2</sub> p < 0.0001) (Fig. 4 and 5). However, both analyses demonstrated similarity between SPSPA and TR population and segregation from FN and RA. Carapace and chelae were characters important to distinguish populations for both males and females. Abdomen has also an important role to differentiate females populations.

For males, the factors loadings (Table 3) were related to carapace and chelae, with particular proportions mainly associated to one unique site, RA. In the I x II plan, which explained 96.68% of the variation, RA crabs were positively associated with all measurements, it may demonstrate the largest size of this population. It is important to notice that basically every measures related to the chelipods were concentrated in one quadrant and it may be the mainly responsible for separating FN population from SPSPA/TR. Gonopod (SMG) was important to separate RA from FN.

For females, the highest factors loadings (Table 3) of the I x II plan, which explained 96.15% of the variation, were mainly related to carapace, chelipod and abdomen values. Chelipod measure (CCL) was the main responsible for differentiating RA and FN from SPSPA/TR on opposite directions. Pereiopod (TPL) and carapace (CL) measures separated RA from FN. Chelipod (CPH) and abdomen (FAW) were especially positively related to RA.

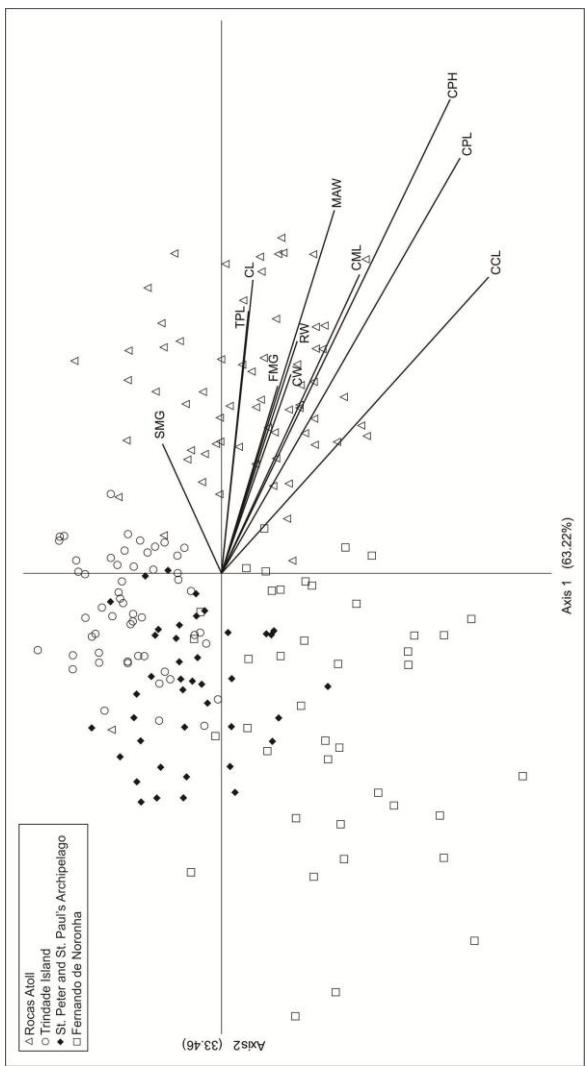


Fig. 4 Scatter plot of canonical variate analysis for *Grapsus grapsus* morphometrics measurements of males. For expansion of abbreviations, see Table 2

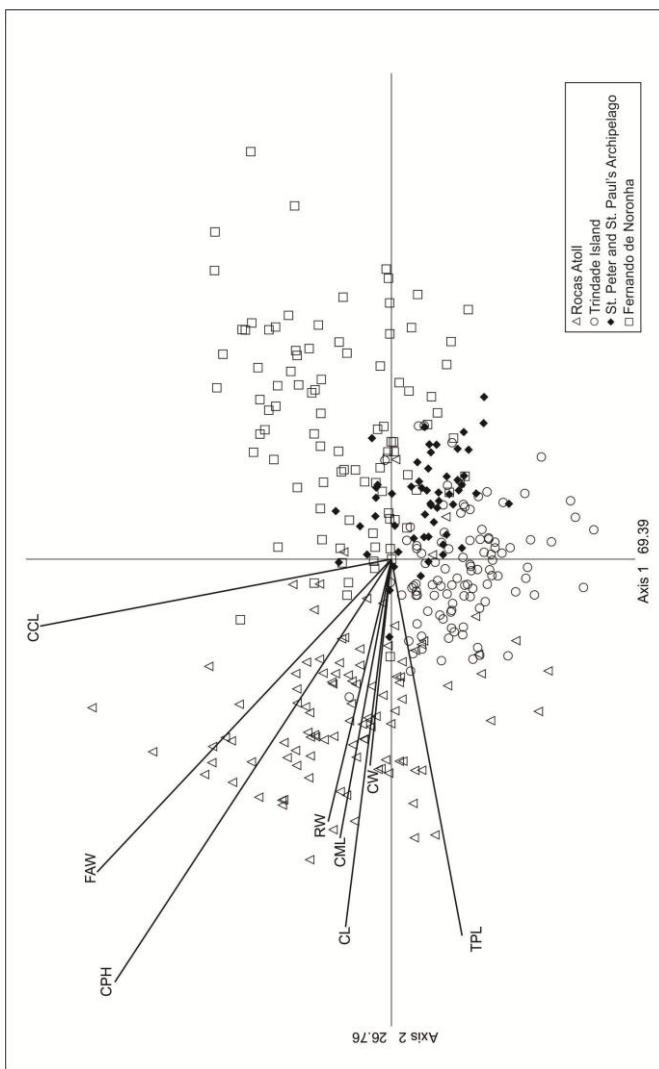


Fig. 5 Scatter plot of canonical variate analysis for *Grapsus grapsus* morphometrics measurements of females. For expansion of abbreviations, see Table 2

Table 3. Factor loadings of CVA on covariance matrices of log transformed measurements of *Grapsus grapsus*

|      | Male                 |                      |                     | Female               |                      |                     |
|------|----------------------|----------------------|---------------------|----------------------|----------------------|---------------------|
|      | Factor 1<br>(63.22%) | Factor 2<br>(33.46%) | Factor 3<br>(3.32%) | Factor 1<br>(69.39%) | Factor 2<br>(26.76%) | Factor 3<br>(3.85%) |
| CW   | -1.918               | 0.158                | 0.182               | 4.597                | 2.410                | -1.017              |
| CL   | 1.507                | 1.632                | 0.750               | -3.787               | -0.093               | -3.143              |
| RW   | -0.340               | -0.472               | -0.089              | 0.076                | 0.394                | 1.310               |
| CML  | -0.167               | 0.675                | -0.376              | -0.185               | 0.865                | 2.232               |
| CPH  | 0.593                | 0.412                | 0.531               | -1.450               | -1.971               | 0.935               |
| CCL  | -0.427               | -1.084               | 0.604               | 1.122                | -1.136               | -0.950              |
| CPL  | 0.370                | -1.780               | -1.091              |                      |                      |                     |
| TPL  | 0.906                | 0.329                | -0.383              | -0.841               | 0.361                | 0.239               |
| MAW* | 0.544                | -0.361               | 0.602               | 0.138                | -1.051               | 0.174               |
| FMG  | -0.291               | -0.486               | -1.026              |                      |                      |                     |
| SMG  | -0.046               | 0.755                | -0.112              |                      |                      |                     |

Numbers in parentheses after factor names correspond to their respective amount of explained variance. For other expansion of abbreviations, see Table 2. \* For female = FAW

Genetic diversity indices are shown in Table 4. A total of 31 haplotypes were found in all islands. The average haplotype ranged from 0.462 to 0.873, therefore except for TR the average value was relatively high and nucleotide diversity varies from 0.001 to 0.004 which means a genetic homogeneity. SPSPA appeared to be the most diverse site with 63.3% of different haplotypes in the sample meaning an average of 0.873 haplotype diversity while TR was the less diverse with only 26.3% of different haplotypes and 0.462 of haplotype diversity.

Table 4. Descriptive statistics on the genetic variability of the studied populations of *Grapsus grapsus*. SPSPA = St. Peter and St. Paul's Archipelago; RA = Rocas Atoll; FN = Fernando de Noronha; TR = Trindade Island

|                       | SPSPA         | FN            | RA            | TR            | TOTAL         |
|-----------------------|---------------|---------------|---------------|---------------|---------------|
| Number of individuals | 11            | 34            | 20            | 19            | 84            |
| Number of haplotypes  | 7             | 13            | 6             | 5             | 31            |
| Haplotype diversity   | 0.873 ± 0.089 | 0.850 ± 0.040 | 0.700 ± 0.073 | 0.462 ± 0.136 | 0.859 ± 0.022 |
| Nucleotide diversity  | 0.004         | 0.003         | 0.002         | 0.001         | 0.004         |

There was substantial differentiation among populations, with nearly half of the genetic variance being found among populations (Table 5). F-statistics obtained with AMOVA revealed significant and high genetic differentiation ( $F_{ST} = 0.504$ ,  $p < 0.0001$ , based on 1000 permutations) and it was used to determine the degree of genetic differentiation between each pair of sites. This pattern seemed to be largely driven by TR population (Table 6), as indicated by the haplotype network (Fig. 6) and the haplotype tree (Fig. 7). TR had only exclusive haplotypes while two haplotypes were shared among SPSPA, FN and RA; two between RA and FN and three between SPSPA and FN. However, TR is not so distant from the others, since there is only one intermediate haplotype that separate it (Fig. 6). In this study, effective population size of each island seemed not to change over time, even though we can notice the smallest size of TR population (Fig. 8).

Table 5. Summary of analyses of molecular variance (AMOVA) with four populations of *Grapsus grapsus*

| Source of variation | df | Sum of squares | Variance components | Variance % |
|---------------------|----|----------------|---------------------|------------|
| Among population    | 3  | 45.521         | 0.726               | 50.40      |
| Within population   | 80 | 57.169         | 0.715               | 49.60      |

$F_{ST}=0.504$ ;  $p<0.0001$

Table 6. Pairwise genetic distance ( $F_{ST}$ ) among four populations of *Grapsus grapsus*

|    | SPSPA  | RA     | FN     |           |
|----|--------|--------|--------|-----------|
| RA | -0.009 |        |        |           |
| FN | -0.011 | 0.014  |        |           |
| TR | 0.720* | 0.791* | 0.665* | *p<0.0001 |

SPSPA = St. Peter and St. Paul's Archipelago; RA = Rocas Atoll; FN = Fernando de Noronha; TR = Trindade Island.

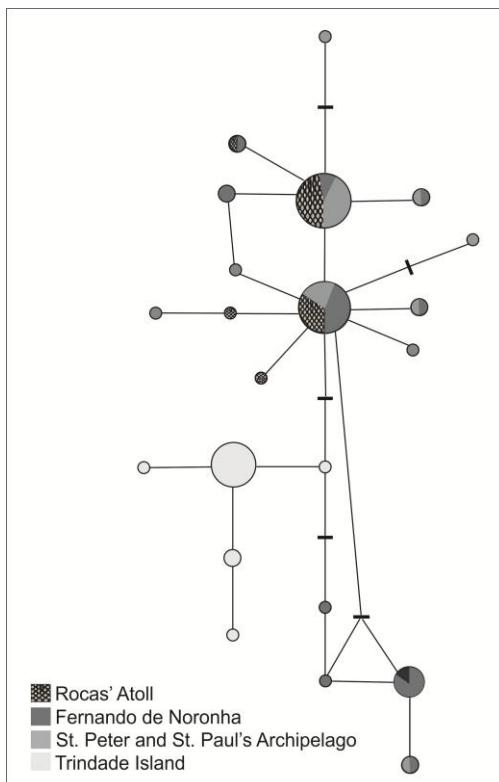


Fig. 6 Haplotype network from 84 *Grapsus grapsus* individuals. Each circle represents a haplotype and their sizes reflect the number of specimens that contain it. Dashes represent missing intermediate haplotypes

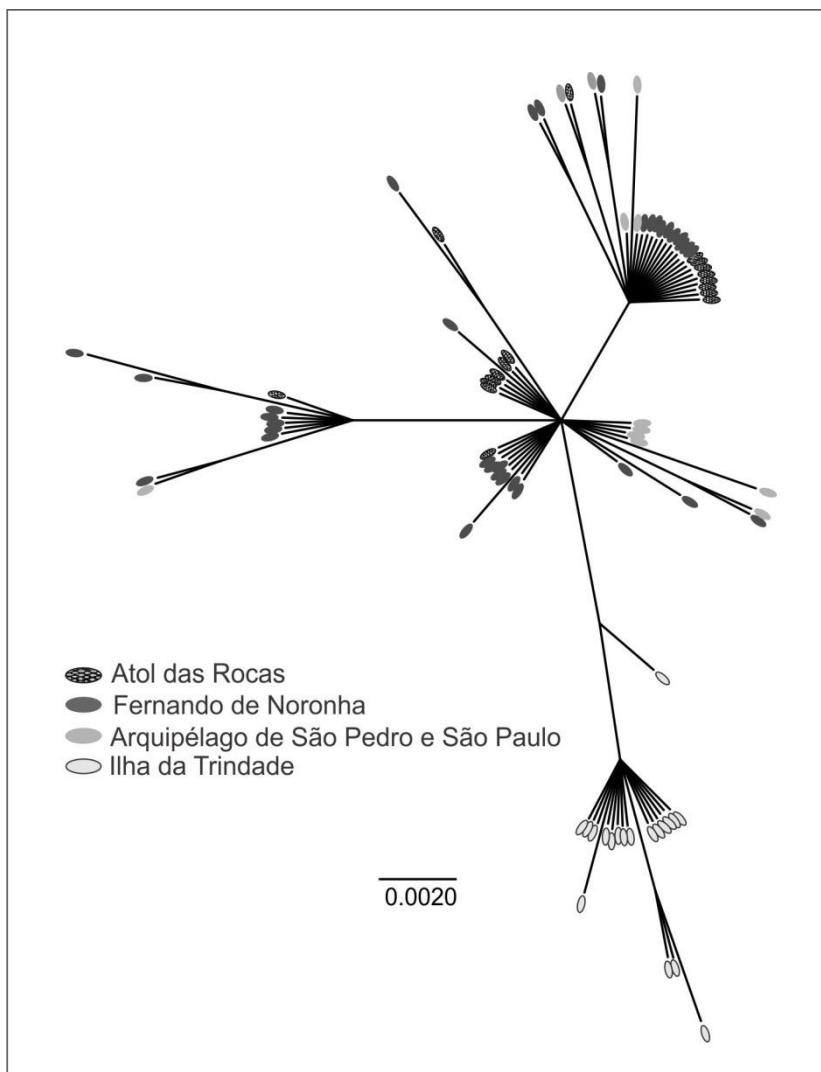


Fig. 7 Unrooted haplotype tree, the final of each branch represents one individual. Specimens at the same branch are more related

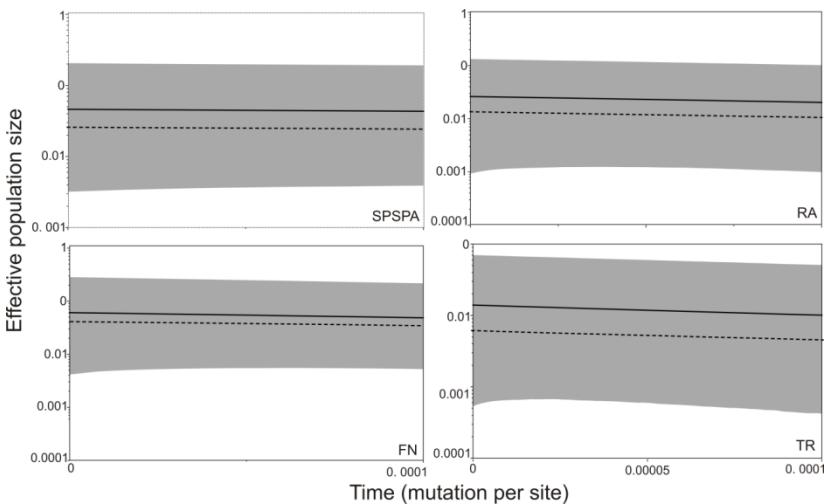


Fig. 8 Bayesian Skyline Plots from *Grapsus grapsus* populations, each plot represents an island. The thick solid line is the mean and the dashed line is the median estimate of effective population size, the gray area shows the 95% confidence limits of the mean. SPSPA = St. Peter and St. Paul's Archipelago; RA = Rocas Atoll; FN = Fernando de Noronha; TR = Trindade Island

## 2.5 DISCUSSION

Our results indicated considerable genetic and morphological divergence among populations of *Grapsus grapsus* in Brazilian oceanic islands. In the first aspect, it appeared to have two populations more similar to each other. According to CVA analysis, Trindade Island and St. Peter and St. Paul's Archipelago were related. Interestingly, both are more than a 1,000 km far from the coast and they are the two islands farther apart from each other. On the other hand, genetic results demonstrated that TR is the only island which did not share any haplotype. Physical and biological factors that promote genetic and morphological differentiation among contiguous populations are still poorly understood (Dawson 2001; Waters et al. 2005). However, these findings may demonstrate that all islands had the same source of founders and TR had a little genetic divergence probably due to the low number of effective population size. In addition, it has restricted connectivity with the other islands, even though not having major morphology changes in relation to SPSPA.

Brian et al. (2006) also found discordance between genetic and morphometric variation in Portunidae crabs. They studied morphology characters of chelae, carapace, pereiopods, abdomen and pleopods. They found some differences among the eight populations studied and there were few differences between male and female. The genetic analyses were made with six enzymes and they did not detect differences among populations. They assumed that these parameters, genetic and morphological, were independent and environment conditions may select a particular genotype. Prior research on crustacean decapods (Fratini and Vannini 2002; Gopurenko and Hughes 2002) had indicated a restriction in gene flow, even between geographically close sites, despite the high potential for dispersal. Both studies were made with the same species but in different areas. According to Gopurenko and Hughes (2002) the genetic differences were due to the marine currents which may explain the difference we found in the present study. However, the opposite genetic pattern, characterized by low levels of population differentiation as a result of high connectivity by larval dispersal, can also be found in crab populations (Cassone and Boulding 2006; Oliveira-Neto et al. 2007).

High mutation rate is common in mtDNA and therefore is expected to present multiple genetic lineages both within and among population (Freeland 2005). In the present study, genetic analyses were performed with mtDNA, and AMOVA analysis showed high levels of genetic differentiation among populations. Apparently, differences found in this study were due to Trindade Island population which presented a more distinct genetic pattern with exclusive haplotypes. Oceanic currents can have different effects on the genetic structure of marine populations. They can be responsible for the dispersion of planktonic larvae, acting as gene-exchange corridors or, alternatively, can constitute an invisible physical barrier to gene flow (Palumbi 1994). Probably, contemporary oceanic superficial currents around TR cannot bring larvae from another island according to their survival time. In addition, the long distance presents high predation risk since decapods larvae are important alimentation resource for a number of marine organisms (Morgan 1990; 1992).

Furthermore, the low nucleotide diversities observed may be result of the relatively short existence of haplotypes, with newly-created haplotypes with a few additional base pair differences not being selected (Cassone and Boulding 2006) or populations were originated by few numbers of haplotypes. In this sense is important to notice the different islands age, all of them were originated from volcanic activities but TR

was the last island to emerge. According to estimations, TR emerged 3.6 million years ago and it is at least 17 million years younger than the others (Almeida 2002a,b; Kikuchi 2002; Campos et al 2009). However, Schubart (2011) believes that Grapsidae amphi-atlantic connection was still possible at least 3 million years ago. Therefore, according to our results, TR might have received larvae from any Atlantic population where the larvae duration could allow them to reach but these connections did not last long.

The Bayesian Skyline Plot revealed almost no change in all population effective sizes over the time, even so TR was the island with the lowest size. It possibly reflected the isolation effect, colonization with few haplotypes and no exchange of individuals with other population over the time. As a result, it was possible to distinguish two groups: one with Equatorial islands (RA, FN and SPSPA) and other with TR. Thus, *G. grapsus* of Equatorial islands seems to freely disperse along them while TR population keeps its viability only through self-recruitment. Some studies corroborate with these findings. Leite et al. (2008) did not find genetic differences among *Octopus insularis* populations that occur in SPSPA, FN and RA. Barroso et al. (2008) also observed a polychaeta species that shared some haplotypes between Equatorial islands. Finally, Rocha (2003) said that reef fishes from TR are more similar to the coast nearby it than to Equatorial islands. Nevertheless, this present study is the first one to compare all Brazilian oceanic islands.

These two groups (Equatorial island x TR) were not applied for morphological patterns since SPSPA and TR were more similar in this aspect. Analyses suggested differences in carapace and chelipod for males and in carapace, chelipod and abdomen for females among SPSPA/TR, RA and FN. Male chelae are used to manipulate females during mate and it can also be an advantage for male fights that are common during this period (Pinheiro and Fransozo 1999). Besides, studies with *Grapsus albolineatus* (Kennishi et al. 1996) showed the chelae morphology was influenced by feeding resources. This crab uses the tip of chelipods to remove microalgae from rock surface and this behaviour is also observed for *G. grapsus* (pers. obs). This latter species has a diversified alimentation that includes algae to cannibalism (Freire et al. 2009a; 2011), thus little differences in chelae morphologies in Brazilian populations could be due to the different availability of food. For females, chelae could also be used to defend themselves against predators and males' unwelcome courtship approach, and protect their brood. The abdomen for females is important because

its size is related to the amount of eggs that a crab can carry (Freire et al. 2009b; 2011). Besides, it is also significant to eggs oxygenation and then, for larvae survivorship and viability (Baeza and Fernandez 2002).

Although there were some differences among populations morphologies, their carapace color patterns were similar. When we compared Brazilian population and Ascension population different color patterns were remarkable. Besides, another trait that we found in all Brazilian populations was the presence of a bifid spine in carpus quelipod which was previously described only for Trindade Island (Ratti 2004). Therefore, based on all specimens analysed here in this study we believe *Grapsus* from TR is *G. grapsus*. Even with mtDNA analyses showing some differences in this population in relation to the other islands, morphological characters including the similarity to SPSPA population and the same color of carapace pattern among all islands kept all Brazilian populations with the same species. Spivak and Schubart (2003) found morphological differences in two *Cyrtograpsus* species, they considered them related to habitat variation and supported the description of these two species as synonym. Besides, we believed the differences in morphology among *G. grapsus* populations were not enough to invalidate interpopulations reproduction of *G. grapsus*.

The genetic versus morphological pattern observed might also indicate that the morphological differentiation found can be an alternative response to habitat-specific selective pressures and not a direct cause of genetic differences (Silva et al. 2010). When phenotypic plasticity rather than constitutive traits takes place, two populations with different phenotypes could have the same genotype. Therefore, adaptive phenotypic plasticity may be important to rising patterns of a species geographic variation among populations. Furthermore, shape analysis plays an important role in biological studies. The phenotype of an organism is related to feeding efficiency, locomotor performance, vulnerability to predators, and reproductive success (Guill et al. 2003). In many crustaceans, the general pattern of growth involves a series of immature instars of generally similar morphology, terminated by a final body shape at which there are distinct morphological changes (Hartnoll 2001).

Finally, both results contributed to understand the connectivity dynamics among Brazilian oceanic islands. These findings can be helpful to the management of Marine Protected Areas. RA population was in the best development situation with the largest individuals observed, with carapace width similar to the Galapagos population, although still smaller than the Pacific coastal sites (reviewed in Freire et

al. 2010), thus it showed the efficacy of this marine reserve. SPSPA population had the smallest crabs although the distant from the coast should help the site preservation. However the high mutilation rate (25%) (Freire et al. 2011) indicated this *G. grapsus* population was under some unfavorable environmental conditions. TR and FN had intermediate individuals sizes, FN is an important touristic place to Brazil and it has already a part of it as a protected area. Of course, given the often wide diversity of taxa (with highly diverse life history strategies) to be protected in an equally wide range of habitat types and oceanographic environments, a single solution is unlikely (Cowen and Sponaugle 2009). However, we could consider *G. grapsus* as a peculiar species, because it has a difficult way of dispersal, as it can only disperse by superficial currents. Therefore, if its connectivity was possible, many other species will also be able to disperse and we believe it could be a model of connectivity for designing marine reserve area. Thus, according to genetic results in this study *G. grapsus* might reach islands up to 700 km a part (SPSPA, FN and RA) but was incapable of arriving at farther islands due to larvae survival time and unfavorable superficial ocean currents. This makes TR an important maintenance spot for *G. grapsus* because it represents a gene pool of different genotypes that are essential to keep the species genetic diversity. Besides, other studies (Alves 2006; Moraes et al. 2006; Floeter et al. 2008) demonstrated TR importance by its endemic organisms. Therefore, a good management to preserve this species and other marine species would be to create a network of Marine Protected Areas among Equatorial islands and a Protected Area around TR, which may include Vitoria-Trindade Ridge for organisms that use seamounts as stepping stone to disperse. Trindade Island is not a Marine Protected Area, therefore the next step should be, through our results and studies alike, let authorities perceive the value of this area and protect it in proper manners.

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### 3 CONCLUSÕES

As conclusões serão apresentadas como um resumo da discussão, no entanto, em português. Desta forma, os dados apresentados podem ser melhor aproveitados por um público mais abrangente, os quais podem se interessar pelo tema.

No presente estudo foram obtidos dados genéticos e morfológicos comparando as populações de *Grapsus grapsus* das diferentes ilhas oceânicas brasileiras entre si. Os resultados obtidos nestes dois aspectos são divergentes. De acordo com a Análise Discriminante da morfometria a Ilha da Trindade e o Arquipélago de São Pedro e São Paulo apresentaram populações com maior similaridade. Por outro lado, os resultados genéticos demonstraram que a Ilha da Trindade é a única ilha que não compartilhou nenhum haplótipo com as outras. Estas descobertas podem demonstrar que todas as ilhas tiveram a mesma fonte de fundadores e a Ilha da Trindade apresenta uma diferença um pouco maior geneticamente provavelmente devido ao baixo valor do tamanho populacional efetivo e difícil conectividade com as outras ilhas, mas não sofrendo grandes mudanças morfológicas quando comparado ao SPSPA. Além disso, diferentes padrões de variação genética e morfológica já foram encontrados em caranguejos braquiúros (Brian et al. 2006).

Aparentemente, as diferenças genéticas encontradas na análise de variância (AMOVA) são relacionadas à população da Ilha da Trindade, que apresenta padrão mais distinto, com haplótipos exclusivos. As ilhas oceânicas brasileiras apresentam diferentes idades, todas elas originadas a partir de atividades vulcânicas, sendo Trindade a última a emergir. De acordo com estimativas, ela surgiu a 3,6 milhões de anos atrás e é pelo menos 17 milhões de anos mais nova do que as outras (Almeida 2002a,b; Kikuchi 2002; Campos et al. 2009). No entanto, Schubart (2011) acredita que a conexão entre os Grapsidae no Atlântico ainda era possível há pelo menos 3 milhões de anos. Portanto, de acordo com os nossos resultados, Trindade pode ter recebido larvas das outras populações do Atlântico, mas esta conectividade não durou muito tempo.

Em relação à dúvida quanto ao status da população de *Grapsus* na Ilha da Trindade, acreditamos que seja realmente *G. grapsus*. Quando observamos os *Grapsus* da Ilha de Ascensão percebemos um padrão de coloração e pintas na carapaça diferentes das observadas nas populações das ilhas brasileiras (ver Anexos). Mesmo com as análises genéticas demonstrando diferenças no mtDNA de TR com relação às outras ilhas, os aspectos morfológicos inclusive a semelhança com a população de

SPSPA e a coloração da carapaça mantiveram todas as populações brasileiras como mesma espécie. Além disso, outra característica observada em todas as populações foi o espinho bifido no carpo do quelípodo (ver Anexo), cuja presença foi previamente descrita como exclusiva para a população de Trindade (Ratti 2004).

Desta forma, as nossas descobertas podem ajudar no manejo de áreas marinhas protegidas, pois de acordo com os nossos dados, *G. grapsus* é capaz de alcançar ilhas com até 700 km de distância, mas incapaz de chegar a ilhas a quase 2.000 km de distância, considerando as correntes marinhas superficiais próximas ao Brasil. Além disso, mostramos a eficácia do Atol das Rocas como reserva, pois apresenta um ambiente favorável para o desenvolvimento ótimo da população, inclusive com tamanho de carapaça similar ao da população de Galápagos, embora ainda menor do que os locais da costa do Pacífico (revisado em Freire et al. 2011). A população do SPSPA apresentou tamanhos menores apesar do isolamento natural, o que proporcionaria um local mais preservado. No entanto, a alta taxa de mutilação (25%) (Freire et al. 2011) nesta população indicou condições ambientais desfavoráveis ao *G. grapsus* deste local. Fernando de Noronha exibiu indivíduos de tamanhos intermediários, apesar do intenso turismo na área existe à área de Parque Nacional Marinho e o tamanho da ilha que favorece diferentes locais de habitação para o caranguejo. A Ilha da Trindade também apresentou tamanhos intermediários de *G. grapsus*, no entanto pouco esforço para conservação é realizada no local. Portanto, reforçamos a importância da proteção da Ilha da Trindade para esta espécie, pois apresenta um estoque genético exclusivo e essencial à manutenção da diversidade genética. Assim, um bom manejo para preservação do *Grapsus grapsus* e de outras espécies marinhas seria incluir a Ilha da Trindade dentro de alguma categoria de Unidade de Conservação, além de manter as outras ilhas protegidas.

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## ANEXOS



Grapsus grapsus em estágio de maturidade/coloração Vermelho em todas as ilhas oceânicas brasileiras. a = Arquipélago de São Pedro e São Paulo; b = Atol das Rocas; c = Ilha da Trindade; d = Fernando de Noronha



*Grapsus adscencionis* da Ilha de Ascensão.  
Foto de RG Hartnoll.



Detalhe no quelípodo com o dente do carpo bífidio,  
característica encontrada em todas as ilhas oceânicas brasileiras.

