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PASSADO E PRESENTE DA ESPONJA *CLATHRINA AUREA* (PORIFERA, CALCAREA) NO ATLÂNTICO OESTE

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Pedro Victor Leocorny Ferreira

Passado e presente da esponja *Clathrina aurea* (Porifera, Calcarea) no Atlântico Oeste

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Biodiversidade e Biologia Evolutiva, Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários para obtenção do título de Mestre em Biodiversidade e Biologia Evolutiva.

Orientadora: Prof.ª Dr.ª Michelle Klautau

Co-orientador: Dr. Thierry Pèrez

Rio de Janeiro

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I – Universidade Federal do Rio de Janeiro

II - Dissertação

“Todos nós temos demônios interiores para lutar. Chamamos esses demônios de ‘medo’, e ‘ódio’, e ‘raiva’. Se você não os domina, então uma vida de cem anos... é uma tragédia. Mas se você os domina, então uma vida de apenas um dia pode ser um triunfo.”

(Yip Man)

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RESUMO

PASSADO E PRESENTE DA ESPONJA *CLATHRINA AUREA* (PORIFERA, CALCARE) NO ATLÂNTICO OESTE

Pedro Victor Leocorny Ferreira

Orientadora: Prof.ª Dr.ª Michelle Klautau

Esponjas são conhecidas por sua baixa capacidade de dispersão e estudos de conectividade têm demonstrado alta estruturação genética de suas populações. Contudo, algumas espécies apresentam ampla distribuição e sua dinâmica de dispersão ainda é pouco conhecida. Assim, o presente trabalho teve como objetivo determinar o grau de conectividade e dispersão das populações de *Clathrina aurea* (Porifera, Calcarea) ao longo do Atlântico Oeste. Utilizando sete loci de microssatélites, encontramos forte similaridade genética entre o Brasil e o Caribe que não foi recuperada nas análises de estruturação com o marcador ITS. Esses resultados suportam a forte influência do Rio Amazonas moldando a dinâmica da conectividade dessas populações ao longo do tempo. Análises de fluxo gênico apontam para uma maior similaridade genética das populações de Antigua e Bequia com a região de Abrolhos, suportando a homogeneidade biogeográfica entre o Caribe e o Nordeste Brasileiro proposta anteriormente com a Província Caribenha. Análises de migrantes mostram uma capacidade de dispersão muito maior do que o esperado para esponjas marinhas. Nossos resultados reforçam a troca de larvas entre Brasil e Caribe e fornecem indícios da importância do corredor de esponjas na manutenção da conectividade de invertebrados marinhos entre essas regiões.

Palavras-chave: esponjas marinhas, Caribe, Brasil, ITS, microssatélite

Rio de Janeiro

2017

ABSTRACT

PAST AND PRESENT SCENARIO OF THE WESTERN ATLANTIC SPONGE *CLATHRINA AUREA*

Pedro Victor Leocorny Ferreira

Supervisor: Prof. Dr. Michelle Klautau

Sponges are known for their low dispersal capability and studies on connectivity have shown high genetic structuration among populations. However, some species present wide distribution and their dispersal dynamics is still not known. Therefore, the present work aims to determine the structure level and dispersion of populations of *Clathrina aurea* along the Western Atlantic. Using seven loci of microsatellites, we found a strong similarity between Brazil and Caribbean not recovered in structure analyses using ITS marker. This results support the strong influence of the Amazon River shaping the dynamic of connectivity of among populations through time. Gene flow analyses reveal higher similarity between populations of Antigua and Bequia with Abrolhos region, supporting the biogeographical homogeneity through the Caribbean and the Northeast region of Brazil. Migrant analyses show a dispersal capability for *C. aurea* much higher than what is expected to marine sponges. Our results reinforce larval exchange between Brazil and Caribbean and give insights of the importance of the sponge corridor on the maintenance of connectiveness of marine invertebrate at these regions.

Keywords: Marine sponges, connectivity, Caribbean, Brazil, Calcarea, ITS, microsatellite.

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LISTA DE ABREVIAÇÕES

AIC: critério de informação Akaike;

BI: *Bayesian Inference*;

$CaCO_3$: carbonato de cálcio;

CAPES: Comissão de Aperfeiçoamento de Pessoal de Ensino Superior;

CNPq: Conselho Nacional de Pesquisa e Desenvolvimento;

COI: citocromo oxidade I;

DNA: ácido desoxirribonucleico;

DNAr: ácido desoxirribonucleico ribossomal;

HCA: hierarchical clustering analyses;

HWE: equilíbrio de Hardy-Weinberg;

ITS: internal transcribed spacer;

LD: desequilíbrio de ligação;

LSU: large subunit ribossomal;

MAFFT: *multiple alignment using fast Fourier transform;*

MCMC: Markov chain Monte Carlo;

ML: Maximum Likelihood;

mtDNA: ácido desoxirribonucleico mitocondrial;

PACOTILLES: *Patterns of Diversity in the Lesser Antilles*;

PC: componente principal;

PCA: análise de components princiapis;

PCR: *polymerase chain reaction;*

SCUBA: *self-contained underwater breathing apparatus;*

SSR: *simple sequence repeats;*

SSU: small subunit ribosomal;

STR; *short tandem repeats;*

VNTR: *variable number of tandem repeats;*

SUMÁRIO

1. INTRODRUÇÃO

1.1. FILO PORIFERA GRANT, 1836

Esponjas (filo Porifera) são os animais mais antigos ainda viventes com origem datada para cerca de 600 milhões de anos atrás (Finks, 1970; Li *et al.*, 1998). São encontradas em quase todos os ambientes aquáticos, inclusive dulciaquícolas, sob as mais diversas condições e nas mais diversas profundidades, latitudes e longitudes.

Os poríferos são sésseis e vivem basicamente de um processo de filtração da água. A partir desse processo, absorvem oxigênio e retêm desde matéria orgânica dissolvida até bactérias e fitoplâncton como forma de alimento (Schmidt 1970; Frost, 1987; Leys & Eerkes-Medrano, 2006). A filtração é um processo ativo – ou mesmo passivo (Leys *et al.*, 2011) – contínuo e unidirecional, onde células flageladas (coanócitos) são responsáveis por promover um fluxo de água que percorre uma série de canais internos presentes no corpo desses animais até chegar a uma ou mais aberturas de saída, chamadas de ósculos (Fig. 1.a; Hentschel *et al.*, 2012). Esse sistema de filtragem da água é o sistema aquífero, que pode ser classificado em cinco diferentes tipos de acordo com a sua organização: asconóide, siconóide, leuconóide, sileibide e solenóide (Fig. 2; Cavalcanti & Klautau, 2011).

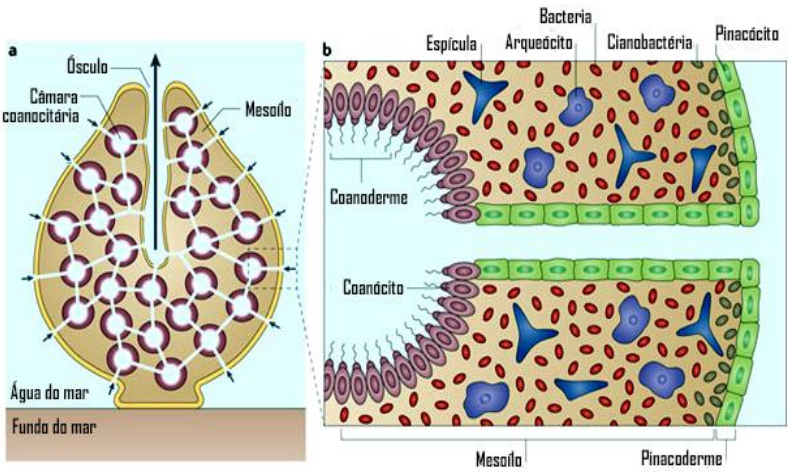

Fig. 1. Visão geral do corpo de uma esponja. **a.** Modelo esquemático mostrando o caminho do fluxo d'água através do corpo em um sistema aquífero leuconóide. **b.** Ampliação esquemática de estruturas internas (modificado de Hentschel *et al.*, 2012).

O corpo das esponjas é relativamente simples e basicamente sustentado por uma matriz extracelular colagenosa, chamada de mesoílo, que abriga não só inúmeros tipos celulares, mas também fibras protéicas (espongina) e elementos inorgânicos (espículas) (Fig. 1.b; Garrone, 1969; Uriz, 2006; Hentschel *et al.*, 2012).

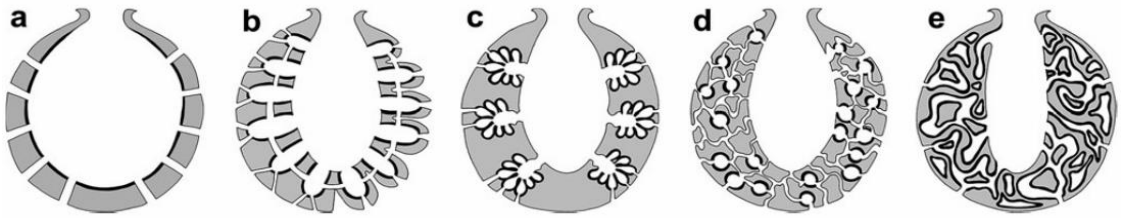

Fig. 2. Representação dos cinco tipos de sistemas aquíferos: **a.** Asconóide; **b.** Siconóide; **c.** Sileibide; **d.** Leuconóide; **e.** Solenóide. Linhas pretas mais espessas representam a coanoderme (retirado de Cavalcanti & Klautau, 2011).

As esponjas são os principais constituintes da fauna bentônica nos mais diversos ambientes marinhos (Hooper & Lévi, 1994) e possuem uma gama de papéis ecológicos. Por exemplo, algumas espécies de esponjas são capazes de promover a consolidação do substrato, facilitando a fixação e o crescimento de muitos organismos; outras espécies são capazes de promover bioerosão em estruturas coralíneas, solubilizando carbonato de cálcio ($CaCO_3$) (*e.g*. Blissett *et al.*, 2006; Carballo *et al.*, 2007; Holmes *et al.*, 2009). Espécies em associação com cianobactérias são consideradas importantes produtoras primárias (Wilkinson, 1983); outras estão associadas a bactérias fixadoras de nitrogênio e possuem importante papel no ciclo e liberação do mesmo na coluna d'água (Wilkinson & Fay, 1979; Diaz & Ward, 1998). Recentemente, de Goeij e colaboradores (2013) mostraram o papel dos poríferos na transformação de matéria orgânica dissolvida em

matéria orgânica particulada, capaz de ser metabolizada por organismos de níveis tróficos superiores, conectando a fauna bento-pelágica e auxiliando na manutenção do ecossistema no qual estão inseridas.

Quanto ao uso de esponjas pela civilização humana, o mesmo tem sido reportado desde a Grécia antiga (Voultsiadou, 2007), embora registros ainda mais antigos indiquem seu uso por civilizações egípcias e fenícias (Chiavarà, 1920). Relatos incluem desde o uso dos poríferos como *snorkel* para mergulho livre (Aristóteles, 350 A.C. a, b), até o uso de suas espículas na confecção de objetos de cerâmica por índios neotropicais (Machado, 1947). Atualmente, possuem um importante papel na saúde humana, pois são fontes ricas de compostos químicos e novos medicamentos com atividades antineoplásicas, antinflamatórias, antimunogênicas e antivirais (e.g. Buchanan & Hess, 1980; McConnell *et al.*, 1994). São também importantes na área de preservação ambiental, onde têm sido estudadas como organismos indicadores da qualidade da água (Selvin *et al.*, 2009) e como potenciais organismos para biorremediação (Milanese *et al.*, 2003; Santos-Gandelman *et al.*, 2014). Na área de engenharia de biomateriais, a organização de seu esqueleto surge como fonte de inspiração e investigação no desenvolvimento de compósitos biomiméticos, arcabouços e moldes (Ehrlich *et al.*, 2007; 2010). Um exemplo é o uso de esponjas (espículas de sílica) como modelo para melhoramento da condução de luz em fibras ópticas (Sundar *et al.*, 2003; Müller *et al.*, 2009).

Todos estes estudos, porém, são possíveis somente devido a esforços para identificação e documentação das espécies existentes ao longo do globo. Atualmente, existem pouco mais de 8.600 espécies válidas para o filo Porifera (van Soest *et al.*, 2017), mas estima-se que possam existir muito mais espécies (van Soeste *et al.*, 2012). Sistematicamente, essas espécies estão divididas em quatro classes distintas: Demospongiae, Hexactinellida, Homoscleromorpha e Calcarea (Fig. 3).

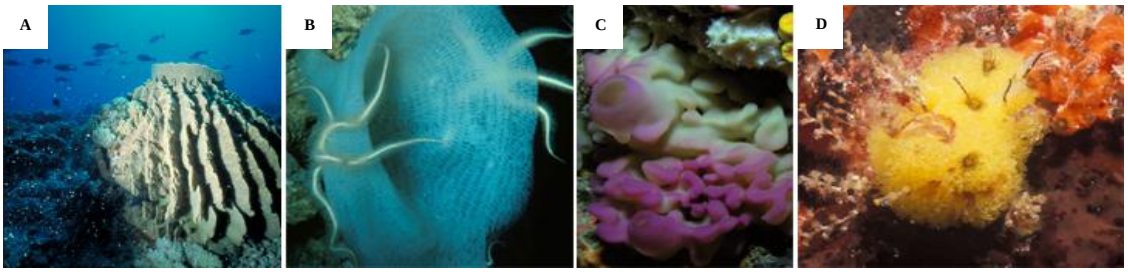

Fig. 3. Representantes das quatro classes do filo Porifera. **A.** Demospongiae (*Xestospongia testudinaria*) em Lesser Sunda Islands, Indonésia. Foto de R. Roozendaal (modificado de van Soest *et al.*, 2012.); **B.** Hexactinellida (*Lefroyella decora*) nas Bahamas (modificado de van Soest *et al.*, 2012.); **C.** Homoscleromorpha (*Oscarella lobularis*) no noroeste do Mediterrâneo (modificado de Boury-Esnault *et al.*, 2013); **D.** Calcarea (*Clathrina aurea*) na Urca do Tubarão, Rio grande do Norte, Brasil (modificado de Lanna *et al.*, 2009).

1.2. CLASSE CALCAREA BOWERBANK, 1864

As esponjas da classe Calcarea são as únicas que possuem seu esqueleto inteiramente formado por calcita (carbonato de cálcio), o que constitui a principal sinapomorfia do grupo. É, também, a única classe dentro do filo onde os cinco tipos de sistemas aquíferos podem ser encontrados. Comumente, são esponjas de tamanho muito pequeno e sem cor, o que, somado ao fato de colonizarem preferencialmente habitats crípticos, como por exemplo cavernas e fendas, faz com que passem despercebidas aos olhos de biólogos e até mesmo esponjólogos em campo. São conhecidas por serem organismos de difícil classificação taxonômica (Manuel *et al.*, 2002) e os caracteres mais importantes utilizados na sua sistemática morfológica são a organização do sistema aquífero, organização do esqueleto e a composição, forma e tamanho de suas espículas. Cerca de 680 espécies foram descritas até hoje (van Soest *et al.*, 2012), divididas em duas subclasses: Calcinea, composta pelas ordens Clathrinida e Murrayonida (10 famílias e 23 gêneros), possuem espículas essencialmente regulares (equiangulares e equirradiadas) e

o núcleo de seus coanócitos é basal e esférico; e Calcaronea, com as ordens Leucosolenida, Lithonida e Baerida (14 famílias e 31 gêneros), apresentam espículas irregulares e o núcleo de seus coanócitos é apical (Fig. 4; Manuel *et al.*, 2002).

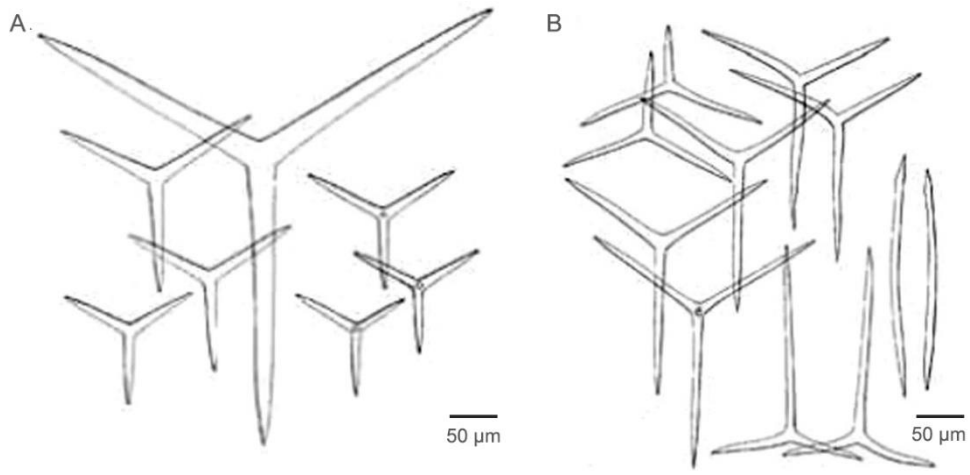

Figura 4. Exemplos de tipos espiculares encontrados nas subclasses (A) Calcinea e (B) Calcaronea (modificado de Manuel *et al.*, 2002).

1.3. CONECTIVIDADE E FERRAMENTAS MOLECULARES

Conectividade, ou a troca de indivíduos entre populações marinhas, é um tópico central em ecologia e genética marinha. O ambiente fluido no qual as populações se encontram oferece uma gama de diferentes maneiras para dispersão de indivíduos e a extensão do sucesso dessa dispersão é um fator determinante na dinâmica das populações, mas pouco compreendido para a maioria das espécies (Cowen *et al.*, 2000). Quando a dispersão é combinada com fatores que levam à dispersão de organismos e à troca de material genético, o conceito de conectividade entre populações emerge (Hellberg *et al.*, 2002). Para espécies bentônicas, a fase larval costuma ser a fase dominante de dispersão e, portanto, foco importante nos estudos de conectividade em ambientes marinhos.

Esponjas não possuem gônadas: uma característica intrínseca de sua gametogênese é a origem de seus gametas a partir da transformação direta de células somáticas (Ereskovsky, 2010). As espécies podem ser tanto hermafroditas quanto gonocóricas e, em sua grande maioria, apresentam desenvolvimento indireto com larvas planctônicas de natação livre. Além disso, existem espécies que podem ser ovíparas, onde a fertilização e o desenvolvimento do embrião é feito externamente, ou vivíparas, onde a fertilização é interna e o embrião é incubado antes de ser liberado como larva (Maldonado & Riesgo, 2008). Suas larvas são conhecidas por serem lecitotróficas; ou seja, se alimentam do próprio vitelo armazenado, resultando em um curto tempo de duração na coluna d'água e a uma baixa capacidade de dispersão que, associada ao seu comportamento filopátrico, revela populações com altos índices de endocruzamento (Maldonado & Berquist, 2002; Maldonado, 2006).

Dado o vasto tamanho dos oceanos e o pequeno tamanho dos propágulos marinhos, determinar se esses propágulos assentam longe de seu local de origem ou próximo aos seus parentais não é uma tarefa trivial. Contudo, migrantes que tiveram sucesso em sua dispersão acabam deixando rastros genéticos que podem ser traçados de maneira indireta. Desta forma, análises moleculares surgem como importantes ferramentas capazes de caracterizar padrões de conectividade e escalas de estruturação genética em ambientes ainda opacos à observação direta de dispersão (Selkoe *et al.*, 2008). Os primeiros estudos com esponjas marinhas da classe Calcarea foram realizados por Solé-Cava *et al.* (1991), evidenciando especiação críptica de populações alopátricas dentro do gênero *Clathrina* com o uso da técnica de eletroforese de isoenzimas. A partir de então, uma série de trabalhos subsequentes foram realizados utilizando esta mesma técnica (*e.g*. Klautau *et al.*, 1994; Klautau & Borojevic, 2001). Porém, com o advento de novas tecnologias, principalmente com a rápida determinação de sequências de DNA pelo

sequenciamento de Sanger (Sanger *et al.*, 1977), fragmentos de DNA ribossomal (DNAr) passaram a ser utilizados em inferências filogenéticas e filogeográficas. O DNAr é formado por repetições de agrupamentos gênicos em tandem, onde subunidades codificantes (SSU, 5.8S e LSU) alternam outras não codificantes (ITS1 e ITS2), chamadas de espaçadores (Long & Dawid, 1980) (Fig 5).

Fig 5. Modelo esquemático do DNAr com as subunidades SSU, 5.8s, LSU e os espaçadores ITS1 e ITS2.

Essas diferentes subunidades dentro do DNAr são heterogêneas quanto a sua taxa de substituição e, portanto, podem refletir relações filogenéticas em diferentes níveis sistemáticos. Por exemplo, o gene SSU é comumente usado em reconstruções ancestrais à nível de filo e classe (*e.g*. Cavalier-Smith *et al.* 1996; Borchiellini *et al.*, 2001), enquanto o gene LSU pode ser usado na resolução de diferentes níveis – especialmente famílias e gêneros – por apresentar taxas de substituição menos conservadas (*e.g*. Medina *et al.*, 2001). Quanto ao gene 5.8S, é raramente utilizado devido à sua curta sequência de nucleotídeos, sendo comumente associado às regiões ITS em estudos filogenéticos (Hillis & Dixon, 1991). Os espaçadores ITS1 e ITS2 possuem taxas evolutivas relativamente rápidas quando comparados com os genes SSU e LSU e, por isso, são amplamente utilizados em estudos filogenéticos e filogeográficos para diversos grupos, desde plantas (*e.g*. Coleman, 2003) até metazoários como nemátodas, insetos e crustáceos (*e.g*. Chu *et al.*, 2001; Hugall *et al.*, 1999; Weekers *et al.*, 2001), sendo os marcadores mais frequentes em estudos de evolução intra e interespecífica de corais e esponjas (van Oppen *et al.*, 2002b).

Microssatélites, também encontrados na literatura como SSR (*Simple Sequence Repeats*), VNTR (*Variable Number of Tandem Repeats*) ou STR (*Short Tandem Repeats*),

são repetições de um até seis pares de base, passíveis de serem encontrados ao longo do genoma de todos os seres vivos. São sequências de DNA neutras – que não estão sob seleção natural – e altamente variáveis, cujo polimorfismo é caracterizado pelo número de repetições dos motivos; logo, resultando em uma variação de tamanho que pode ser observada em géis eletroforéticos de alta resolução (Selkoe & Toonen, 2006). Além disso, a variação genética nos vários *loci* de microssatélites é caracterizada pela alta taxa mutacional, o que resulta em uma alta heterozigosidade e múltiplos alelos por *locus*, necessários para estudos de genética de populações. Até o presente momento, microssatélites foram desenvolvidos para apenas 11 espécies de esponjas: *Halichondria panicea* (Pallas, 1766) (Knowlton *et al.*, 2003), *Crambe crambe* (Schmidt, 1862) (Duran *et al.*, 2004), *Scopalina lophyropoda* Schmidt, 1862 (Blanquer *et al.*, 2005), *Xestospongia muta* (Schmidt, 1870) (Richards, 2010), *Spongia officinalis* Linnaeus, 1759 (Dailianis *et al.*, 2011), *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004 (Guardiola *et al.*, 2012), *Stylissa carteri* (Dendy, 1889) (Giles *et al.*, 2013), *Clathrina aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991 (Padua *et al.*, 2013), *Cliona delitrix* Pang, 1973 (Chaves-Fonnegra *et al.*, 2015), *Petrosia* (*Petrosia*) *ficiformis* (Poiret, 1789) (Taboada *et al.*, 2015) e *Poecillastra laminaris* (Sollas, 1886) (Zeng *et al.*, 2016).

Diferentes marcadores moleculares podem revelar histórias evolutivas diferentes de acordo com a sua natureza. Por exemplo, marcadores neutros nos mostram o papel do fluxo gênico e deriva gênica moldando as populações, enquanto marcadores sobre seleção natural nos informam sobre processos adaptativos locais (Avise, 2004). O mesmo acontece com marcadores que possuem taxas evolutivas diferentes: marcadores como o ITS – que possuem uma taxa mutacional mais lenta – são capazes de nos oferecer introspecções referentes a um cenário histórico das populações estudadas (Avise 2000), enquanto marcadores como microssatélites – que possuem uma rápida taxa mutacional –

nos revelam um cenário atual da estruturação de populações em fina escala e eventos demográficos recentes (Hewitt, 2004).

CLATHRINA AUREA SOLÉ-CAVA, KLAUTAU, BOURY-ESNAULT, BOROJEVIC & THORPE, 1991

Clathrina aurea é uma esponja amarela com sistema aquífero asconóide e um corpo de anastomose frouxa e irregular (Fig 6). Seu esqueleto é desorganizado e composto apenas por triactinas com actinas cilíndricas – às vezes, levemente onduladas – e pontas arredondadas. A espécie é comumente encontrada em águas rasas e ambientes protegidos da luz, como tocas, fendas e cavernas (Klautau & Borojevic, 2001). A espécie possui uma ampla distribuição ao longo da costa brasileira, com registro para diversos estados: Rio Grande do Norte (Muricy *et al.*, 2008; Lanna *et al.*, 2009), Pernambuco (Muricy & Moraes 1998; Santos *et al.*, 2002; Moraes *et al.*, 2006; Muricy & Hajdu, 2006), Bahia (Rossi *et al.*, 2011), Rio de Janeiro (Solé-Cava*et al.*, 1991; Muricy *et al.*, 1993; Klautau *et al.*, 1994; Muricy & Silva, 1999; Klautau & Borojevic, 2001; Klautau & Valentine, 2003; Muricy & Hajdu, 2006; Santos *et al.*, 2010; Padua *et al.*, 2013), São Paulo (Muricy & Hajdu, 2006; Lanna *et al.*, 2007) e Santa Catarina (Padua *et al.*, 2013). *Clathrina aurea* era considerada endêmica da costa do Brasil até ser reportada para a costa do Peru (Azevedo *et al.*, 2015), onde um estudo filogeográfico propõe que essa espécie teria chegado à região por transporte antropogênico (Cóndor-Luján*et al.*, *in prep*). Mais recentemente, coletas realizadas pela expedição PACOTILLES (“Patterns of Diversity in the Lesser Antilles”) durante o ano de 2014 encontraram indivíduos morfologicamente semelhantes a *C. aurea* ao longo das ilhas caribenhas das Pequenas Antilhas.

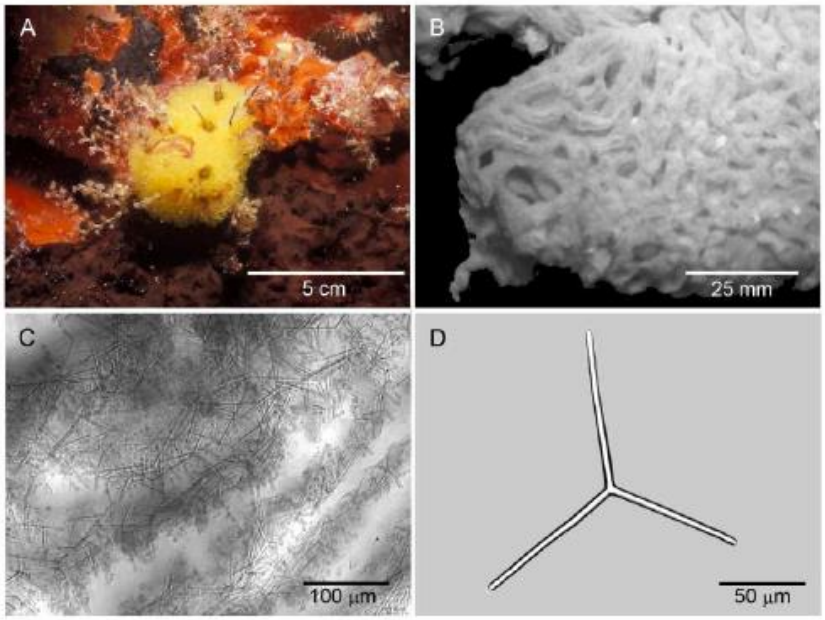

Fig 6. *Clathrina aurea*. (A) Foto *in vivo*; (B) Espécime fixado; (C) Corte tangencial do esqueleto; (D) Triactina (retirado de Lanna*et al.*, 2009).

A ocorrência da espécie nesta região é surpreendente, dada a distância entre o ponto mais ao norte do Brasil para o qual *C. aurea* é conhecida (Bacia Potiguar, Rio Grande do Norte) com as ilhas mais ao sul das Pequenas Antilhas. Além disso, ainda há a presença do Rio Amazonas entre esses locais com uma grande descarga de água doce, já conhecida como barreira à conectividade de diversas espécies marinhas (Vermeij, 1978; Leão, 1986; Sarver *et al.*, 1998; Rocha *et al.*, 2001). Esponjas são organismos sésseis e conhecidos por possuírem baixa capacidade de dispersão (Mariani *et al.*, 2000; Uriz *et al.*, 2008; Ereskovsky, 2010), o que levanta alguns questionamentos sobre o cenário de distribuição de *C. aurea*: Seriam as populações brasileiras e caribenhas co-específicas? Qual o grau de conectividade entre essas populações? Seria o Rio Amazonas uma barreira à conectividade dessas populações? Se há fluxo gênico, como ele é mantido? Qual a capacidade de dispersão da espécie?

Dado o presente panorama, a conectividade entre populações de *C. aurea* será estudada utilizando diferentes marcadores moleculares (ITS e Microssatélites) para responder a essas perguntas.

OBJETIVOS

OBJETIVO GERAL

- Avaliar o grau de conectividade de populações de *Clathrina aurea* ao longo do tempo no Atlântico Oeste;

OBJETIVOS ESPECÍFICOS

- Estimar a diversidade genética de *C. aurea* no Brasil e no Caribe;
- Avaliar o grau de estruturação e o fluxo gênico entre as populações de *C. aurea*;
- Inferir a capacidade de dispersão e a direção do fluxo gênico entre as populações de *C .aurea*;

Past and present scenario of the Western Atlantic sponge *Clathrina aurea*

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Running title: Connectivity of *Clathrina aurea* from the Lesser Antilles

ABSTRACT

Sponges are known for their low dispersal capability and studies on connectivity have shown high genetic structuration among populations. However, some species present wide distribution and their dispersal dynamics is still not known. Therefore, the present work aims to determine the structure level and dispersion of populations of *Clathrina aurea* along the Western Atlantic. Using seven loci of microsatellites, we found a strong similarity between Brazil and Caribbean not recovered in structure analyses using ITS marker. This results support the strong influence of the Amazon River shaping the dynamic of connectivity of among populations through time. Gene flow analyses reveal higher similarity between populations of Antigua and Bequia with Abrolhos region, supporting the biogeographical homogeneity through the Caribbean and the Northeast region of Brazil. Migrant analyses show a dispersal capability for *C. aurea* much higher than what is expected to marine sponges. Our results reinforce larval exchange between Brazil and Caribbean and give insights of the importance of the sponge corridor on the maintenance of connectiveness of marine invertebrate at these regions.

KEYWORDS

Marine sponges, connectivity, Caribbean, Brazil, Calcarea, ITS, microsatellite.

INTRODUCTION

Sponges are the principal components of the Caribbean reefs and constitute a great part of its biomass with a high diversity of species (Díaz & Rützler 2001). They are known

for a vast gamma of important ecological roles, ranging from promoting substrate consolidation to food connection between bentho-pelagic fauna, thereby being the major responsible for changing the ecosystem they are inserted in (Wulff, 2001; Bell, 2008; Goeij *et al*., 2013;). In order to comprehend how sponges are able of changing ecosystems, however, it is first important to better understand their population dynamics by assessing the extent to which populations are connected and their dispersal capabilities (Hellberg 2007; Jones *et al*., 2007). This allows delineation of population boundaries and identification of small genetic clusters, what is vital for conservation priorities (Wulff, 2001; Palumbi, 2004; Jones, 2009).

It has been long hyphotesized that marine populations are capable of maintaining connectivity through a vast geographical range, facing weak barriers to dispersal (Avise, 1998; Palumbi, 2004). However, several evidences have been mounted at increasing rates contradicting this concept of broad larval dispersal and raising prevailing views of closed-extent marine populations (*e.g*., Cowen, 2000; Swearer *et al*., 2002, Jones *et al*., 2005, Almany *et al*., 2007; Cowen *et al*., 2007). Although the phylum Porifera remains poorly studied regarding population connectivity, the few existent studies indicated low dispersal capability of its larvae, strongly correlated to a short lifespam and a philopatric behaviour (Bergquist & Sinclair 1968, 1973; Ilan & Loya 1990; Meroz & Ilan 1995; Lindquist *et al*., 1997; Mariani *et al*., 2000; Maldonado, 2006). This results in high levels of population structure for many widespread species even in historical (phylogeography) and recent (population genetics) time scale analyses (*e.g*. Duran *et al*., 2004; Woerheide *et al*., 2008; López-Legentil & Pawlik. 2009; DeBiasse *et al*., 2010; Becking *et al*., 2013; Chaves-Fonnegra *et al*., 2015; Richards *et al*., 2016 ; Riesgo *et al*. 2016).

Mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA) markers, especially the Cytochrome Oxidase I (COI) and the Internal Transcribed Spacers (ITS), have been used to unravel phylogeographic structure and demographic history of many Demospongiae species (Duran *et al*., 2004; DeBiasse *et al*., 2010; Xavier *et al*., 2010; Becking *et al*., 2013) and some few Calcarea (Woerheide *et al*., 2002; 2008). Due to the nature of these markers, it is possible to estimate the degree of connectivity among populations in a historical (or phylogenetical) scenario, allowing the identification of the past events that shaped them. On the other hand, microsatellite markers present high mutation rates and, consequently, they are useful for population genetics studies as they reflect a recent (or ecological) scenario of population connectivity. Up to date, microsatellite loci have been

developed for nine Demospongiae – *Halichondria panicea* (Pallas, 1766) (Knowlton *et al*., 2003), *Crambe crambe* (Schmidt, 1862) (Duran *et al*., 2004), *Scopalina lophyropoda* Schmidt, 1862 (Blanquer *et al*., 2005), *Xestospongia muta* (Schmidt, 1870) (Richards, 2010), *Spongia officinalis* Linnaeus, 1759 (Dailianis *et al*., 2011), *Stylissa carteri* (Dendy, 1889) (Giles *et al.*, 2013), *Cliona delitrix* Pang, 1973 (Chaves-Fonnegra *et al*., 2015), *Petrosia* (*Petrosia*) *ficiformis* (Poiret, 1789) (Taboada *et al.*, 2015) and *Poecillastra laminaris* (Sollas, 1886) (Zeng *et al.*, 2016) – and two Calcarea species – *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004 (Guardiola *et al*., 2012) and *Clathrina aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991 (Padua *et al*., 2013).

Clathrina aurea Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991 is a yellow, calcareous sponge commonly found in light protected environments and widely distributed along the Brazilian coast (over a distance of about 3,500 km) (Muricy *et al*., 2006; Lanna *et al*., 2009). Azevedo *et al*. (2015) reported this species for the Peruvian coast and phylogeographical studies suggest its introduction due to anthropogenic transport (Cóndor-Luján *et al*., *in prep*). More recently, *C. aurea* was also found in the Lesser Antilles. This wide distribution is surprising, considering the low capability of dispersal known for other sponge species. Therefore, our aims were to evaluate the degree of connectivity between Brazilian and Caribbean populations of *C. aurea* in different time scale analyses and infer the dispersal capability of this species.

MATERIALS AND METHODS

Sample collection

Caribbean specimens were sampled by SCUBA diving in different sites along the Lesser Antilles during the PACOTILLES ("PAtterns of diversity and COnnectivity in Lesser AnTILLES") expedition (Figure 7). A total of 235 specimens were analyzed and 92 were identified as *Clathrina aurea*. In regard to representatives of the Brazilian populations, DNA sequences and genotypes were obtained from Genbank database or provided by Padua *et al*. (*in press*).

Specimens identification

For taxonomic identification were used morphological and molecular approaches. For morphology, slides of dissociated spicules and skeletal sections were made following

standard protocols (Woerheide & Hooper, 1999; Klautau & Valentine, 2003). Tangential sections of cortical and atrial skeletons were also made and spicules were measured at the base of each actine (width) and from tip to base (length) using a light microscope with an ocular micrometer.

DNA Extraction, alignment and phylogeny

For molecular analyses, DNA was extracted from ethanol-preserved specimens using a QIAamp® DNA MiniKit (QIAGEN). The Internal Transcribed Spacer (ITS) region of the nuclear DNA was amplified by polymerase chain reaction (PCR) using the following primers: fwd 5'-TCATTTAGAGGAAGTAAAAGTCG-3' and rv 5'-GTTAGTTTCTTTTCCTCCGCTT-3' (Lôbo-Hajdu *et al*., 2004). PCR mixes per sample contained 5.55 µL Milli-Q water, 3.0 µL 5x Green GoTaq® Flexi Buffer (PROMEGA), 0.75 µL bovine serum albumin (from 10 mg/mL solution), 1.5 µL dNTPs (from 2 mM solution), 0.5 µL of each primer (from 10 µM solution), 1.5 µL $MgCl_2$ (from 25 mM solution) and 0.2 µL GoTaq® Flexi Polymerase (PROMEGA; from 5U solution). Reaction steps included 5 min at 95 º C, 35 cycles of 1 min at 50 º C, and 1 min at 72 º C, followed by 5 min at 72 º C. Purified PCR products were then sequenced (forward and reverse strands) with BigDye Terminator v3.1 in an ABI 3500 sequencer (Applied Biosystems). Sequences were edited using the software Chromas 2.6. and BLAST searches (http://www.ncbi.nlm.nih.gov/blast) were conducted to confirm their biological source.

The final alignment was conducted using the MAFFT online software (Katoh & Standley, 2013) using an auto strategy method with score matrix 200 PAM/k = 2, gap opening penalty = 1.53 and offset value = 0. A maximum likelihood (ML) tree was generated using the software MEGA 6 (Tamura *et al*., 2013) and a Bayesian (BI) tree was computed using the software MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The best-fit model of nucleotide substitution for our dataset was chosen by the software jModelTest 2.0 (Guindon & Gascuel, 2003; Darriba *et al*., 2012) and was the GTR+G, as predicted by the Akaike information criteria (AIC). ML analysis was performed with pairwise deletion, bootstrap with 1000 pseudo-replicates and a subtree-pruning-regrafting (SPR) heuristic tree search. The Bayesian Metropolis-coupled Markov chain Monte Carlo estimation of phylogeny was performed with 1 million simulations in two independent runs, each run consisting of four chains and sampling

every 100 generations. BI data was checked using the software Tracer v.1.5 (Rambaut & Drummond, 2014).

Past timescale analyses (ITS): haplotype network and structure inference

Number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and neutrality tests were calculated in DnaSP (Librado & Rozas, 2009). Haplotype network was constructed in NETWORK 4.6 (fluxus-engineering.com) using a median-joining algorithm (Bandelt *et al*., 1999). Levels of genetic structure among subpopulations were characterized using Φst in ARLEQUIN 3.5 (Excoffier & Lischer, 2010) and values were plotted graphically on a heatmap constructed under R software (The R Foundation for Statistical Computing, 2005). Genetic divergence (p distance) was calculated using the software MEGA 6 and hierarchical clustering analyses (HCA) were conducted with these values under R software. Mantel test was carried out using the R package "ade4" (Dray & Dufour, 2007). Differentiation between populations was assessed by Bayesian inference, using the GENELAND software (Guillot *et al*., 2005a; Guillot *et al*., 2005b; Guillot *et al*., 2008; Guillot, 2008; Guillot & Santos, 2010; Guedj & Guillot, 2011) under spatial and uncorrelated allele frequency model with one independent Markov Chain running for 1,000,000 MCMC iterations with sampling increment of 100.

Present timescale analyses (Microsatellites): population structure assessment

Seven loci of microsatellites (Cau_A7, Cau_B2, Cau_C2, Cau_D1, Cau_D8, Cau_E6 and Cau_G3) developed by Padua *et al*. (2013) were used. PCR mixes per sample contained 2.9 µL Milli-Q water, 2.0 µL 5x Green GoTaq® Flexi Buffer (PROMEGA), 0.5 µL bovine serum albumin (from 10 mg/mL solution), 1.0 µL dNTPs (from 2 mM solution), 0.2 µL of forward primer (from 10 µM solution), 0.8 µL of reverse primer (from 10 µM solution), 1.0 µL $MgCl_2$ (from 25 mM solution) and 0.2 µL GoTaq® Flexi Polymerase (PROMEGA; from 5U solution). Reaction steps included four stages: (1) 3 min at 95 º C; (2) 5 cycles of 30 secs at 95 º C and 30 secs at 54 º C, followed by 45 secs at 72 º C; (3) 35 cycles of 30 secs at 92 º C and 30 secs at 54 º C, followed by 55 secs at 72 º C; and (4) 20 min at 72 º C. PCR products were genotyped with GeneScan™ 600 LIZ® dye in ABI 3500 (Applied Biosystems) and determined using the software GeneMapper®. The package GenAlEx (Peakall & Smouse, 2012) was used to assess allelic frequencies, values of observed and expected heterozygosity and to test for Hardy-Weinberg (HWE). Linkage disequilibrium (LD) between pairs of locus and computation of inbreeding

coefficient (F_{IS}) were conducted in FSTAT 2.9.3.2 (Goudet, 1995). FREENA software (Chapuis & Estoup, 2007) was used to estimate the frequency of null alleles and to calculate F_{ST} values corrected with ENA method for any positive bias introduced by the presence of null alleles (1000 iterations). Genetic structuration was assessed by distance methods – HCA and Principal Component Analyses (PCA) – and also by Bayesian inferences – using the software STRUCTURE (Pritchard *et al*., 2000) and GENELAND.

PCA analyses were performed on a matrix of Cavalli-Sforza and Edwards (1967) genetic distances using INA correction method (Chapuis & Estoup, 2007), where eigenvalues and eigenvectors of the sample covariance matrix were computed by applying the "prcomp" function of the R statistical package.

In STRUCTURE analyses, the number of genetic clusters (K) was estimated under partitioning (K = 1–12) and then calculated *ad hoc* statistically by mean values of Ln probability. Runs were performed using 10 independent Markov chains for each value of K, and consisted of 1,000,000 Markov chain Monte Carlo (MCMC) iterations (including 500,000 MCMC steps burn-in). *A priori* analyses were conducted under admixture model and *a posteriori* analyses with ancestry model LOCPRIOR were also performed under admixture (Hubisz *et al*., 2009). STRUCTURE HARVESTER (Earl & von Holdt, 2011) was used to estimate Ln probabilities and permutations of clusters were conducted under CLUMPP program (Jakobsson & Rosenberg, 2007). Visualization of CLUMPP clustering outcomes was performed using DISTRUCT 1.1 (Rosenberg, 2004).

GENELAND analyses were conducted incorporating spatial coordinates, uncorrelated allele frequency and using null allele model – the latter used to correct for any upward bias on the estimation of K due to the presence of null alleles (Guillot *et al*., 2008). Four independent Markov Chain were run for 1,000,000 MCMC iterations with sampling increment of 100.

Past and contemporary gene flow estimation

In order to assess the extent of dispersal between *C. aurea* populations, as defined by the clustering inferences, we used different approaches depending on the considered timescale.

For ITS marker, migration rates and asymmetry in gene flow were estimated in coalescent-based approach implemented in MIGRATE-n (Beerli & Felsenstein, 2001;

Beerli, 2006). A Bayesian inference was used to estimate the parameter $M(M = m/u$, where m is the migration rate, and u is the mutation rate) which describes the mutation-scaled long-term migration rate between populations, and to estimate θ ($\theta = 4N_e u$) as the mutation-scaled effective population size. Analyses were inferred under DNA sequenced model and search parameters of Metropolis sampling Markov chains consisted of 1 long chain with a static heating scheme (1.0, 1.5, 3.0 and 1,000,000.0), an increment of 100, 50,000 recorded steps and burn-in of 10,000. Starting values of M and θ were randomly generated from uniform distributions.

For Microsatellite markers, migration of individuals between each population were assessed by two different methods: (1) using GENECLASS 2 (Piry *et al*., 2004) to identify first-generation migrants using the criteria of Rannala and Mountain (1997) and simulated likelihood inferred by re-sampling procedure of Paetkau *et al.* (2004; 1,000 simulated individuals), with a threshold (p-value) of 0.01; and (2) using MIGRATE-n with a Brownian microsatellite model and search parameters of Slice sampling Markov chains consisting of 1 long chain with static heating scheme (1.0, 1.5, 3.0 and 1,000,000.0), an increment of 100, 500,000 recorded steps, burn-in of 50,000 and starting values of M and θ randomly generated from uniform distributions.

RESULTS

Specimens identification

From the 235 collected specimens, 92 were identified as *Clathrina aurea* by morphological evaluation. Identifications were corroborated by molecular analyses: final alignment was conducted with 731 sites and all specimens analyzed nested within the *Clathrina aurea* clade with high posterior probability values, as shown in the phylogenetic tree (Figure 8). Moreover, the genetic distance of *Clathrina aurea* (0–0.5%) felt in the intraspecific range of other *Clathrina* species (0–2%), also confirming that Brazilian and Caribbean populations of this species are conspecific (Table 1).

Historical timescale structuration

In total, 151 sequences were studied among 11 different localities. High number of haplotypes and overall haplotype diversity (h = 21 and Hd = 0,7975, respectively) were found, with a total of 18 variable and nine singleton sites. Haplotype diversity (Hd) within sampling locations ranged from 0.000 (Bequia, Guadeloupe and Mayreau) to 0.802

(Ilhabela) and nucleotide diversity (π) ranged from 0.00000 (Bequia, Guadeloupe and Mayreau) to 0.00274 (Ilhabela). Neutrality tests were non-significant for all populations (Table 2). Mantel test showed no significant correlation between geographic and genetic distances (r = 0.09483; p = 0.6826).

The haplotype network was congruent in its topology with the phylogeny estimated by the ML tree, having showed three main haplotypes that allowed a division into three groups (Figure 9). The most common haplotype (H1) was found in the pink group, which had a total of eigth different haplotypes. That group reunites only specimens from the Caribbean Sea. The blue group is centred around the second most frequent haplotype (H10) and it contains sequences from all the Brazilian localities. The green group is composed also only of Brazilian sequences, however, it includes mainly specimens from Arvoredo – South of Brazil – and some from Cagarras and Ilhabela (Southeast of Brazil). No haplotypes were shared between the Brazilian and the Caribbean sampling localities.

For clustering analyses, GENELAND results suggest the presence of the same three distinct genetic clusters also visually delimited in the haplotype network: (i) Arvoredo; (ii) Brazil, except for Arvoredo; and (iii) Caribbean. Estimation of Φ_{st} values are in accordance with clustering results found so far, showing no structuration within the Caribbean but high levels of structuration in the Brazilian coast. High pairwise values were found when comparing Arvoredo with other Brazilian localities and when comparing Brazilian and Caribbean localities (Figure 10a). Hierarchical clustering analyses

Recent timescale structuration

All the three *loci* amplified showed high levels of polymorphism with an overall number of alleles ranging from 24 (Cau_D1) to 48 (Cau_D8). Private alleles were found in all localities, except for Mayreau (Table 3). The three analysed loci showed no LD. Inbreeding coefficient (F_{IS}) values per population were non-significant after Bonferroni correction for most localities, suggesting deficit of heterozygosites (Table 4). This was confirmed by deviation of HWE in most localities. Moreover, analysis with Microchecker suggested the possible presence of null alleles within all the three loci. Mantel test also showed no significant correlation between geographic and genetic distances (r = 0.1041; p = 0.3146).

Average values for F_{st} with ENA correction method within each locus ranged from 0.087 to 0.159 and showed almost no difference when compared to standard F_{st} ones. The Brazillian localities (Arvoredo, Cabo Frio, Cagarras and Ilhabela) show high genetically differences from the Caribbean ones (Antigua, Guadeloupe, Martinique, Mayreau, Saint Martin and Saint Vincent); however, Abrolhos was more similar to the Caribbean than with Brazilian localities, being close related to Bequia and Guadeloupe (Figure 10b). Futhermore, within Brazil, Arvoredo showed the highest divergence, whereas among the Caribbean, Saint Martin was the most genetically divergent locality.

The PCA analyses were conducted with Cavalli-Sforza & Edwards (1967) genetic distances for each pair of populations, suggesting six genetic clusters in the total data set: (i) Guadeloupe; (ii) Martinique; (iii) Abrolhos, Bequia and Saint Vincent; (iv) Mayreau and Saint Martin; (v) Arvoredo; and (vi) Cabo Frio, Cagarras and Ilhabela (Figure 12). PC1 explained 44.9% of the variation found in the pairwise distance matrix, whereas PC2 and PC3 explained 12.8% and 8.7%, respectively. PC1 and PC2 were able to separate the data into two main clusters: Brazil and Caribbean. PC3, however, did not separate these two main groups, but the genetic clusters within Brazil and Caribbean were recovered.

Regarding analyses with the software STRUCTURE, membership coefficients (Q) were estimated for different values of K in *a priori* analyses (Figure 13). The analyses recovered two main clusters for all values of K: (i) Brazil; and (ii) Caribbean. Substructuration within these clusters showed better resolution in the *a posteriori* analyses – especially regarding the Caribbean group – considering the most probable value of K (K = 5), as showed by Ln probabilities (Figure 13d): (i) Arvoredo; (ii) Cabo Frio, Cagarras and Ilhabela; (iii) Mayreau and Saint Martin; (iv) Guadeloupe and Martinique; and (v) Antigua, Bequia and Abrolhos. Corroborating F_{st} and PCA analyses, individuals from Abrolhos showed consistent membership to the Caribbean cluster (v) and the structuration of the Brazilian south region (Arvoredo) was also recovered.

Bayesian inference using the software GENELAND incorporating spatial model showed a more conservative result, indicating structuration of individuals into three clusters: (i) Caribbean; (ii) Brazil, except for Arvoredo; and (iii) Arvoredo – the same populations found using ITS marker, but clustering individuals from Abrolhos within the Caribbean.

Historical Dispersal Pattern

MIGRATE-n indicated very low flow of individuals between the population clusters estimated by GENELAND ($N_eM \leq 10$; Figure 14a) with the highest flow of effective migrants per generation (N_eM =12.0) found between clusters (i) and (ii).

Recent Dispersal Pattern

With a threshold (p-value) of 0.01, 14 individuals within the data set were identified as first-generation migrants from Abrolhos (n = 1), Bequia (n = 1), Cabo Frio (n = 1), Cagarras (n = 1), Guadeloupe (n = 2), Ilhabela (n = 1), Martinique (n = 2), Saint Martin (n = 4), and Saint Vincent (n = 1). Only a single individual was found to have migrated from Brazil (Abrolhos) to the Caribbean (Saint Martin), with all the other migrations being within Brazil and within the Caribbean.

Migration rates inferred by MIGRATE-n indicated low flow of individuals between population clusters estimated by the STRUCTURE (Figure 14b). Nonetheless, highest flow of effective migrants per generation (N_eM =33.4) was found between clusters (iii) and (v), reinforcing the connection between Brazil (Abrolhos) and the Caribbean (Mayreau and Saint Martin).

DISCUSSION

Our results reveal the large influence of the Amazon River shaping the dynamic of populations of *Clathrina aurea* along the Western Atlantic. Ecological timescale analyses show a strong connectivity between Brazilian and Caribbean populations but, in the past scenario for our model species, these two populations are structured with almost no effective migrants between them. As proposed by Rocha (2003) for reef fishes, the Amazon River might be a permeable barrier to connectiveness due to oscillations on the sea-level and this seems the best hypotheses to explain the pattern found in the present study. Our microsatellite data reflect a present time of interglacial interval (high sea levels) where the deep outer shelf in the Amazon has low sedimentation and normal salinity, allowing the genetic maintenance between Brazilian and Caribbean populations of *C. aurea*. On the other hand, ITS data reflect a past period of glacial maxima (low sea levels) where high sedimentation, high salinity and reduction of the continental shelf break, combined with the discharge of the plume, reinforces the river as a barrier and explains the genetic split found between these two populations. The Amazon River is a well-known barrier in the formation of sister species of different reef fishes in the Western Atlantic (*e.g. Lythrypnus mowbrayi* and *L. brasiliensis* (Greenfield, 1988); *Thalassoma*

bifasciatum and *T. noronhanum* (Rocha *et al*., 2001)) and known as the responsible for the formation of geminate species pairs of corals, gastropods and crustacea (Vermeij, 1978; Leao, 1986; Sarver *et al*., 1998). It reflects the bases for allopatric speciation where a barrier that divides a species spatially prevent gene flow between them (Barton, 1998). Our results show that Brazilian and Caribbean populations of *C. aurea* are conspecific, but during the last glacial period, connectivity between these populations must have been deprived in function to the presence of the Amazon River, allowing genetic differences to be accumulated. Four out of seven loci tested for Caribbean populations in the present study failed to amply during PCR. As Padua *et al*. (2013) developed these seven polymorphic loci of microsatellite for individuals sampled from Brazilian populations, amplification failure must reflect variations in flaking regions for primer annealing accumulated during times of low sea levels; therefore, supporting our present hypothesis for the connectivity of these two populations through time.

Several studies of reef fishes have demonstrated that the Amazon River can be crossed through deep sponge bottoms, known as the "sponge corridor" (Collette & Rüztler, 1977). It is constituted of extensive carbonate structures harbored by a rich and diverse benthic community of sponges and other filter feeder species, with living assemblages of mesophotic and deep reef-associated organisms (Moura *et al*., 2016). Despite its sponge diversity, however, no specimens of *C. aurea* were found in the region. Up to date, *C. aurea* is registered only for shallow waters, and the reef structures and rhodolith beds that composes the sponge corridor are largely located in deep regions (>40 m); therefore, it could not use the Amazon reef system as a stepping stone. This agrees with Mantel's non-significant tests carried out in the present study and suggests the species must rely on other ways to surpass this barrier, such as rafting or larval dispersal (Luiz *et al*., 2011). Our analyses of structure and migration results indicates that larvae of *C. aurea* have high dispersal capability, crossing distances over 5,000 km that separates Brazil from Caribbean in order to maintain genetic homogeneity between these two populations, but this result goes against several studies on connectivity between sponges' population that reveal high genetic structure among different species (*e.g.*: *Paraleucilla magna* (Blanquer & Uriz, 2010; Guardiola *et al*., 2012); *Crambe crambe* (Duran *et al*., 2004; Chaves-Fonnegra *et al*., 2015); *Xestospongia mutua* (Richards *et al*., 2016); *Ircinia fasciculata* (Riesgo *et al*., 2016)). The phylum Porifera holds the prevailing view of limited larval dispersal, mainly due to their philopatric behaviour and by larvae's short lifetime in the

water column (Mariani *et al*., 2000; Uriz *et al*., 2008; Ereskovsky, 2010); however, several methods for sponges to produce energy storage structures have been described, thereby being capable of increasing larvae's lifespan (Sciscioli *et al*., 1991; 1994). For example, lipid droplets and yolk granules are known structures involved in nourishment of eggs and have been reported in two species within the family Clathrinidae (*Borojevia aspina* e *B. brasiliensis*), but could not be observed in *C. aurea* (Lanna & Klautau, 2016). Very little is still known about the reproduction of *C. aurea* (Lanna & Klautau, 2016) and further studies may help better understand its larvae dispersal capability.

High dispersal of *C. aurea* was also revealed in fine geographic scales: within Brazil, Cabo Frio and Ilhabela are separated by nearly 340 km; and in the Caribbean, Saint Martin and Mayreau represents the north and southern outermost islands in the Lesser Antilles, respectively, separated by approximately 620 km. In both cases, analyses with ITS and microsatellite markers clustered these distant localities into one unique population. Regarding Caribbean populations, it is surprisingly not to see islands near to each other clustering together: for example, Mayreau is more close to Bequia than Saint Martin, separated by only 8 km. Despite that, they do not constitute a single population by our structure analyses. Several ecological factors are known to shape population differentiation, especially surface currents (e.g. Silberman *et al*., 1994; Shulman & Bermingham, 1995; Galindo *et al*. 2006). The Lesser Antilles seems to have a complex current system (Stalcup & Metcalf, 1972); however, studies on oceanographical and ecological characterization of the region are still very scarce (Holcombe & Moore, 1977; Duncan *et al*., 1982) and no study on marine connectivity along the islands has been conducted so far; thus, giving no insights to explain the major factors driving population differentiation of *C. aurea* within the Lesser Antilles. For broader geographic scales, populations of Abrolhos showed more genetic similarity to Caribbean than Brazilian populations, and this connectivity is also supported for a high number of effective migrants ($N_eM = 33.4$) between them. Our results reflect the biogeographical homogeneity between Brazil and the Caribbean once proposed by Ekman (1953) with the Caribbean Province, revealing Abrolhos as the southern limit of distribution for Caribbean populations of *C. aurea*.

Analyses revealed significant deviation from HWE for populations of Guadeloupe, Martinique, Saint Vincent and within Brazil, but no deviation was found on exact tests considering Brazilian and Caribbean localities as two different populations ($p < 0.001$),

suggesting that it must be reflex of poor sampling. Still, conspicuous differences between values of observed and expected heterozygosity were observed within populations. *Clathrina aurea* presents a lifespan ranging from weeks to one year (Orton, 1914, 1920; Johnson, 1979; Cavalcanti *et al*., 2013; Padua *et al*., 2016), with an average of 4.7 months, also enhanced by association to azooxanthellate scleractinian corals (Padua *et al*., 2016; Ribeiro *et al*., 2016). Although its lifespan seems short when compared to demosponges that usually have a lifespan of months (Ereskovsky, 2000), decades (Mercado-Molina *et al*., 2011) or even hundreds of years (Lehnert & Reitner, 1997; Woerheide *et al*., 1997), studies show high recruitment rates for this species (Padua *et al*., 2016) that could result in overlapping generations and, therefore, explain the bias between heterozygosity values. F_{IS} within Caribbean populations showed high positive values – although not significant – which could have resulted for technical reasons such as nonamplifying alleles (*e.g*. null alleles) (Shaw *et al*., 1999; Miller & Waits, 2003; Wandeler *et al*., 2003; Shinde *et al*., 2003). Indeed, the three loci used in this study were suggested to present null alleles for all populations. It is known that the presence of null alleles has the potential to artificially inflate measures of differentiation among populations by reducing the genetic diversity within them (Chapuis & Estoup, 2007). However, our results revealed high allelic diversity for all loci (ranging from 24 to 48 alleles) and high values of expected heterozygosity, similar to what have been reported for other different species of marine sponges so far (*e.g*. Guardiola *et al*., 2012; Chaves-Fonnegra *et al*., 2015; Richards *et al*., 2016). Moreover, population differentiation measured with F_{st} values showed similar genetic structure when we compare the fixation index calculated for ITS with microsatellite maker, except for the similarity between Abrolhos and Caribbean populations revealed by microsatellites but not by ITS, due to the influence of the Amazon River shaping its connectiviness. Several other studies on population genetics show no consistent differences regarding structuration using loci with potential null alleles for sponges (*e.g*. Calderón *et al*., 2007; Guardiola *et al*., 2012; Chaves-Fonnegra *et al*., 2015; Richards *et al*., 2016; Riesgo *et al*., 2016) and even other marine invertebrates (*e.g*. Shaw *et al*., 1999; Launey *et al*., 2001; Hedgecock *et al*., 2004; Reece *et al*., 2004; Miller *et al*., 2014), thereby suggesting that the presence of null alleles may not affect population differentiation for sponges as it was previously thought.

Our study presented a high degree of genetic structure among populations of *Clathrina aurea* found throughout the Western Atlantic. Past and present timescale analyses

revealed different patterns of structuration explained by the permeability of the Amazon River as a barrier through time in the connectivity between Brazilian and Caribbean populations. Furthermore, genetic clustering and migrant analyses showed strong insights for a high dispersal capability of *C.aurea* through long geographic distances.

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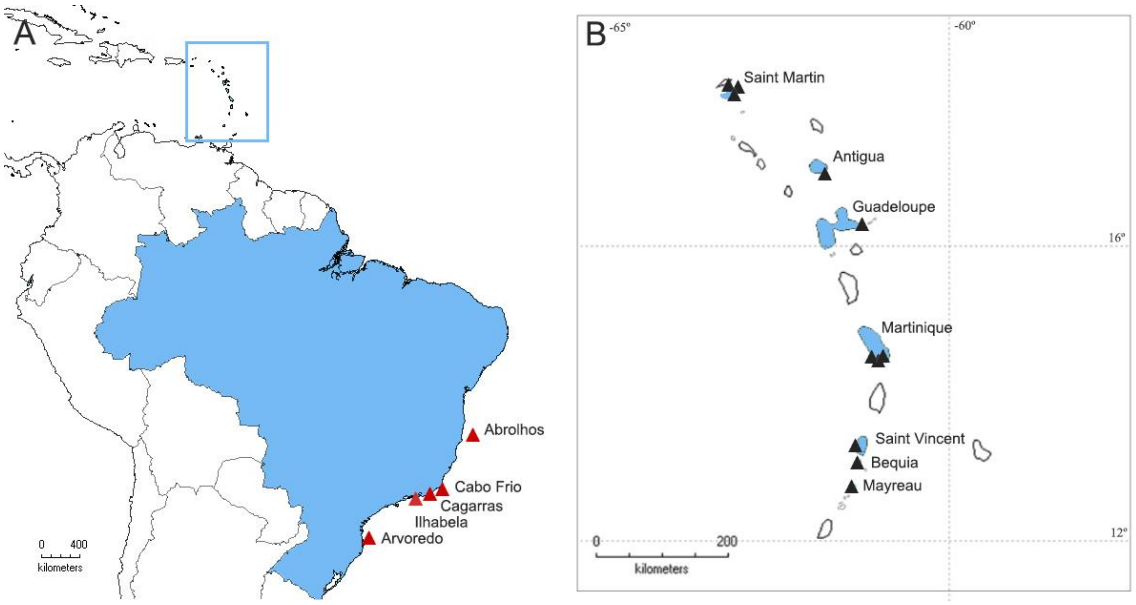

Figure 7. Map showing sampling locations of the analyzed material. (A) Brazilian populations. (B) Detail of the Lesser Antilles sampling localities. Red triangles represent sequences obtained from Genbank database and black triangles represent sampled populations.

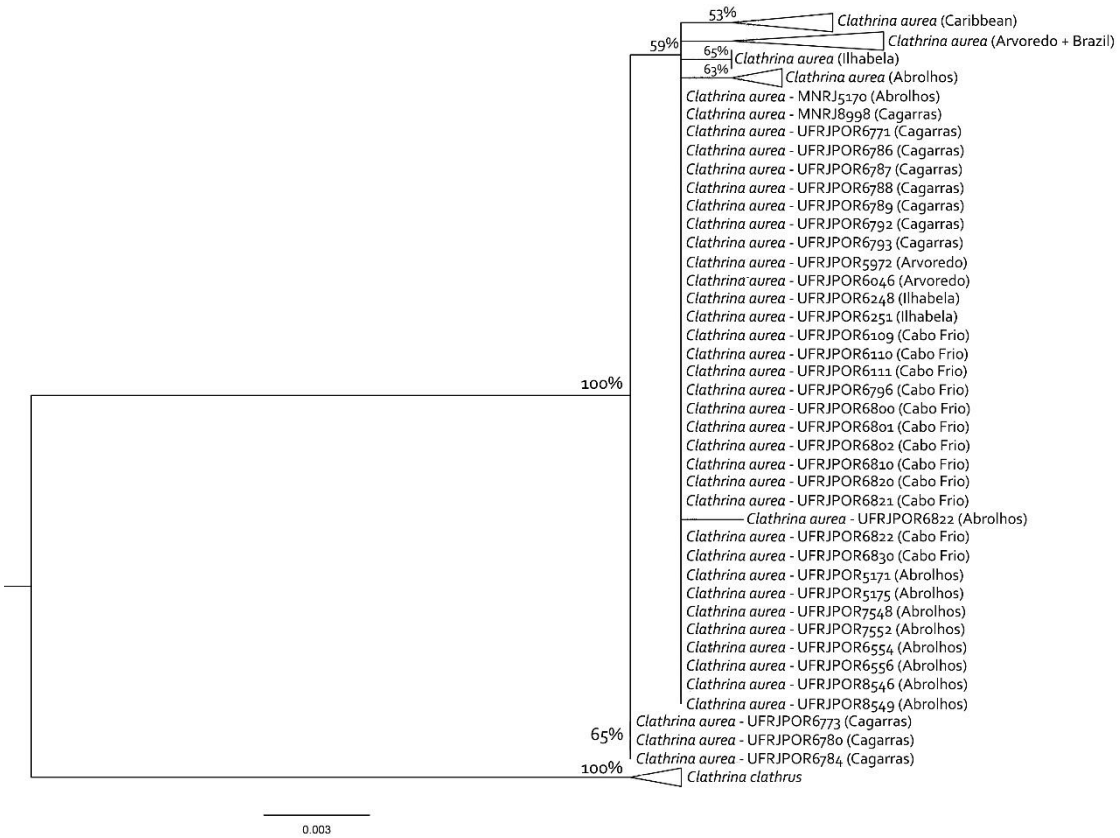

Figure 8. Maximum likelihood tree built with 731 bp of ITS marker, using a GTR+G correction model. Numbers represent support values (bootstrap, 1000 pseudo-replicates).

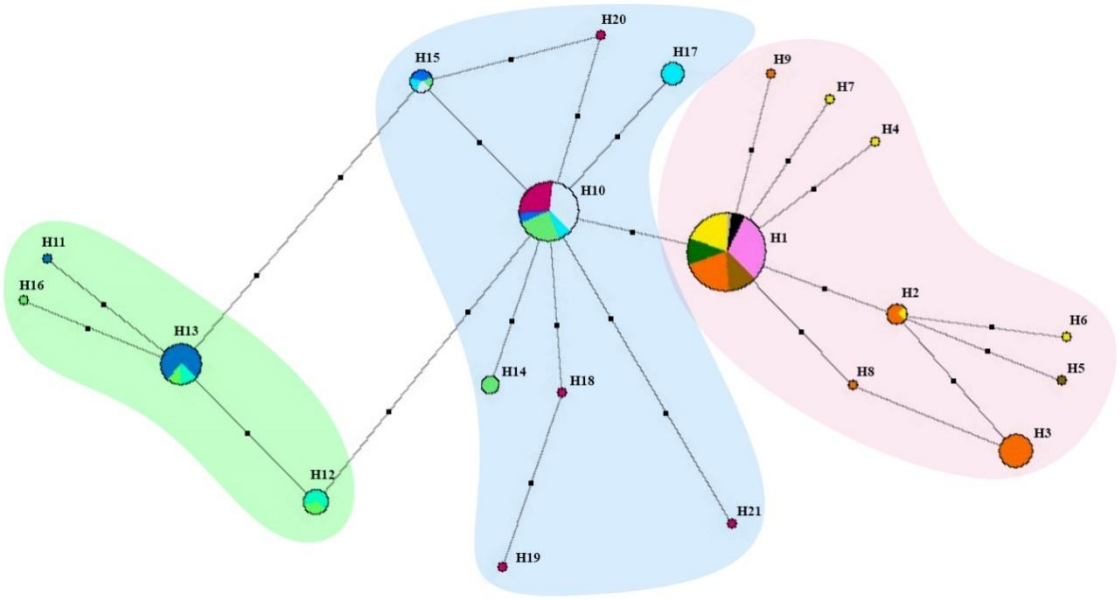

Figure 9. Median-joining haplotype network. Each circle represents a different haplotype: from H1 to H21. Different colours represent the following localities: ● Saint Martin; ● Bequia; ● Guadeloupe; ● Antigua; ● Saint Vincent; ● Martinique; ● Mayreau; ● Abrolhos; ● Cagarras; ● Arvoredo; ○ Cabo Frio; ● Ilhabela. The size of circles is proporcional to the relative haplotype frequency in the data set. Squared dots correspond to mutation steps between haplotypes. Colored contours delimit haplogroups.

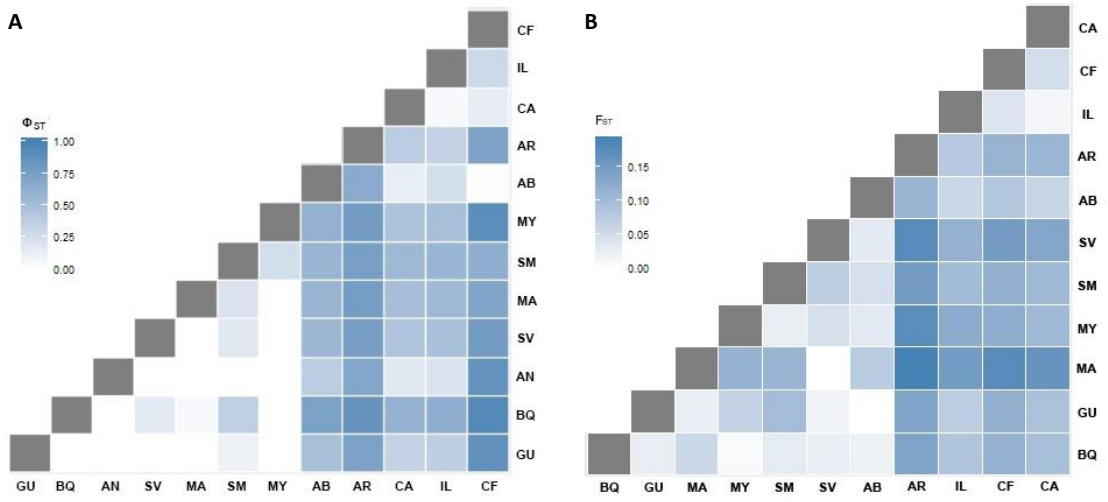

Figure 10. Heatmap showing pairwise (A) Φ_{st} values and (B) F_{st} values with ENA correction method. AN: Antigua; BQ: Bequia; GU: Guadeloupe; MA: Martinique; MY: Mayreau; SM: Saint-Martin; SV: Saint-Vincent; AB: Abrolhos; AR: Arvoredo; CA: Cagarras; CF: Cabo Frio; IB: Ilhabela.

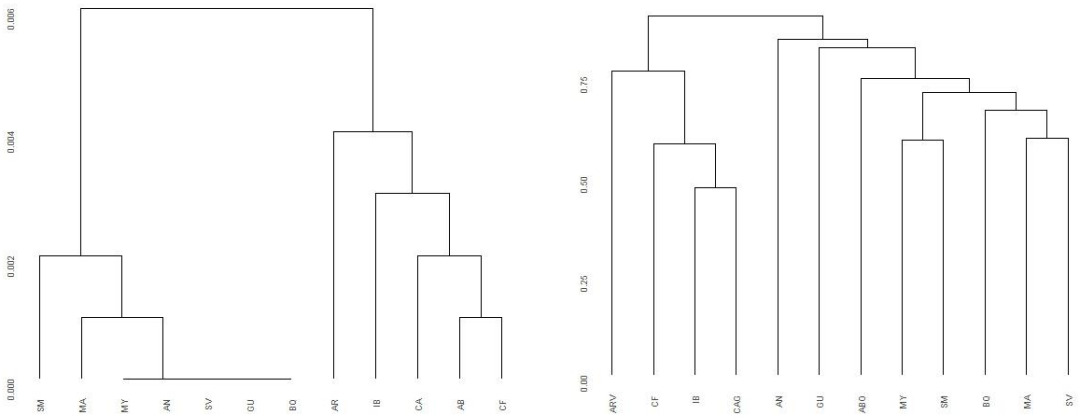

Figure 11. Dendrograms of (A) p-distances calculated with ITS marker, and (B) Cavalli-Sforza & Edwards (1967) genetic distances for three loci studied of microsatellites.

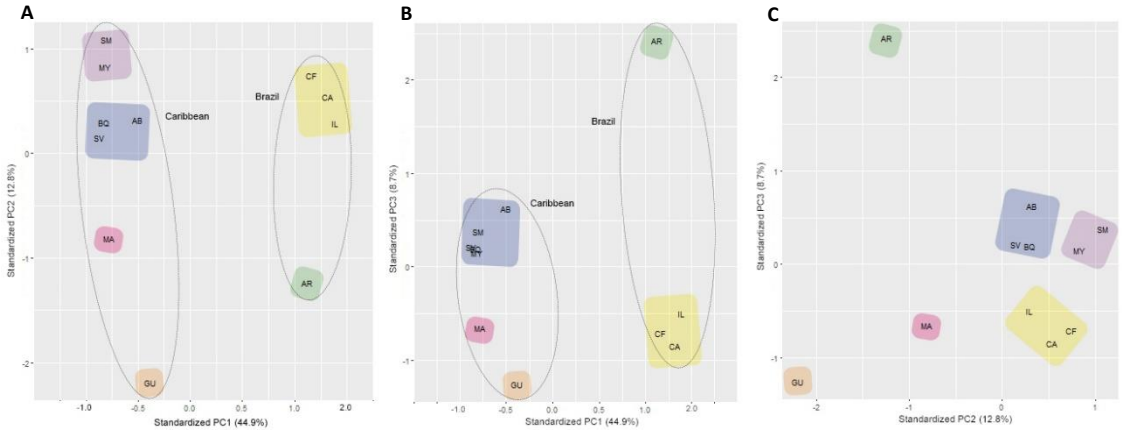

Figure 12. Principal Component Analyses (PCA) using Cavalli-Sforza & Edwards (1967) genetic distances for each pair of localities. (A) PC1 vs PC2 biplot; (B) PC1 vs PC2 biplot; (C) PC2 vs PC3 biplot. AB: Abrolhos; AR: Arvoredo; BQ: Bequia; CA: Cagarras; CF: Cabo Frio; GU: Gaudeloupe; IL: Ilhabela; MA: Martinique; MY: Mayreau; SM: Saint Martin; SV: Saint Vincent.

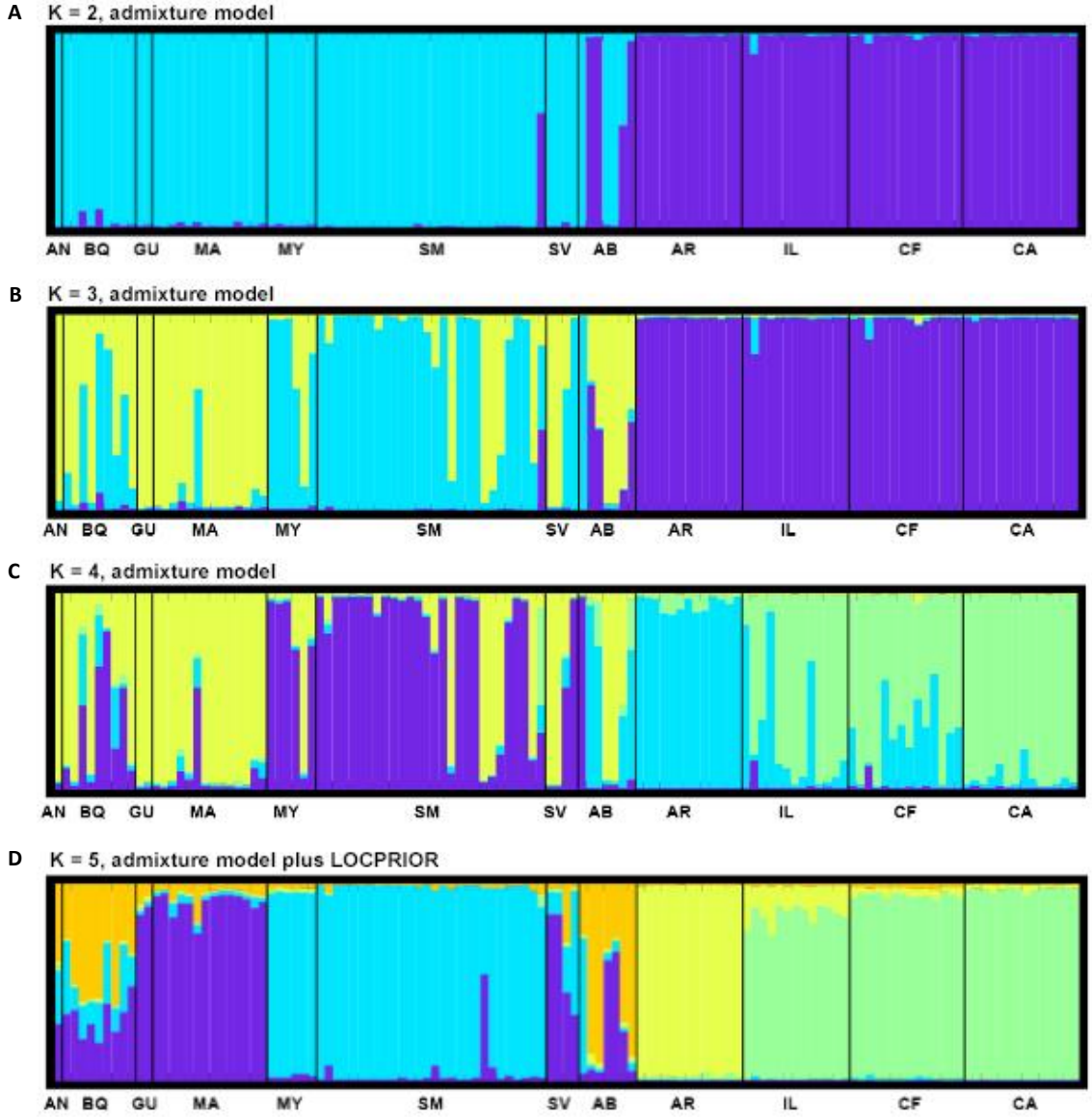

Figure 13. Assignment of individuals in different genetically homologous groups (*K*) in (A–C) *a priori* and (D) *posteriori* analyses in STRUCTURE. Individuals are represented by a vertical bar partitioned into *K*-coloured segments showing the proportion of membership coefficient for different genetic clusters. Vertical lines split individuals from the same localities:(AN) Antigua; (BQ) Bequia; (GU) Guadeloupe; (MA) Marinique; (MY) Mayreay; (SM) Saint Martin; (SV) Saint Vincent; (AB) Abrolhos; (AR) Arvoredo; (IL) Ilhabela; (CF) Cabo Frio;(CA) Cagarras.

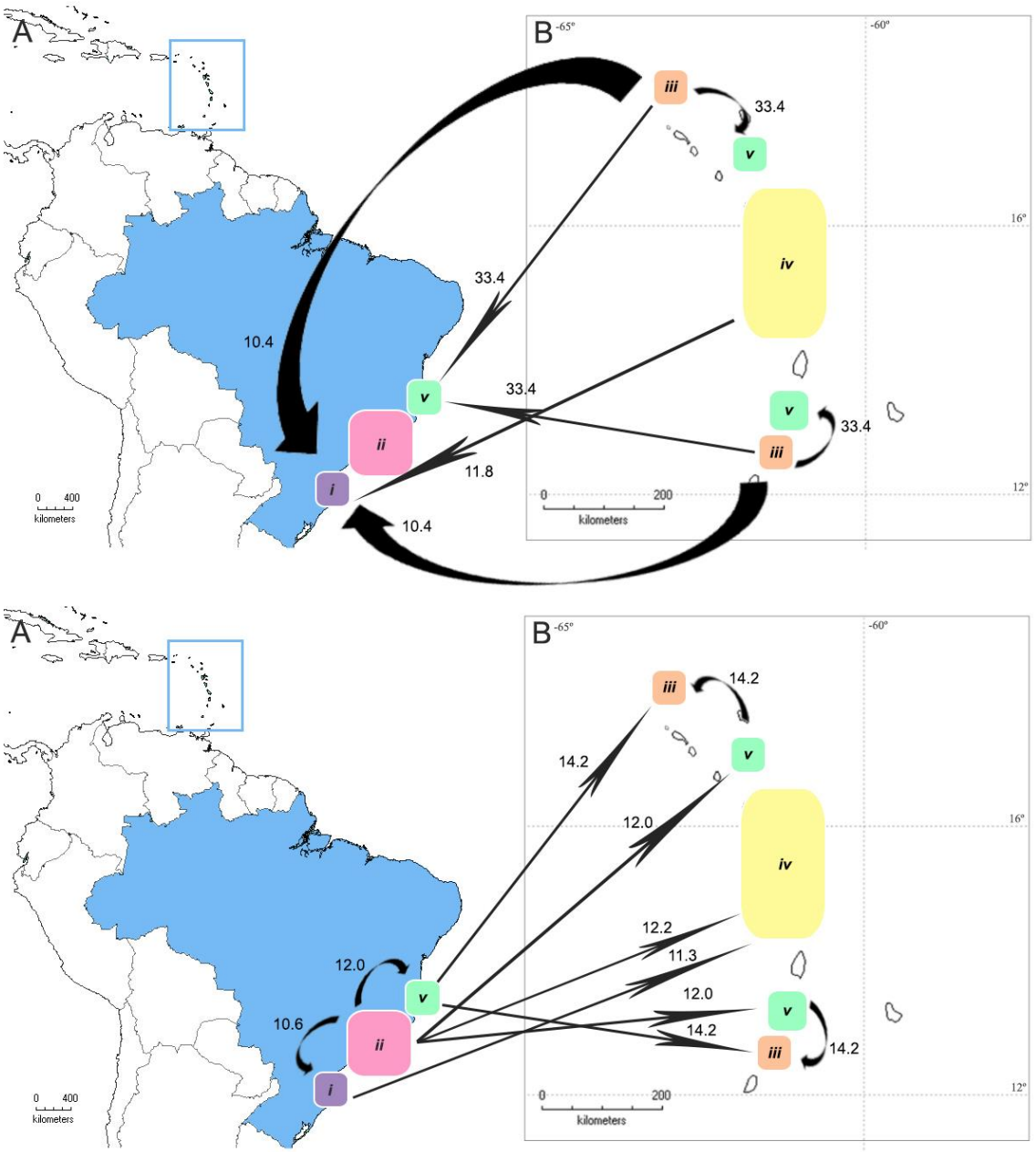

Figure 14. Summary of the estimates of gene flow based on Bayesian inference of migration rates and population sizes by the software MIGRATE-n among population clusters of *Clathrina aurea*. Arrows represent directions of migration and values near arrowheads correspond to the effective number of migrants per generation ($N_eM \geq 10$) for a 97.5% interval of confidence considering all loci. Populations are coded as follows: (i) Arvoredo; (ii) Cabo Frio, Cagarras and Ilhabela; (iii) Mayreau and Saint Martin; (iv) Guadeloupe and Martinique; and (v) Abrolhos, Antigua, Bequia and Saint Vincent.

Table 1. Mean *p* distances calculated between group localities. Lower diagonal represents genetic distances and upper diagonal represents standard deviation (1000 bootstrap).

	AN	BQ	GU	MA	MY	SM	SV	AB	AR	CA	CF	IB
AN		0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.003	0.001	0.001	0.002
BQ	0.000		0.000	0.000	0.000	0.001	0.000	0.001	0.003	0.001	0.001	0.002
GU	0.000	0.000		0.000	0.000	0.001	0.000	0.001	0.003	0.001	0.001	0.002
MA	0.000	0.000	0.000		0.000	0.001	0.000	0.001	0.003	0.002	0.001	0.002
MY	0.000	0.000	0.000	0.000		0.001	0.000	0.001	0.003	0.001	0.001	0.002
SM	0.001	0.001	0.001	0.002	0.001		0.001	0.001	0.003	0.002	0.001	0.002
SV	0.000	0.000	0.000	0.001	0.000	0.002		0.001	0.003	0.001	0.001	0.002
AB	0.002	0.002	0.002	0.003	0.002	0.003	0.003		0.002	0.001	0.000	0.001
AR	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.004		0.002	0.002	0.002
CA	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.002	0.003		0.001	0.001
CF	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.001	0.004	0.002		0.001
IB	0.003	0.003	0.003	0.004	0.003	0.005	0.004	0.003	0.003	0.003	0.002	

AN: Antigua; BQ: Bequia; GU: Guadeloupe; MA: Martinique; MY: Mayreau; SM: Saint-Martin; SV: Saint-Vincent; AB: Abrolhos; AR: Arvoredo; CA: Cagarras; CF: Cabo Frio; IB: Ilhabela.

Table 2. Genetic diversity indices for individual collection sites and neutrality tests.

	n	S	h	Hd (±sd)	π (±sd)	Neutrality test			
						Tajima's D	Fu and Li's D	Fu and Li's F	Fu's Fs
Antigua	1	0	1	0.000	0.00000	*	*	*	*
Bequia	18	0	1	0.000	0.00000	*	*	*	*
Guadeloupe	2	0	1	0.000	0.00000	*	*	*	*
Martinique	15	4	5	0.476	0.00093	-1.51811	-1.47017	-1.69382	-2.677
Mayreau	6	0	1	0.000	0.00000	*	*	*	*
Saint Martin	27	3	5	0.675	0.00157	0.98412	-0.22854	0.13426	-0.514
Sait Vincent	7	2	2	0.286	0.00082	-1.23716	-1.29591	-1.37408	0.856
Abrolhos	13	5	5	0.538	0.00129	-1.57943	-1.60955	-1.82099	-2.036
Arvoredo	18	4	4	0.477	0.00151	-0.28539	0.21103	0.08706	-0.009
Cabo Frio	13	1	2	0.154	0.00022	-1.14915	-1.36547	-1.48111	-0.537
Cagarras	17	5	6	0.757	0.00243	0.46297	0.43863	0.51133	-1.029
Ilhabela	14	4	5	0.802	0.00274	1.71352	1.16427	1.48985	-0.036

(*): Could not be calculated due the presence of single haplotype

Table 3. Summary of genetic variation per loci for each sampling locality.

	AN	BQ	GU	MA	MY	SM	SV	AB	AR	CF	CA	IB	CARIBBEAN	BRAZIL
A7														
n_A	1	5	1	11	6	28	2	6	11	14	14	11	54	56
p_A	0	0	1	3	0	1	1	2	3	1	0	1	9	16
H_O	0.000	0.000	1.000	0.636	0.833	0.429	0.000	0.333	0.818	0.786	0.786	0.818	0.463	0.750
H_E	0.000	0.800	0.500	0.897	0.736	0.870	0.500	0.819	0.868	0.903	0.865	0.909	0.913	0.949
D1														
n_A	1	9	2	14	6	28	4	7	13	14	14	13	64	61
p_A	0	0	0	0	0	1	0	3	1	1	0	1	4	18
H_O	0.000	0.444	0.000	0.357	0.167	0.500	0.000	0.571	0.846	0.857	0.786	0.846	0.375	0.803
H_E	0.000	0.673	0.500	0.446	0.625	0.781	0.375	0.847	0.716	0.791	0.827	0.831	0.723	0.910
D8														
n_A	1	8	2	8	0	25	5	5	12	14	14	12	47	57
p_A	0	6	0	4	0	5	0	1	4	1	1	2	22	21
H_O	1.000	0.375	0.500	0.625	0.000	0.240	0.000	0.400	1.000	1.000	1.000	1.000	0.340	0.947
H_E	0.500	0.883	0.625	0.828	0.000	0.820	0.444	0.760	0.833	0.829	0.875	0.899	0.925	0.931
Mean														
n_A	1.0	7.2	1.7	11.0	4.0	27.0	3.0	6.0	12.0	14.0	14.0	12.0	55	58
H_O	0.332	0.273	0.500	0.538	0.332	0.390	0.000	0.595	0.888	0.881	0.857	0.888	0.393	0.833
H_E	0.167	0.784	0.542	0.724	0.454	0.824	0.440	0.809	0.806	0.841	0.856	0.880	0.854	0.930
HWE	-	*	NS	NS	*	***	NS	NS	NS	NS	NS	NS	***	***

n_A: number of different alleles; p_A: number of private alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; HWE: probability of deviation from Hardy–Weinberg equilibrium. Significant values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: non-significant. AN: Antigua; BQ: Bequia; GU: Guadeloupe; MA: Martinique; MY: Mayreau; SM: Saint-Martin; SV: Saint-Vincent; AB: Abrolhos; AR: Arvoredo; CA: Cagarras; CF: Cabo Frio; IB: Ilhabela;

Table 4. FIS values computed for each sampling locality per locus. Bold values indicate significant values.

	BQ	GU	MA	MY	SM	SV	AB	AR	CA	CB	IB	CARIBBEAN	BRAZIL
A7	**1.000**	*	0.372	0.000	**0.521**	1.000	0.649	0.104	0.147	0.166	0.128	**0.500**	**0.218**
D1	0.390	1.000	0.221	0.793	**0.376**	1.000	0.392	-0.143	0.022	-0.047	0.086	**0.487**	**0.125**
D8	**0.618**	0.500	0.307	*	**0.717**	1.000	0.556	-0.158	-0.069	-0.170	-0.106	**0.647**	**-0.009**
All	**0.698**	0.750	**0.315**	0.354	**0.541**	1.000	**0.532**	-0.058	0.035	-0.011	0.035	**0.550**	0.112

DISCUSSÃO

Os marcadores utilizados para o presente estudo refletem tempos evolutivos diferentes dentro do cenário de conectividade de *Clathrina aurea* ao longo do Atlântico Oeste e revelam uma grande influência do Rio Amazonas moldando essas populações. O Rio Amazonas é localizado na região nordeste brasileira, conhecido como uma forte barreira à dispersão de diversos organismos marinhos (Miloslavich *et al*., 2011). Contudo, oscilações no nível do mar entre intervalos glaciais seriam responsáveis por promover a permeabilidade do Rio Amazonas à conectividade dessas populações – hipótese levantada por Rocha (2003) para explicar a conectividade de populações de diversas espécies de peixes recifais reportados tanto para a costa brasileira quanto para o Caribe. Essa oscilação explicaria as falhas de amplificação em quatro dos sete *loci* estudados no presente trabalho, refletindo o acúmulo de diferenças genéticas durante o período em que as populações brasileiras e caribenhas de *C. aurea* estiveram separadas (máxima glacial), mas também explica a similaridade genética atual dessas mesmas populações como reflexo da permeabilidade da barreira durante os períodos interglaciais.

Análises de migrantes mostram um alto fluxo entre as populações brasileiras e caribenhas de *C. aurea*, possível de acontecer devido a presença do "corredor de esponjas", localizado abaixo da descarga de água doce do Rio Amazonas (>40m) (Collette & Rüztler, 1978; Moura et *al*., 2016). Apesar disso, a espécie só é conhecida em águas rasas, o que sugere que suas larvas sejam capazes de cruzar longas distâncias para manter a conectividade observada entre as duas populações. De fato, análises de estruturação e migração do presente trabalho sugerem que larvas da espécie sejam capazes de cruzar distâncias maiores que 5.000 km que separam o Brasil do Caribe (Pequenas Antilhas), indo de encontro a outros estudos de diferenciação genética em esponjas (*e.g*.: *Paraleucilla magna* (Blanquer & Uriz, 2010; Guardiola *et al*., 2012);

Crambe crambe (Duran *et al*., 2004b; Chaves-Fonnegra *et al*., 2015); *Xestospongia mutua* (Richards *et al*., 2016); *Ircinia fasciculata* (Riesgo *et al*., 2016)). Pouco se conhece sobre a reprodução da espécie de *C. aurea* (Lanna & Klautau, 2016) e mais estudos se fazem necessário para melhor compreender a capacidade de dispersão larval da espécie.

Dentro das Pequenas Antilhas, Saint Martin e Mayreau representam o limite norte e sul, respectivamente, das ilhas caribenhas estudadas e estão separadas por cerca de 620 km. Análises de estruturação genética reúnem essas duas localidades em uma única população, apesar de não reunirem ilhas muito mais próximas umas das outras (*e.g*. Bequia e Saint Martin, separadas por apenas 8km). Diversos fatores oceanográficos e ecológicos são conhecidos por moldar a diferenciação genética de populações (Galindo *et al*., 2006), contudo, essa caraterização das Pequenas Antilhas é muito escarça (Holcombe & Moore, 1977; Duncan *et al*., 1982) e nenhum estudo de conectividade marinha é conhecido para a região, o que dificulta introspecções para explicar o padrão de conectividade para *C. aurea* observado na região. Quanto a população brasileira, Abrolhos mostrou maior similaridade genética com as ilhas caribenhas do que com localidades brasileiras, refletindo homogeneidade biogeográfica entre o nordeste brasileiro com o Caribe como proposto por Ekman (1953).

Desvios do Equilíbrio de Hardy-Weinberg nas populações estudadas sugerem uma baixa amostragem populacional e a conspícua diferença entre os valores de heterozigosidade observada com a esperada pode ser resultado da sobreposição de gerações devido à alta taxa de recrutamento da espécie (Padua *et al*., 2016; Ribeiro *et al*., 2016). Resultados inferem a presença de alelos nulos para os três *loci* utilizados no presente estudo; porém, uma alta diversidade alélica e de heterozigosidade esperada, somada aos valores de F_{ST} similares aos cálculados com o marcador ITS, sugerem que eles não estejam afetando a diferenciação populacional encontrada – como já foi

observado em diversos outros estudos de conectividade com esponjas (*e.g*. Calderón et al., 2007; Guardiola *et al*., 2012; Chaves-Fonnegra *et al*., 2015; Richards *et al*., 2016; Riesgo *et al*., 2016).

O presente trabalho revela uma alta estruturação das populações de *C. aurea* ao longo do Atlântico Oeste, revelando a influência do Rio Amazonas na dinâmica populacional da espécie ao longo da região. Além disso, análises de migração e estruturação genética sugerem uma alta capacidade de dispersão larval de *Clathrina aurea* ao longo de longas distâncias.

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