Programa de Pós-graduação em Diversidade Animal
Universidade Federal da Bahia

Ueslei da Conceição Lopes

Estudo morfológico e molecular de *Geodia gibberosa* Lamarck, 1815 (Astrophorida, Geodiidae)

Salvador
2012

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Dissertação apresentada ao Instituto de Biologia da Universidade Federal da Bahia para a obtenção do Título de Mestre pelo Programa de Pós-Graduação em Diversidade Animal, na Área de Zoologia.

Orientadora: Carla M. M. da Silva
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De acordo com o regimento geral da UFBA e com o regimento interno deste programa de pós-graduação, foram iniciados os trabalhos da Comissão Examinadora, composta pelos professores Dra. Carla Maria Menegola da Silva (presidente), Dra. Gisele Lobo Hadju e Dra. Angela Maria Zanata às 09 horas do dia 29 de Junho de 2012.
O mestrando fez a apresentação oral da dissertação durante 45 minutos. Após o encerramento das argüições, às 12 horas, a Comissão Examinadora pronunciou-se pela sua APROVAÇÃO, conforme parecer em anexo.
Esta Ata será assinada pelos membros da Comissão Examinadora e deste Colegiado, para compor o processo de emissão do diploma.

Salvador, 29 de Junho de 2012.

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COLEGIADO

Dedicatória

Por todo carinho, confiança, apoio e
amor, dedico esse trabalho
aos meus pais.

Epígrafe

"O sucesso nasce do querer,
da determinação e persistência em se
chegar a um objetivo. Mesmo não atingindo o alvo,
quem busca e vence obstáculos, no mínimo fará coisas admiráveis."

José de Alencar.

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Índice

Resumo

Geodia gibberosa é uma das espécies que apresentam as maiores distribuições geográfica e batimétrica em comparação com suas congêneres. Com variações significativas na morfologia externa e no conjunto espicular, é considerada uma espécie com alto grau de polimorfismo e, muitas vezes, de difícil identificação. Tais características têm levado diversos pesquisadores a questionar os limites de sua variabilidade e a levantar hipóteses acerca da existência de espécies crípticas. Com o presente estudo, se objetivou avaliar o *status* taxonômico dessa espécie, aliando informações de morfologia e de biologia molecular, a qual empregou a utilização dos marcadores mitocondriais COI (subunidade I da citocromo c oxidase) e ATP6 (subunidade 6 da ATP sintase). Embora tenham sido observadas variações na morfologia das espículas e registradas categorias de megaescleras nunca vistas no holótipo, não foi possível encontrar nenhum padrão que possibilitasse a separação de *G. gibberosa* em espécies morfologicamente distintas. Contudo, através das ferramentas moleculares, foi determinada a existência de, pelo menos, três clados fortemente suportados, possibilitando a aceitação da hipótese de que *G. gibberosa* representa um complexo de espécies. Acrescido a isso, no presente trabalho, é descrita uma nova espécie de *Geodia* para a costa da Venezuela, com a apresentação de uma chave taxonômica para as 12 espécies nominais agora válidas que ocorrem no Caribe, e registrada a primeira ocorrência de *Pachymatisma johnstonia* para o oceano Atlântico ocidental.

Palavras-chave: Variação morfológica, marcadores mitocondriais, Demospongiae, *Geodia*, complexo de espécies, *Pachymatisma*.

Abstract

Geodia gibberosa is a species with one of the largest geographic and bathymetric distributions when compared to its peers. With significant variations in both external morphology and espicule repertoire, it is considered a species with a high degree of polymorphism and often difficult to identify. These characteristics have led several researchers to question the limits of its variability and led them to postulate hypotheses about the existence of cryptic species. The aims of the present study was to evaluate the taxonomic status of this species, combining information from morphology and molecular biology, which used the mitochondrial markers COI (subunit I of cytochrome c oxidase) and ATP6 (ATP synthase subunit 6). Although variations in the morphology of the spicules were observed, and new categories of megascleres were registered for the holotype, it was not possible to find any pattern that could allow us the split *G. gibberosa* into morphologically distinct species. However, through the molecular approach, it was determined the existence of at least three highly supported clades, enabling the acceptance of the hypothesis that *G. gibberosa* represents a species complex. Added to this, in the present study, we describe a new species of *Geodia* for the coast of Venezuela, with a taxonomic key to the 12 nominal species that occur in the Caribbean up to now, and the first occurrence of *Pachymatisma johnstonia* for western Atlantic Ocean.

Key words: Morphological variation, mitochondrial markers, Demospongiae, *Geodia*, species complex, *Pachymatisma*.

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ATP6 – Subunidade 6 da ATP sintase

BCT - Bocas Del Toro

BI - Inferência bayesiana

bp - Pares de base

CNPGG - Colección Nacional del Phylum Porifera Gerardo Green, Universidad Nacional Autónoma de Mexico, Mexico

COI - Subunidade I da Citocromo c Oxidase

DNA - Ácido desoxirribonucléico

dNTP - Deoxinucleotídeos fosfatados

FIOCRUZ - Fundação Oswaldo Cruz: Centro de Pesquisas Gonçalo Muniz, Salvador

FL - Flórida

MCN - Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil

MNHN - Muséum National d'Histoire Naturelle, Paris, França

MNRJ - Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brasil

mtDNA - DNA mitocondrial

ML - Máxima verossimilhança

NCBI - National Center for Biotechnology Information

NJ – Neighbor Joining

PCR - Reação em Cadeia da Polimerase

PEG - Polietilenoglicol

UFBA-POR - Coleção de Porifera, Museu de Zoologia, Universidade Federal da Bahia

UFC - Universidade Federal do Ceará, Fortaleza

UFSCar - Universidade Federal de São Carlos, São Carlos, São Paulo, Brasil

UNDP/FAO - Programa das Nações Unidas para o Desenvolvimento/ Organização das Nações Unidas para Agricultura e Alimentação

USNM - Smithsonian Institution, National Museum of Natural History, EUA

ZMA POR - Instituut voor Systematiek en Populatiebiologie, Zoölogisch Museum, Porifera Collection, Holanda

ZMBN - Universitetsmuseet i Bergen, Universitetet i Bergen, Noruega

Introdução geral

1. O GÊNERO *GEODIA*

Lamarck (1815) descreveu um novo gênero da família “Alcyons” e lhe atribuiu o nome *Geodia* em alusão a “aberturas maiores que poros, reunidas em uma faceta orbicular”, cujo aspecto de crivo isolado se assemelhava às cavidades das rochas, conhecidas como ‘geodos’. O autor notou que as espécies pertencentes a esse gênero, “apresentam forma subglobosa, são côncavas e internamente ocas”. São também caracterizadas pela grande quantidade de fibras que lhes conferem resistência e, mesmo quando secas, ocorre a manutenção da forma. Em sua superfície são encontrados poros esparsamente distribuídos, além do crivo isolado, cujas aberturas não se destinam à entrada de água na esponja (LAMARCK, 1815).

Posteriormente, esse gênero foi incluído na família “Geodiadae”, juntamente com *Caminus* Schmidt, 1862, *Cydonium* Fleming, 1828 (sinônimo de *Geodia*), *Erylus* Gray, 1867, *Pachymatisma* Johnston, 1842 e *Triate* Gray, 1867 (sinônimo de *Erylus*), levando-se em conta, além da morfologia externa, as categorias espiculares (GRAY, 1867). Desse modo, as esponjas “massivas, com uma cavidade central coberta por membrana reticulada ou perfurada, e que possuem espículas alongadas com dois ou três ramos recurvados nas extremidades, e microescleras dispostas sobre a superfície”, passaram a ser classificadas como *Geodia*.

A revisão mais recente da família considerou seis gêneros válidos e destacou que as espécies pertencentes a *Geodia* são distinguidas por apresentar óxeas longas, triênios regulares radialmente dispostos na superfície (ou próximas a ela), e por um conjunto de espículas pequenas constituído por esterrásteres globulares e euásteres. Contudo, é o padrão de organização cribiporal de poros e ósculos (Figura 1) o caráter que diferencia *Geodia* dos demais, dentro de Geodiidae (URIZ, 2002).

Esse táxon, que data do Cretáceo (RIGBY & SMITH, 1992), conta atualmente com 145 espécies nominais e pode ser considerado, em termos quantitativos, o grupo taxonômico mais significativo da família, sendo seguido pelos gêneros *Erylus* e *Isops* Sollas, 1880, com 66 e 20 espécies, respectivamente (VAN SOEST *et al.*, 2012). Sua expressiva

distribuição, com ampla ocorrência nos oceanos Atlântico, Índico e Pacífico, conta com alto grau de diversidade em regiões tropicais e subtropicais e raras espécies em águas frias (VAN SOEST, 1994), podendo atingir profundidades superiores a mil metros (SILVA, 2002).

Análises filogenéticas baseadas em biologia molecular com Geodiidae fizeram ressurgir as subfamílias Geodiinae e Erylinae, outrora descritas por Sollas (1888), e mostraram esta primeira sendo fortemente sustentada, tanto com marcador nuclear quanto com mitocondrial. Contudo, mesmo *Geodia* se mostrando monofilético, esses estudos evidenciaram que os gêneros *Isops* e *Sidonops* Sollas, 1889 seriam seus sinônimos juniores (constituindo o que Cárdenas *et al*. (2009b) chamou de "complexo *Isops-Sidonops-Geodia*") e que espécies dentro do gênero, a exemplo de *Geodia gibberosa* Lamarck, 1815, ainda careceriam de amplo estudo taxonômico (CÁRDENAS *et al*., 2009a; CÁRDENAS, 2010).

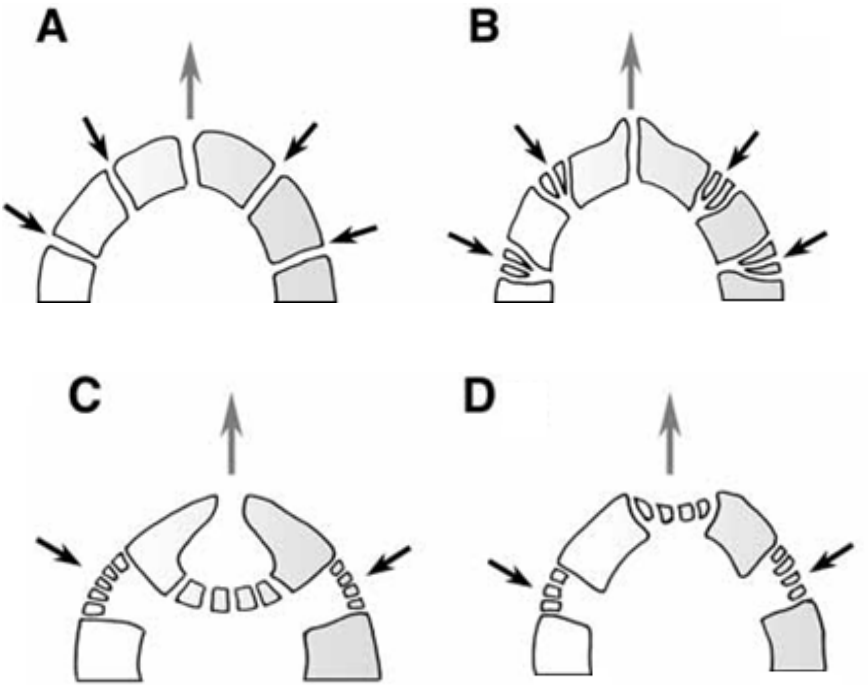


Figura 1. Representação esquemática do arranjo dos canais inalantes e exalantes em diferentes gêneros de Geodiidae. A, *Erylus* e *Isops*. B, *Caminus*, *Pachymastima* e *Sidonops*. C-D, *Geodia* (*Geodia*) e *Geodia* (*Cydonium*), respectivamente. Adaptado de Uriz (2002).

1.1. A ESPÉCIE *GEODIA GIBBEROSA* LAMARCK, 1815

Descrita para a costa da Guiana Francesa, *Geodia gibberosa*, constitui a espécie-tipo do gênero. Usualmente essa espécie é utilizada em estudos de química de produtos naturais, tendo sido encontrada uma ação neurotóxica proveniente de seus extratos (RANGEL *et al.*, 2001), além de diversos lipídios (NAKANISH *et al.*,1953; CARBALLEIRA & RODRIGUEZ, 1991; ROD'KINA, 2005). Produtos derivados de organismos marinhos têm atraído cada vez mais a atenção da comunidade científica e, de certo modo, encorajado novas pesquisas devido ao possível potencial econômico (MENDOLA, 2003).

Acreditava-se que a disposição radial das espículas de *G. gibberosa* pudesse configurar um mecanismo de proteção mecânico à espécie, mas estudos de ecologia indicaram o contrário (CHANAS & PAWLIK, 1995; DUNLAP & PAWLIK, 1998) e mostraram que, além de se tratar de uma espécie extremamente palatável, sendo considerada o alimento preferido de algumas tartarugas (e.g., *Eretmochelys imbricata* (Linnaeus, 1766)) (PAWLIK *et al*., 1995) e de peixes (e.g., *Sparisoma aurofrenatum* (Valenciennes, 1840) e *Sparisoma chrysopterum* (Bloch & Schneider, 1801)) (DUNLAP & PAWLIK, 1996; 1998), *G. gibberosa* carece de defesas químicas (ENGEL & PAWLIK, 2005). Essa suposta vulnerabilidade, no entanto, é atenuada pela alelopatia promovida pela espécie, a qual estimula o crescimento de outras esponjas, e.g. *Tedania ignis* (Duchassaing & Michelotti, 1864), *Lissodendoryx isodictyalis* (Carter, 1882) e *Haliclona (Reniera) tubifera* (George & Wilson, 1919), garantindo-lhe proteção indireta contra a predação (ENGEL & PAWLIK, 2000; 2005).

Dos representantes de *Geodia* que ocorrem no Atlântico ocidental, *G. gibberosa* é a espécie que apresenta as maiores distribuições geográfica e batimétrica. Há registros de sua ocorrência partindo da Carolina do Norte, nos Estados Unidos, a São Sebastião em São Paulo, Brasil, com profundidades que variam de 0,1 m (Salvador, Brasil) a 33 m (costa da Carolina do Sul, EUA) (presente estudo). Outros trabalhos ainda expandem essa distribuição, registrando-a na costa ocidental africana (TOPSENT, 1918; LÉVI, 1959) no Pacífico oriental (DE LAUBENFELS, 1936a). Amplitudes similares a estas são vistas apenas em *Geodia neptuni* Sollas, 1889 e *Geodia corticostylifera* Hajdu *et al.*, 1992, cuja distribuição vai do México ao Rio de Janeiro, e da Jamaica a São Paulo, respectivamente (SILVA, 2002). Essa espécie pode ocorrer tanto em mangues (HECHTEL, 1965; PULITZER-FINALI, 1986; WULFF, 2000) quanto em costões rochosos (WIENDENMAYER, 1977) e em

recifes (PAWLIK *et al*., 1995), assumindo tamanhos distintos de acordo com o tipo de ambiente (CÁRDENAS *et al*., 2009a).

G. gibberosa é descrita na literatura como uma espécie polimórfica. Suas formas vão de espessa e incrustante a um padrão maciço e globoso, nas cores branca, marrom, verde e preta, possuindo ósculos uniporais e poros cribriporais (CÁRDENAS *et al*., 2009a), além de registros esporádicos de categorias espiculares que não constam de sua descrição original (WIENDENMAYER, 1977; SILVA, 2002; MURICY & HAJDU, 2006; MURICY *et al*., 2008). Tais peculiaridades fazem dessa espécie um táxon, muitas vezes, de difícil identificação, tanto no ambiente quanto em laboratório, e mostram que "estudos abrangentes se fazem necessários visando avaliar os limites de sua variabilidade" (HAJDU *et al*., 1992). Um reflexo disso pode ser evidenciado com as sinonimizações realizadas até o momento (SILVA, 2002).

Schmidt (1870) criou o gênero *Pyxitis* e transferiu *G. gibberosa* para o táxon recém-criado. Von Lendenfeld (1903) redefiniu *Geodia* e sinonimizou *Cydonium* Fleming e *Pyxitis* Schimidt. Hechtel (1965), por sua vez, criou os subgêneros *Geodia* Lamarck e *Cydonium* Fleming, baseando-se nas disposições e características dos poros e ósculos. Porém, a utilização de tais arranjos como caracteres diagnósticos passou a ser contestada, assim como a validade desses e de outros subgêneros, os quais seriam sinônimos de *Geodia* (DE LAUBENFELS, 1936b; HAJDU *et al*., 1992; SILVA, 2002). *Geodia cariboea* Duchassaing & Michelotti, 1864, que foi descrita como "grande e amplamente fixada, com superfície não porosa, levemente reticulada e com o crivo de ósculos ausente", é considerada sinônima de *G. gibberosa* (DE LAUBENFELS, 1936b; HECHTEL, 1965). Das 12 espécies de *Geodia* descritas por Bowerbank (1872; 1873a; 1873b; 1874), que também analisou o espécime-tipo, quatro (*Geodia tumulosa*, *Geodia media*, *Geodia dysoni* e *Geodia reticulata*) foram sinonimizadas por apresentarem basicamente o mesmo conjunto espicular, com algumas diferenças no arranjo de poros e ósculos (CARTER, 1882; DE LAUBENFELS, 1936a; HECHTEL, 1965; HAJDU *et al*., 1992). As espécies *Sidonops stromatodes* e *Geodia media* var. *leptoraphes*, ambas descritas por Uliczka (1929), assim como *Geodia flexisclera* Pulitzer-Finali, 1986 também foram consideradas sinônimas de *G. gibberosa* (HAJDU *et al*., 1992).

Dado o polimorfismo apresentado por essa espécie, Cárdenas *et al*. (2009a) levantaram a hipótese de que poderiam existir duas ou mais espécies crípticas em

G. gibberosa e sugeriram que estudos morfológicos, combinados com análises moleculares, fossem feitos a fim de testá-la. Em seu trabalho de filogenia molecular da ordem Astrophorida, Cárdenas (2010) levantou mais uma vez a hipótese, indicou o parafiletismo de *G. gibberosa* (Figura 2) e recomendou fortemente a realização de um amplo estudo taxonômico com ênfase na morfologia externa e no esqueleto, uma vez que, em seu estudo, o mesmo não encontrou diferenças espiculares (CÁRDENAS, 2010).

1.2. TAXONOMIA INTEGRATIVA

Devido ao número reduzido de caracteres morfológicos que podem ser utilizados na determinação das espécies, não raramente encontramos grupos de poríferos cuja identificação é conflitante. Seus limites, enquanto unidades taxonômicas, são dificilmente estabelecidos, acarretando a subestimação do número real de espécies conhecidas (KLAUTAU *et al*., 1999; KNOWTON, 2000).

Tal como evidenciado em *G. gibberosa*, as variações de forma, tamanho e coloração, exibidos por muitos invertebrados marinhos sésseis e bentônicos (e particularmente por poríferos), têm implicações taxonômicas diretas (LÓPEZ-LEGENTIL *et al.*, 2010). Estas podem enviesar outras linhas de pesquisa que igualmente dependem de identificações acuradas (e.g. estudos de química de produtos naturais, de ecologia, assim como a criação de áreas prioritárias para a conservação), ou provocar efeitos cascata em virtude da replicação de falhas previamente cometidas (BORTOLOUS, 2008; LOCKE & COATES, 2008).

Sequências de DNA têm sido constantemente empregadas e a sua utilidade em estudos taxonômicos está bem estabelecida (CÁRDENAS, 2010). Nesse contexto, as sequências de nucleotídeos surgem como uma fonte independente de informações que podem ajudar a fornecer as respostas que não estão sendo obtidas unicamente por meio da morfologia comparativa (MEIER, 2008). Na literatura é encontrada uma infinidade de exemplos nos quais marcadores nucleares e mitocondriais são utilizados a fim de estimar os níveis de diversidade e distâncias genéticas entre indivíduos e populações de espécies (FOLMER *et al*., 1994; LÔBO-HAJDU *et al*., 2004).

Porém, embora as moléculas venham contribuindo substancialmente para resolução de problemas nos níveis intra e interespecífico, é importante salientar que o DNA está longe de ser a solução para todos os problemas de ordem taxonômica e o seu resultado não deve ser o único levado em consideração (TAUTZ *et al.*, 2003; LÔBO-HAJDU, 2006).

A taxonomia de Porifera atual vivencia uma espécie de renascimento; uma fase que é marcada pelo uso de diferentes fontes de informação (morfologia externa, espículas, aspectos embriológicos e do desenvolvimento, geográficos, bioquímicos, ecológicos, etc.) que, integradas, podem conferir maior robustez e confiabilidade aos resultados obtidos (PADIAL *et al*., 2010).

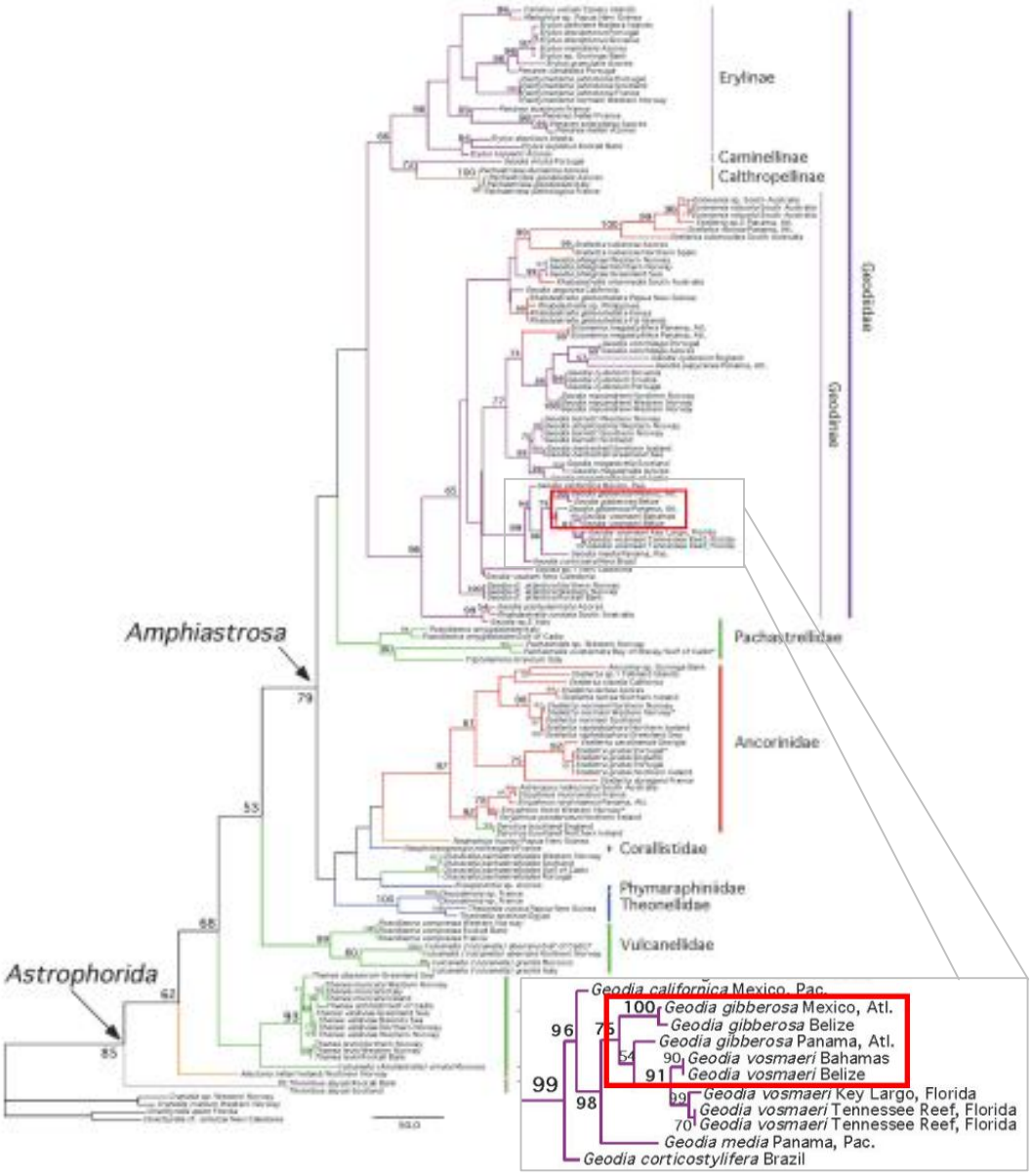


Figura 2. Filogenia molecular da ordem Astrophorida com os marcadores COX 1 e 28S, pelo método da máxima verossimilhança, com destaque para o parafiletismo de *G. gibberosa*. Adaptado de Cárdenas (2010).

2. OBJETIVOS

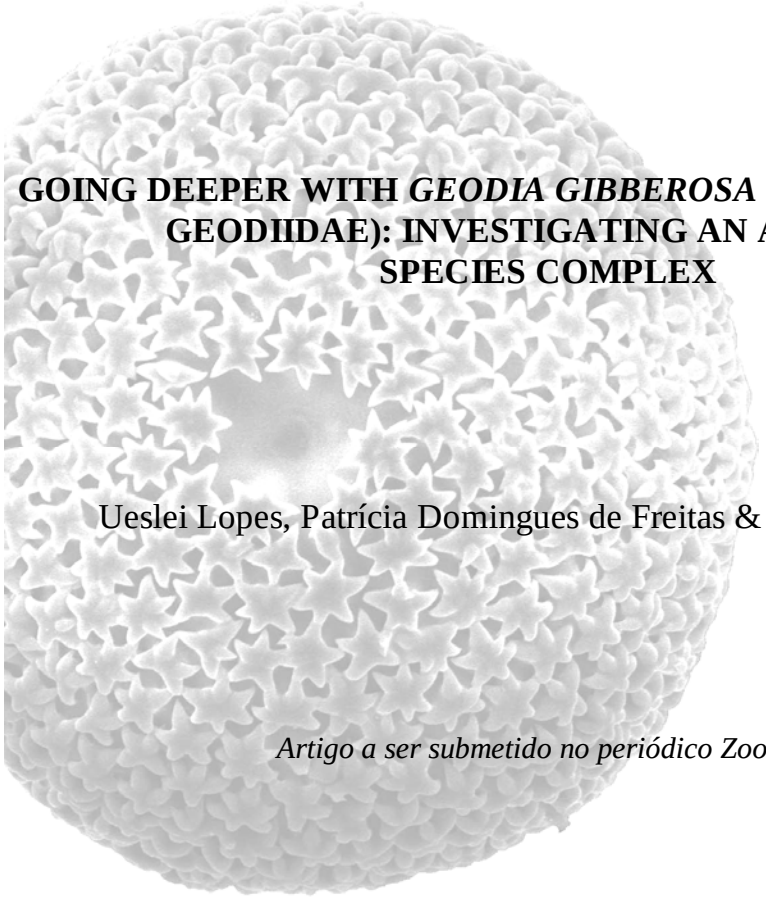
2.1. OBJETIVO GERAL

Caracterizar o polimorfismo de *G. gibberosa* Lamarck, 1815 e testar, por meio das análises morfológica e molecular, a hipótese desta representar um complexo de espécies.

2.2. OBJETIVOS ESPECÍFICOS

- Realizar amplo estudo taxonômico da espécie *G. gibberosa*, contemplando diversas localidades ao longo da costa brasileira e Caribe;
- Verificar o *status* taxonômico da espécie;
- Testar marcadores moleculares;
- Avaliar a variabilidade morfológica e molecular de distintas populações, visando a subsidiar estudo filogeográfico.

Capítulo 1



GOING DEEPER WITH *GEODIA GIBBEROSA* (ASTROPHORIDA, GEODIIDAE): INVESTIGATING AN ALLEGED SPECIES COMPLEX

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Going deeper with *Geodia gibberosa* (Astrophorida, Geodiidae): investigating an alleged species complex

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Abstract

Diverse studies applying molecular techniques have been revealing the existence of cryptic species of sponges, especially among those considered cosmopolitan or wide-distributed and with scarce morphologic features. *Geodia gibberosa* Lamarck, 1815 is registered for a large extension of the Western Atlantic Ocean, but contrary to many other species of poriferans, it presents a rich spicule repertoire and a significant variability in gross morphology, which brings difficulties to taxonomists to delimit its boundaries. We used COI and ATP6 mitochondrial markers and the classic taxonomic method of morphology to estimate the variability within *G. gibberosa*, aiming to test the hypothesis of the existence of cryptic species. Three distinct clades were found with both molecular markers, but no distinguishable morphologic pattern that could allow us to separate species was evidenced. Although our results suggested a low variability for the COI and ATP6 genes, these outcomes have matched with previous studies with poriferans, supportting the status of *G. gibberosa* as a species complex.

Key words: Marine sponge, Demospongiae, morphological variation, redescription, mitochondrial markers, cryptic species.

Resumo

Diversos estudos empregando técnicas moleculares têm detectado a presença de espécies crípticas em esponjas, especialmente naquelas consideradas cosmopolitas ou amplamente distribuídas e que de carecem caracteres morfológicos. *Geodia gibberosa* Lamarck, 1815 tem sido registrada ao longo do oceano Atlântico ocidental, mas diferentemente de outras espécies, esta possui um conjunto espicular rico e significativa variabilidade em sua morfologia externa, que culminam por dificultar a delimitação da espécie. Nesse trabalho foram utilizados os marcadores mitocondriais COI e ATP6, assim como os métodos clássicos de taxonomia morfológica, visando estimar a variabilidade dentro de *G. gibberosa* e, desse modo, testar a hipótese de existência de espécies crípticas. Três clados distintos foram obtidos com ambos os marcadores, mas nenhum padrão morfológico, que permitisse a separação de espécies, foi evidenciado. Mesmo os resultados sugerindo baixa variabilidade dos marcadores mitocondriais, estes se mostraram compatíveis com outros trabalhos já desenvolvidos com poríferos e permitem afirmar que *G. gibberosa* representa um complexo de espécies.

Palavras-chave: Esponjas marinhas, Demospongiae, variação morfológica, redescrição, marcadores mitocondriais, espécie crípticas.

1. Introduction

The poriferans are the most basal organisms within Metazoa and correspond to a highly diverse group of marine benthic communities, playing important roles in the coral reef system functioning (Bell, 2008). Yet, diverse species have been attracting the attention of the academy because of the pharmacological properties of their chemical compounds (Joseph & Sujatha, 2011; Sinko *et al*., 2012) and its potential application on human health (Frota Junior *et al*., 2012). Many of these organisms are usually characterized by the lack or paucity of proper morphologic traits, which increases significantly the difficulties for taxonomists to establish the borders of the species. The taxonomy of sponges based on morphologic features includes color, form, texture, type and size of the spicules as well as their skeletal arrangement, but they are not that reliable since these traits can vary under the influence of the environment (Bell *et al*., 2002; Carballo, 2006). Moreover, they may lead taxonomists to poor descriptions or misidentifications, and consequently bring serious implications to other types of studies which require the usage of well-identified specimens. The advent of the molecular techniques, however, has been responsible for the increasing number of species complex and cryptic species detection among poriferans (Solé-Cava, *et al*., 1992; Klautau *et al*., 1999; Xavier *et al*. 2010; Escobar *et al*., 2012; de Paula *et al*., 2012). It has been reported that wide distributed species, or so-called cosmopolitan ones, would actually be fruit of an overconservative taxonomy, where morphologically similar but evolutionary distinct species would be artificially named as the same, resulting on the underestimation of the real sponge biodiversity (Klautau, *et al*. 1999; Plotkin & Boury-Esnault, 2004).

Geodia gibberosa Lamarck, 1815 correspond to the type species of the genus and was the first Geodiidae described. The name of the genus came from the similarity between the rock cavities (also known as ‘geodes’) with the oscules which were grouped in depressions. In the literature, this taxon is considered as a wide distributed species, being registered in many different habitats and depths through the Western Atlantic Ocean (Silva, 2002). Contrary to many species of sponges, *G. gibberosa* does not present scarcity of features, but in contrast it is described by presenting significant morphologic plasticity, which led taxonomists to doubt its real boundaries (Hajdu *et al*., 1992). As an alternative to explain the polymorphism of this species, Cárdenas *et al*. (2009) postulated the hypothesis that *G. gibberosa* would represent a species complex, comprised by two or more cryptic

species, and suggested that a study allying both morphologic and molecular analyses should be conducted to test this.

Given that, we, thus, aimed with the present work (1) to develop a taxonomic study based on morphologic characters of the samples, obtained from almost the entire species' geographic distribution, and (2) concomitantly apply molecular markers to verify if *G. gibberosa* represents a species complex or simply corresponds to a species with remarkable variable morphologic features.

2. Materials and methods

2.1. Sampling and fixation

Specimens were collected by C. Menegola, J. C. Fernandéz, U. Lopes, J. G. B. De Marchi and V. Cedro in the cities of Salvador (Pituba, 13°00'32" S, 38°27'37" W; Barra, 13°00'36" S, 38°31'43" W), Maceió (Guaxuma, 09°35'29" S, 35°40'00" W), São Miguel dos Milagres (09°16'6.47" S, 35°21'40.27" W), João Pessoa (Ponta do Cabo Branco, 07°08'23" S, 34°47'47" W), and in Panama (Bocas del Toro, 09°16' 37.8" N , 82°10'16.2" W) in the intertidal zone. Vouchers of the collected samples were preserved in 96% ethanol in the field and the fixative was replaced two times at least, once after six hours and another later than 24 hours, so that the water could be removed completely (Cárdenas, personal communication). Vouchers were stored at -20° C until the DNA extraction. The entire specimens were preserved in 80% ethanol and deposited at the Porifera Collection at Museu de Zoologia of Universidade Federal da Bahia (UFBA-POR) for further morphologic analysis. Digital photos were taken both *in situ* and *ex situ* whenever possible. Materials from other localities were provided by some researchers as donation or by loaning from diverse institutions. Spicule measures of all the samples examined in the present study, as well as its respective localities, are listed in the Table 1.

2.2. Morphologic analysis

Dissociated spicules and thick sections mounts were obtained according to Mothes de Moraes (1985). Each dimension of 30 spicules per spicule type was measured unless otherwise noted (N: number of spicules measured) in the Table 1. Spicule measurements

were made with a Zeiss Axioplan 2 Imaging and Zeiss Axiovision release 4.5 software, and are given in µm as the following format: minimum – *mean* – maximum. Triaenes' measures are given in the subsequent order: shaft length/shaft width; cladome length/clad length/clad width. Rhabdome and clad width were measured at the base of these structures. Measures of microscleres are: total diameter/ray length/center diameter/ray width. For better observing details and ultrastructures, the spicules were coated with gold in the Denton Vacuum LLC Desk IV and later photographed with JEOL-JSM 6390LV scanning electronic microscopy at Centro de Pesquisas Gonçalo Muniz (Fundação Oswaldo Cruz, Salvador). Draws of megascleres were made with Adobe® Illustrator® CS5.

2.3. DNA extraction, PCR amplification and sequencing

Fragments from the choanosomal region of 44 samples were used to perform the DNA extraction, which followed a modified protocol of Sambrook, Fristch & Maniatis (1989) with phenol:chloroform:isoamyl alcohol (25:24:1). For a 25µl PCR reaction, it was used 50 – 100 ng of DNA template, 1 unit of Platinum® *Taq* polymerase (Invitrogen), 2,5µl of 10x PCR buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl), 2 mM of $MgCl_2$, 200 µM of dNTP, 0,4 µM of each primer, and 15 µl of sterile distilled water. The primers developed by Rua *et al.* (2011), ATP6porF (5'GTA GTC CAG GAT AAT TTA GG-3') and ATP6porR (5'-GTT AAT AGA CAA AAT ACA TAA GCC TG-3') were used to amplify over 450 bp sequence of the subunit 6 of ATP synthase (ATP6) gene. Approximately 550 bp of the subunit I of the cytochrome c oxidase (COI) was amplified using the universal primer pair LCO 1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO 2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'), which were described by Folmer *et al.* (1994). All PCR thermal cycles were ran in an Applied Biosystems Veriti® Thermal Cycler, under the following conditions: ATP6, 1 cycle [94 °C/3 min], 35 cycles [93 °C/1 min, 54 °C/1 min, 72 °C/1 min], 1 cycle [72 °C/7 min]; COI, 1 cycle [94 °C/5 min], 5 cycles [94 °C/30 s, 46 °C/45 s, 72 °C/1 min], 35 cycles [94 °C/30 s, 50 °C/45 s, 72 °C/1 min], 1 cycle [72 °C/10 min].

In addition, two specific internal primers for the COI partition were designed to amplify short fragments of 350 bp, especially for those samples of poriferans loaned from museums. They were manually designed and their quality was checked at OligoCalc (www.basic.northwestern.edu/biotools/oligocalc.html) (Kibbe, 2007). Primers and the conducted amplification conditions are described as the following: PorF1 (5'-CGG GTA

TGA TAG GTA CAG GGT T-3') and PorR1 (5'-TGA ATG TGC TTG TAC GCT CG-3'), 1 cycle [94 °C/5 min]; 35 cycles [94 °C/1 min, 60 °C/45 s, 72 °C/30 s]; 1 cycle [72 °C/10 min]. Second pair: PorF2 (5'-TCA GCT TTT GTT GAA CAA GG-3') and PorR2 (5`-CTT CTG GGT GTC CAA ARA AYC A-3'), 1 cycle [94 °C/5 min]; 35 cycles [94 °C/1 min, 48 °C/45 s, 72 °C/30 s]; 1 cycle [72 °C/10 min]. Excess dNTPs and unincorporated primers were removed from the PCR products with PEG, based on the protocol described by Lis & Schleif (1975) and further sequenced by *Macrogen, Inc*., in South Korea (www.macrogen.com). Additional sequences were obtained from the GenBank (www.ncbi.nlm.nih.gov): *G. gibberosa* (HM592723.1, EU442209.1).

2.4. Phylogenetic and distance analysis

Sequences were aligned with ClustalX v.2 according the default parameters (Larkin *et al.*, 2007) and edited with Bioedit v. 7.0.9.0 (Hall, 1999). Phylogenetic reconstructions based on the nucleotide sequences data set were performed under Maximum Likelihood (ML), Neighbor Joining (NJ) and Bayesian Inference (BI) using, respectively, PAUP* 4.0b 10 (Swofford, 2002), MEGA 5 (Tamura *et al*., 2011) and MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) softwares. The best-fit evolutionary model selected for both genes for ML and BI was HKY+G and was estimated for each independent gene under the Hierarchical Likelihood Ratio Tests (hLRTs) by Modeltest 3.7 (Posada & Crandall, 1998), which was implemented in PAUP*. The robustness of branches was verified with 1000 bootstrap replicates and values >80 were considered high enough to support the clades in ML and NJ reconstructions. ML trees were calculated using heuristic searches and a tree bisection and reconnection (TBR) branch swapping algorithm, and NJ analysis was performed with uncorrected *p*-distances with complete deletion of bases containing missing data and gaps. Four Markov chains were run for 3 million generations and sampled every 300 generations, with burn-in of 9001. In BI, posterior probabilities superior to 0.95 were consistent for the supporting of the clades. Additional sequences of different species of *Geodia* were obtained from GenBank and used to calculate *p*-distances among clades in MEGA 5 with 1000 bootstrap replicates, intending to estimate values of genetic distances inside the genus. *Geodia vosmaeri* (Sollas, 1886) and *Pachymatisma johnstonia* Bowerbank in Johnston, 1842 were chosen as outgroup based on the latest molecular phylogeny of Geodiidae (Cárdenas *et al*., 2010). Also, COI sequences that were submitted by researchers with a track record in *Geodia* (or related species) were collected

from GenBank to estimate the genetic distances within the genus. Nevertheless, only two sequences of *Geodia vosmaeri* for ATP6 gene were available at GenBank, which hampered the chances to avaluate the genetic distances within *Geodia*.

2.5. Abbreviations

CNPGG (Colección Nacional del Phylum Porifera Gerardo Green, Universidad Nacional Autónoma de Mexico, Mexico); FIOCRUZ (Fundação Oswaldo Cruz: Centro de Pesquisas Gonçalo Muniz, Salvador); UFBA (Universidade Federal da Bahia, Salvador); UFBA-POR (Porifera Collection, Museu de Zoologia, Universidade Federal da Bahia); UFC (Universidade Federal do Ceará, Fortaleza); UFSCar (Universidade Federal de São Carlos, São Carlos); MCN (Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil); MNHN (Muséum National d'Histoire Naturelle, France); USNM (Smithsonian Institution, National Museum of Natural History, USA); MNRJ (Museu Nacional do Rio de Janeiro, Brazil); ZMA POR (Instituut voor Systematiek en Populatiebiologie, Zoölogisch Museum, Porifera Collection, Holanda); ZMBN (Universitetsmuseet i Bergen, Universitetet i Bergen, Norge).

3. Morphologic results

Systematic description (obtained from Cárdenas *et al*., 2009)

Genus *Geodia* Lamarck, 1815

Geodia gibberosa Lamarck, 1815

Synonyms (from Cárdenas *et al*., 2009).

Geodia gibberosa Lamarck, 1815, 334.

Pyxitis gibberosa Lamarck, 1815 Schmidt 1870, 70.

Geodia (Geodia) gibberosa Lamarck, 1815 Hechtel 1965 68, pl. VIII, fig. 2.

Geodia cariboea Duchassaing de Fonbressin and Michelotti, 1864 (in part) 105, pl. XXV, fig. 8.

Geodia tumulosa Bowerbank, 1872 628, pl. XLVII.

Geodia media Bowerbank, 1873 (non *G. media* von Lendenfeld, 1910) 13, pl. II.

Geodia dysoni Bowerbank, 1873 14, pl. III.

Geodia reticulata Bowerbank, 1874 300, pl. XLVI, figs. 14–20.

Sidonops stromatodes Uliczka, 1929 54, figs. 51–56, pl. I, fig. 10.

Geodia media var. *leptoraphes* Uliczka, 1929 56, figs. 57–67, pl. I, fig. 11.

Geodia flexisclera Pulitzer-Finali, 1986 76, figs. 10–11.

Holotype. MNHN DT–608, dry, French Guiana

Material examined. *Geodia gibberosa*, MNHN DT–608, schizoloholotype (MCN 4757), French Guyana; USNM 33314, off South Carolina, USA, 32°49’24” N/ 78°39’12” W, South Carolina Marine Resources coll., 12.II.1981, 33m depth; ZMAPOR 17978, Georgia, USA, 31°36’00” N/ 80°47’25” W, R. Ruzicka coll., 23.VIII.2004; ZMAPOR 17983, Georgia, USA, 31°24’4” N/ 80°52’8” W, R. Ruzicka, 27.VIII.2004; USNM 48248, off South Georgia, 31°23’42”N/ 80°53’06” W, Georgia Marine Resources for Minerals Management Service Coll., 04.III.1981, 17m depth; USNM 42560, Naples, Florida, USA, 25°46’01” N/ 82°23’49” W, Continental Shelf Associates coll., 12.II.1982, 26.1m depth; CNPGG 078, Yucatán, Mexico, Justo Sierra coll., 00.IV.1985; CNPGG 1203, Yucatán, Mexico, Justo Sierra coll., 25.IV.1985; CNPGG 1191, Yucatán, Mexico, Justo Sierra coll.; 18.X.1985; USNM 32866, Columbus Cay, Belize, I. Macintyre coll., 26.IV.1979, 10-15m depth; USNM 32872, Carrie Bow Cay, Blue Hole, Belize, G. Hendler coll., 21.III.1979, 18-27m depth; UFBA-POR 3627, Bocas del Toro, Panama, Carla Menegola coll., 29.VII.2010, 0.1 m; ZMBN 77928 and ZMBN 81780, Bocas del Toro, Paco Cárdenas coll., 0.5-1m depth; UFBA-POR 3061, Archipeago Sabana-Camagüey, Cuba, 23°15’58” N/ 80°55’59” W, Z. Marcos coll., 16.III.2001, 1m depth; UFBA-POR 3062, Arquipélago Sabana-Camagüey, Cuba, 22°04’13” N/ 81°33’39” W, P. Alcolado coll., 15.VIII.1980, 1m depth; UFBA-POR 3071, Norte da Cayeria de San Felipe, Cuba, 21°57’56” N/ 83°34’05” W, P. Alcolado coll., 20.X.1968, 22m depth; UFBA-POR 3622, UFBA-POR 3623 and UFBA-POR 3624, Oeste de Grand Cul de Suc, Guadeloupe (France) 16°20’30” N; 61°41’18”W, P. Alcolado coll., 2010, 0.5m depth; ZMAPOR 17737, Martinica, France, 15°59’36” N/ 61°24’12” W, P. T. Michaelis coll., 16.VI.2002; UFBA-POR 4140, Praia dos Dois Coqueiros, Caucaia, Ceará, 03°41’30”S/ 38°36’10”W, C. Licarião and T. Soraya colls., 07.X.2010; MNRJ 14755, Praia dos Dois Coqueiros, Caucaia, Ceará, 03°41’30”S/

38°36’10”W, S. Salani coll., 15.I.2010; MNRJ 9148, MNRJ 9149, MNRJ 9150, MNRJ 9151 and MNRJ 9154, Maracajaú, Rio Grande do Norte, Brazil, 24.IX.2004; UFBA-POR 4041, UFBA-POR 4042, UFBA-POR 4043 and UFBA-POR 4044, Ponta do Cabo Branco, Paraíba, Brazil, U. Lopes and J.G.B. DeMarchi colls., 19.III.2011, 0.1 - 0.5m depth; UFBA-POR 3273, São Miguel dos Milagres, Alagoas, Brazil, C. Menegola coll., 15.II.2010, 0.5m depth; UFBA-POR 4045, Guaxuma, Maceió, Alagoas, Brazil, V. Cedro coll., 19.II.2011; UFBA-POR 4046, UFBA-POR 4047, UFBA-POR 4048, UFBA-POR 4049, Pituba, Salvador, Bahia, Brazil, Coll. U. Lopes, 02.V.2011, 0.1-0.5m depth; MNRJ 8396, Salvador, Bahia, Brazil, E. Hajdu and C. Santos colls., 07.VI.2004, 5 - 12,5m depth; UFBA-POR 964, Capitania dos Portos, Salvador, Bahia, Brazil, 02.VI.1988; UFBA-POR 4150, Mar Grande, Veracruz, Bahia, Alexandre Borges coll., 12°58’22” S/ 38°36’33”W, 0-1m depth; UFBA-POR 1817, Pedra Pequena, Veracruz, Bahia, Brazil, 09.VI.2004; MNRJ 287, São Sebastião, São Paulo, Brazil, 40°45’3.6” N/ 73°59’ 0.24” W, E. Hajdu and G. Muricy, 24.I.1996, 10 - 15m depth.

Description. External morphology of the samples. Mostly massive with tendency to become lobate, but globose and encrusting shapes were found (Figure 1 A-F). Color is brownish-green in mangroves, and varies from light brown to whitish with beige choanosome in rocky shores. After fixation the color changes to brownish yellow, grey, purple or remains whitish, and the oscular regions are usually darker than the rest of the body (Figure 1 D-H). The surface is usually smooth to the touch and micro-hispid around the oscules. The sponges have a hard consistency and its interior is compact. Oscules (1 - 2 mm) are uniporal, individualized by its sphincter, circular or ovoid, and frequently clustered in lobes (diameter = 12 mm) (Figure 1G-H, K). Pores can be both uni- or cribriporal (0.1 - 0.2 mm) and do not follow any distributional pattern throughout the surface (Figure 1 I-J).

Skelenton. The cortex (175 - 1150 μm) is subdivided into a thin ectocortex of acanthospheroxyaster II, usually irregular, and a thick endocortex of sterrasters. A third thin layer of sponging fibers (60 - 75 μm), located right under the endocortex, may occur. Bellow the cortex, bundles of oxeas and triaenes are radially organized in the choanosome, becoming a bit confused towards the inner portion. Strongyloxeas are found in the choanosome, but they are mostly positioned around the oscules. Abundant acanthoxyasters and acanthospheroxyasters II, as well as developing and mature sterrasters, are scattered

throughout the choanosome while acanthospheroxyasters I are rare and placed under the cortex (Figure 1 K-L).

Spicules. Megascleres. (a) choanosomal oxea (Figure 2A-D), stout or slender, straight or slightly curved, with acerate, blunt or hastate tips; (b) cortical strongyloxea (Figure 2E), fusiform, slender, mostly straight with hastate tip; (c) ortotriaene often transitional to plagiotriaene (Figure 2F-G). Rhabdome, stout or slightly curved with hastate or blunt tip. Cladome with cladi mostly forward directed, directed at right angles to the rhabd or bent; (d) anatriaene (Figure 2H), choanosomal, rare. Rhabdome, long, slender, slightly curved. Cladome, minute, with short cladi sharply downward curved; (f) protriaene (Figure 2I), choanosomal, rare. Rhabdome, long, slender, slightly curved with hastate tip. Cladome with slender cladi sharply curved forward from the rhabdome; (g) plagiotriaene (Figure 2J), choanosomal, rare, slender. Rhabdome, mostly straight with hastate tip. Cladome, cladi forward directed with hastate tips.

Microscleres. (a) sterraster (Figure 3A-C), mostly ovoid or spherical with 3 - 10 smooth star-like branched tubercules; (b) acanthoxyaster (Figure 3D), minute center, 5 - 10 rays with up to 30 spines; (c) acanthospheroxyaster I (Figure 3E), rare, medium center, 13 - 14 rays with up to 20 spines; (d) acanthospheroxyaster II (Figure 3F), robust center with minute spined rays.

Habitat of the collected samples. Panama: on mangrove roots, ca. 1m depth. Brazil: on rocky shores, in crevices or under rocks, 0.1 - 0.5m depth.

Ecology. *G. gibberosa* seems to be very palatable to turtles and parrotfishes (Pawlik *et al*., 1995; Dunlap & Pawlik, 1996; 1998; Wulff, 2000) which explains the fact of this species usually be found in hidden habitats. In the Caribbean, *G. gibberosa* is usually found in mangroves while the Brazilian individuals are frequently found in crevices or under rocks. They are usually associated to algae, *Palythoa* sp. and other invertebrates, such as echinoderms, polychaetans and sponges.

Geographical distribution. USA, North Carolina (Wells *et al*., 1960), South Carolina (Schmidt, 1870), Florida (Little, 1963; Engel & Pawlik, 2005), Georgia (Freeman *et al*., 2007), Texas (Riggs *et al*., 1998), California (Sim & Bakus, 1986); Mexico (Topsent, 1889; Winfield & Ortiz, 2010); Belize (Rützler *et al*. 2000); Cuba (Alcolado, 1976; Sardiñas & Alcolado, 2004; Alcolado *et al*. 2004; Díaz & Rützler, 2009); Jamaica (Bowerbank, 1872; Hechtel, 1965; Wulff, 2000; Díaz & Rützler, 2009); St. Thomas (Duchassaing & Michelotti, 1864); Tortola (Duchassaing & Michelotti, 1864) ; Dominican Republic

(Bowerbank, 1873); Puerto Rico (Bowerbank, 1873; Sollas, 1888; Pulitzer-Finali, 1986); St. John (Uliczka, 1929); Barbados (Uliczka, 1929; van Soest & Stentoft, 1988); Bahamas (de Laubenfels, 1949; Wiedenmayer, 1977); Bermudas (de Laubenfels, 1950); Guadeloupe (Díaz & Rützler, 2009); Martinique (Bowerbank, 1873; Sollas, 1888); Honduras (Bowerbank, 1873); Panama, Atlantic coast (de Laubenfels, 1936; Wulff, 2000; Cárdenas *et al*. 2009); Panama, Pacific coast (de Laubenfels, 1936); Colombia (Díaz & Zea, 2008); Curaçao (Arndt, 1927; van Soest, 1981); Saba (Silva, 2002); Venezuela (Carter, 1882; Wulff, 2000; Díaz & Rützler, 2009); St. Vicent (Carter, 1882); French Guiana (Lamarck, 1815); Brazil, Ceará, Dois Coqueiros Beach (present study); Rio Grande do Norte, Maracajaú (Muricy *et al*. 2006); Paraíba, Ponta do Cabo Branco (present study); Alagoas, São Miguel dos Milagres (present study); Alagoas, Maceió (present study); Bahia, Salvador, Praia da Pituba (present study); Bahia, Salvador, Capitania dos Portos (present study); Bahia, Veracruz, Pedra Pequena (present study); Bahia, Veracruz, Mar Grande; São Paulo, São Sebastião (Rangel *et al*., 2001) (Figure 4); São Tomé, West Africa (Topsent, 1918; Lévi, 1959).

Bathymetrical distribution. From 0.1m (Salvador, Bahia, Brazil) to 33m (off South Carolina) (present study).

Remarks. This is the first study with *G. gibberosa* which included samples from almost the entire species geographic distribution, encompassing the region from South Carolina, USA, to São Sebastião, Brazil. Specimens with the massive, encrusting and globular morphotypes were analyzed, but the former shape seemed to be more common. Massive and encrusting samples were both found in crevices or under rocks. The oscules are uniporal, and pore arrangements are both uni- and cribriporal, reinforcing previous suggestions that the arrangement of the incurrent and excurrent canals does not seem to be a reliable character to separate genera in Geodiidae (Hajdu *et al*., 1992; Silva, 2002; Cárdenas *et al*., 2009). The three-layered cortex once noticed by Hechtel (1965) and Wiedenmmeyer (1977) were seen in the specimens from Guadeloupe (France) (UFBA-POR 3622, UFBA-POR 3623 and UFBA-POR 3624), but the layer of fibers was gradually discontinuous. The measurements of the spicules were in accordance with previous studies of the species (Sollas, 1888; Wells *et al*., 1960). Additional categories of triaenes, such as ana-, pro-, meso- and promesotriaenes have already been observed in *G.gibberosa* (Hechtel, 1965; van Soest & Stent, 1988; Silva 2002 and Cárdenas *et al*., 2009), but this is the first work which registers anatriaenes to the holotype. Although the rhabdome of these spicules

were broken, it is clearly different from those seen in *Geodia papyracea* Hechtel, 1965 (which are minute and more abundant), and its cladi are much shorter when compared to those in *Geodia glariosa* Sollas, 1886, *Geodia australis* Silva & Mothes, 2000 and *Geodia riograndensis* Silva & Mothes, 2000. Rare protriaenes were found in 25% of the samples analyzed, but was not seen in the holotype which probably is related to the small size of the fragment obtained from the museum. Furthermore, rare and slender plagiotriaenes are noticed for the first time to the species, being present in 28 specimens of the 44 observed (including the holotype). This spicule differs from the transitional variations of orthotriena essentially in size, with the rhabdome lengh at least two times smaller, and the form of the cladome, which cladi are upward directed. The recurrent description of a second category of oxea (Sollas, 1888; Hechtel, 1965; van Soest & Stentoft, 1988; Muricy *et al*., 2006; Muricy *et al*., 2008; Hajdu *et al*., 2012) should be properly switched to cortical strongyloxea, since this fusiform spicule has a blunt end. Additionally, the term strongylaster should be standardized as acanthospheroxyaster II in accordance to the prominent spine at their tips and the robust sphere-like center presented by the spicule. Also, it was noticed that the acanthospheroxyaster II varies significantly in morphology, but not in size, which allows us to assume that, as soon as the spicule gets mature, the center increases and rays tend to be minute (Figure 3F). That, however, cannot be affirmed for sure once no study of spicule formation was developed in the present work.

4. Molecular results

The resulting dataset for COI partition comprised 32 sequences of 551 bp, which 45 sites were parsimony informative. Although the ATP6 showed less variation than COI with 29 parsimony informative sites, this dataset only consisted of 10 sequences of 422 bp. Four distinct clades within *G. gibberosa* were found for the COI partition (Figure 5), but given we did not manage to amplify the DNA of all individuals for ATP6 (Figure 6), just three groups and one specimen of the outgroup (I, II, III and *P. johnstonia*) could be verified and will be used here only for comparison. The posterior probabilities as well as the values of bootstrap for ML and NJ methods are written above each branch, for values higher than 50%, when in congruence with the BI trees. All individuals of the Clade I were collected in the coast of Salvador city, except the specimen A121, which was sampled in the

Ilha dos Frades (domain of the same city). Clade II comprises specimens from the U.S.A., Mexico, Guadeloupe and Northeastern Brazil (from Maceió to Caucaia), but also includes one sample from Salvador, Ilha de Vera Cruz and São Sebastião each. A specimen with a very thick cortex from Mexico (A314) presented the highest divergence value among the clades of the COI tree and was kept apart from the Clade III, which comprises individuals from Panama, exclusively. The sample A316 was just amplified with the ATP6 and clustered in Clade II in the ATP6 topology. The well-supported Clade IV includes specimens from Belize and Georgia. Genetic divergence values found for both genes were quite similar, ranging from 3.6% - 6.4%, and 2.8% - 5.8% in COI and ATP6 tree, respectively. Maximum intra-group divergence was detected in the Clade IV with 0.7% for COI, and 0.3% for ATP6 in the first clade. Divergence between ingroup and outgroup for COI was over 10% whereas for ATP6 it was 8.4% (Table 2).

5. Discussion

Unlike many poriferans which lack proper morphologic characters (Erwin & Thacker, 2007; Redmond & McCormack, 2008; Erpenbeck *et al*., 2012), species of Astrophorida possess a significant spicule repertoire (Cárdenas *et al*., 2011), and this fact theoretically should facilitate their identification/characterization. Our morphologic results describe *G. gibberosa* as possessing at least three different morphotypes, with six different categories of megascleres and four of microscleres, but some of these spicules somehow differ significantly in abundance. Diverse studies have been demonstrating that sponge morphologic features, such as gross morphology and spicule sizes may change under the influence of environmental factors (Mercurio *et al*., 2000; Bell *et al*., 2002; Meroz-Fine *et al*., 2005; Cavalcanti *et al*., 2007; Massaro *et al*., 2012), which could explain the variability addressed to *G. gibberosa* hitherto. But, specimens collected in nearby localities were both variable in gross morphology (e.g. those from João Pessoa, which were massive and encrusting) and even in spicule repertoire (e.g. the samples from Salvador) so that any pattern that could allow us to separate species morphologically, thus, was not evidenced.

A plausible explanation for the infrequency of some categories of spicules such as ana- and protriaenes could be related to its non-structural function, differently from oxeas,

orthotriaenes and sterrasters which play an important role in this subject. Moreover, Burton (1949), in his study about the development of *Geodia barretti* Bowerbank, 1958, noticed that these triaenes remained the last ones to be produced by that species (what might occur to *G. gibberosa* as well). Regarding what we call secondary triaenes (here represented by the ana-, pro- and the minute plagiotriaenes), it is important to note that, in all 38 specimens where these spicules were present, there was often a combination of just two of each categories. This stresses, in our opinion, the complementary function of these spicules to provide mechanical reinforcement of the choanosome and support of the cortex, particularly the ectocortex. On the other hand, the scarcity of acanthospheroxyasters I and the minute plagiotriaene should be further investigated.

In the contrary, our molecular results based on the mitochondrial DNA (mtDNA) suggest that *G. gibberosa* represents a species complex, comprised by three distinct species, at least. The genetic distances for COI fragment observed in the present work were not as high as those found by Blanquer & Uríz (2007) (up to 22%), but they matched with previous studies with *Cliona celata* Grant, 1826 developed by Xavier *et al*. (2010) and de Paula *et al*. (2012) (*p*-distance varied between 6% and 8% and between 2% and 8%, respectively), and also were quite similar to the divergence values detected in other invertebrates (Carmona *et al*., 2011; Neusser *et al*., 2011; Hemery *et al*., 2012). The values of genetic divergences found in the genus (uncorrected *p*-distance between 1.1% to 8.2%) (see Appendix A) may be considered insufficient to separate species, since diverse authors have already stressed the slow evolution of the COI partition for some basal marine invertebrates (Wörheide, 2006; Huang *et al*., 2008; Bucklin *et al*., 2010). However, the low intraspecific distances within the clades (less than 1%) followed by the high support of the branches and the congruence between both topologies encourage us to accept the Cárdenas's hypothesis (2009).

Even our molecular results clearly suggesting the split of *G. gibberosa* into distinct genetic groups, it is curious to realize that the Clade II remained distributed through the same geographic extension occupied by the so-called *G. gibberosa*, whereas clades I and III are restricted to one single area each. This observation is contrary to the current consensus about statuses of broadly distributed species, especially when we consider the low dispersal capacity of the sponge larvae (Maldonado, 2006). Maybe this pattern presented by the Clade II could be explained by the maintenance of an uninterrupted gene flow, or this distribution could be a fruit of anthropic actions. Its occurrence in some places in Salvador

and in São Sebastião could also support the latter hypothesis, given that these regions represent important Brazilian harbor areas. Both Clade I and II seem to coexist in the same region, but the high distances between the former and the other clades might be an evidence of reproductive isolation (Xavier *et al*., 2010), or its occurrence in one single area could be an effect of subsampling. Regarding the restricted distribution of the Panamanian clade, which comprises only individuals from mangrove, Duran & Rützler (2006) already pointed out that environmental differences and disparities of ecological interactions could indicate great potential for ecological speciation between habitats. The Yucatán samples were clustered in different clades and the sample 314 presented the highest genetic divergences. We suggest that the population of that locality should be proper investigated in the future, as well as more collections should be provided to stablish the status of the Clade IV.

6. Conclusion

Additionally to the variation in gross morphology already described in the literature, our results revealed that *Geodia gibberosa* possesses officially more spicule categories than expected, and that a rich spicule repertoire does not necessarily represent a synonymous of easy identification, since any morphological pattern could be established to separate it into different species. On the other hand, via molecular analysis, it was possible to conclude that at least three genetically distinct species comprise the so-called *G. gibberosa* and made possible to realize how the diversity within this complex was underestimated. In an era of a technologic advancement and the resurrection of a new taxonomy, we not only encourage the usage of molecular techniques in solving questions like this, but also stimulate the application of other sources (e.g. embryological, histological, biochemical, etc.) with the intention to get closer to the real extant biodiversity.

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Table 1. Micrometries of the spicules of *Geodia gibberosa* Lamarck, 1815. Triaenes measures are shaft length/shaft width; cladome length/clad length/clad width. Measures of microscleres are total diameter/ rays length/center diameter/ rays width. Measures are in µm (N=30). Means are in *italic*. n.o.. =not observed. Acanthoxy.= acanthoxyaster. Acantho. I= acanthospheroxyaster I. Acantho. II= acanthospheroxyaster II.
(*) specimens with amplified DNA.

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
-	French Guiana	Holotype MNHN DT-608 (Schyzoholoty-pe MCN 3639)	1411 - *1656.2* - 1907/ 16 - *22.8* - 31	111 - *209.1* - 315/ 3 -*5.4* -8	1150 - *1316.7* - 1600/ 26 - *31* - 37.6/ 300 - *380* - 440/ 212.8 - *230.5* - 239.4/ 21.3 - *23.9* - *26.6*	- - -/ 10.6 - 10.6 - 10.6/21.3 - 22.6 - 23.9 / 5.3 - 6.7 - 8/ 4 - 5.8 - 7 [N=2]	n.o.	58 (broken)/ 8 / 40 / 24/ 5.3 [N=1]	66 - *82.3* – 98	18 - *23.1* - 38/ 8 - *10.3* -17/ 2 - *3.5* -9/ 1 - 1.2 - 2	n.o..	4 - *5.8* - 7
A336	Off South Carolina, USA	USNM 33314	990 - *1251.7* - 1720/ 12.5 - *18.3* - 27.5	175 - *244.6* - 312.5/ 2.5 - *4.6* - 7.5	900 - *1073.3* - 1200/ 15 - *22.5* - 30/ 320 - *472.5* - 620/ 160 - *232.1* - 287.5/ 12.5 - *19.8* - 30 [N=12]	n.o.	n.o.	470 - *630* - 790/ 10 - *11.3* - 12.5/ 100 - *115* - 130/ 37.5 - *52.5* - 67.5/ 7.5 - *8.8* - 10 [N=2]	42.5 - *47* - 52.5	9.6 - *26.9* - 32.2/ 8.4 - *12.5* - 15.4/ 2.8 - *2.9* - 4.2/ 1.4 - *1.4* - 1.4	11.2 - *12.6* - 14 [N=2]	4.2 - *6.3* - 11.2
A326	Georgia, USA	POR 17978	780 - *1112.7* - 1400/ 10.6 - *20.2* - 29.3	172.9 - *214* - 247.4/ 2.7 - *5.6* - 8	670 - *937.3* - 1250/ 10.6 - *22.2* - 31.9/ 150 - *397.7* - 600/ 79.8 - *208.3* - 319.2/ 10.6 - *18.5* - 26.6	1330 - *1590* - 1810/ 8 - *10.6* - 16/ 30 - *35* - 40/ 13.3 - *13.3* - 13.3/ 5.3 - *10* - 13.3 [N=4]	n.o.	430 / 18.6/ 130/ 79.8/ 16 [N=1]	39.9 - *50* - 66.5	17.3 - *21.9* - 27/ 7.6 - *10.7* - 13/ 2.2 - 3.7 - 5.4/ 1.1 - *1.4* - 2.2	n.o.	4.3 - *5.4* - 6.5

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A327*	Georgia, USA	POR 17983	740 - 1077.7 - 1460/ 8 - 15.3 - 21.3	172.9 - *218* - 255.4/ 2.7 - *5.4* – 8	600 - *863.3* - 1200/ 13.3 - *19.1* - 29.3/ 140 - *372.5* - 540/ 87.8 - *201.4* - 316.5/ 10.6 - *16.3* - *23.9* [N=24]	n.o.	n.o.	320 - *423.3* - 500/ 10.6 - *12.4* - 13.3/ 80 - *93.3* - 100/ 47.9 - *57.6* - 66.5/ 8 - *8* – 8	37.2 - *45.2* - 53.2	10.8 - *21.1* - 31.3/ 5.4 - *10* - 19.4/ 2.2 - *3.5* - 5.4/ 1.1. - *1.1* - 1.1	10.8 - *11.9* - 14 [N=4]	4.3 - *5.7* - 8.6
A340*	Off Georgia, USA	USNM 48248	930 - *1155.7* - 1540/ 13.3 - *20* -26.6	178.2 - *231.8* - 274/ 2.7 - *6.2* – 8	800 - *1021.1* - 1200/ 21.3 - *24.5* - 31.9/ 260 - *404.4* - 560/ 159.6 - *222.3* - 279.9/ 18.6 - *22.5* - 26.6 [N=9]	n.o.	n.o.	340 / 5.3/ 50/ 23.9/ 5.3 [N=1]	42.6 - *53.8* - 63.8	10.8 - *15.6* - 21.6/ 5.4 - *24.5* - 31.9/ 1.1 - *2.4* - 5.4/ 1.1 - *1.2* - 2.2	8.6 - *10.5* - 14 [N=4]	4.3 - *5.4* - 6.5
A335*	Naples, Florida, USA	USNM 42560	1120 - *1269.7* - 1470/ 16 - *25.4* - 31.9	220.8 - 268.4 - 335.2/ 5.3 - *7.1* – 8	780 - *1187.3* - 1600/ 21.3 - *30* - 45.2/ 200 - *422.7* - 620/ 101.1 - *224.2* - 345.8/ 16 - *23.5* - 31.9 [N=11]	n.o.	n.o.	n.o.	55.9 - *67.5* - 75.4	16.2 - *20.4* - 23.8/ 5.4 - *9.2* - 10.8/ 2.2 - *2.8* - 5.4/ 1.1 - *1.1* - 1.1	n.o..	4.3 - *5.2* - 6.5
A314*	Yuccatán, Mexico	CNPGG 078	1260 - *1805.8* - 2158/ 20 - *34.2* - 45.2	276.6 - *373.4* - 505.4/ 5.3 - *9.8* - *13.3*	1000 - *1526.4* - 1850/ 29.3 - *40.9* - 47.9/ 420 - *630.7* - 950/ 186.2 - *348.3* - 500/ 29.3 - *35.7* - 40 [N=14]	n.o.	n.o.	n.o.	79.8 - *104.5* - 114.4	14 - *21.6* - 29.2/ 5.4 - *9.7* - 13/ 2.2 - *3.6* - 5.4/ 1.1 - *1.3* - 2.2	n.o.	5.4 - *6.8* - 10.8

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A315*	Yuccatán, Mexico	CNPGG 1203	1060 - *1381.3* - 1660/ 12.5 - *26.4* - 42.5	137.5 - *201* - 257.5/ 2.5 - *3.9* – 5	850 - *1317.8* - 1550/ 23.9 - *40.6* - 55.9/ 220 - *489.6* - 660/ 106.4 - *252* - 359.1/ 29.3 - *36.8* - 47.9 [N=23]	n.o.	n.o.	700 - *742.5* - 800/ 16 - *18.6* - 23.9/ 100 - *117.5* - 150/ 50.5 - *62.5* - 79.8/ 13.3 - *16.6* - 21.3 [N=4]	60 - *70.6* - 77.6	18.4 - *23.4* - 32.4/ 7.6 - *11.1* - 16.2/ 2.2 - *3.5* - 4.3/ 1.1 - *1.1* - 2.2	n.o.	4.3 - *6.3* - 8.6
A317	Yuccatán, Mexico	CNPGG 1191	620 - 1066.7 - 1340/ 16 - *35.1* - 71.8	266 - *455.4* - 620/ 5.3 - *11.1* - 13.3	810 - *1193.6* - 1450/ 13.3 - *15.6* - 21.3/ 180 - *257.9* - 340/ 53.2 - *78.5* - 106.4/ 10.6 - *13.3* - 23.9 [N=14]	1370 - *1462.5* - 1500/ 21.3 - *27.3* - 37.2/ 100 - *112.5* - 120/ 45.2 - *63.8* - 79.8/ 16 - *20.6* - 26.6 [N=4]	155 0 - *1975* - 2400/ 21.3 - *27.3* - 37.2/ 100 - *112.5* - 120/ 45.2 - *63.8* - 79.8/ 2.7 - *4* - 5.3 [N=2]	n.o.	55.9 - *72.8* - 106.4	16.2 - *25.2* - 64.8/ 5.4 - *11.6* - 32.4/ 2.2 - *3.1* - 7.6/ 1.1 - *1.1* - 2.2 [N=20]	14 - *16.2* - 18.4 [N=4]	5.4 - *8.2* - 13
A341*	Belize	USNM 32866	690 - *1174.5* - 1390/ 10.6 - *17.9* - 31.9	207.5 - *278.5* - 361.8/ 2.7 - *4.8* – 8	400 - *961.9* - 1400/ 13.3 - *23.9* - 42.6/ 160 - *315.2* - 600/ 79.8 - *170.4* - 311.2/ 10.6 - *20.2* - 34.6	n.o.	n.o.	310 - *370* - 400/ 2.7 - *8* - 10/ 30 - *46.7* - 60/ 18.6 - *31.9* - 39.9 [N=3]	85.1 - *95.8* - 105.6	16.2 - *23* - 32.4/ 7.6 - *10.8* - 16.2/ 1.1 - *3.1* - 5.4/ 1.1 - *1.1* - 2.2	10.8 - *10.8* - 10.8 [N=2]	3.2 - *6* - 8.6
A342	Belize	USNM 32867	890 - *1156.2* - 1370/ 13.3 - *21.7* - 29.3	186.2 - *253.2* - 305.9/ 5.3 - *6.8* – 8	1040 - *1317.5* - 1630/ 18.6 - *29.8* - 45.2/ 440 - *576* - 700/ 212.8 - *295.7* - 489.4/ 16 - *25.8* - 34.6	n.o.	1800 - *2010* - 2220/ 8 - *9.3* - 10.6/ 140 - *195* - 250/ 39.9 - *67.8* - 95.8/ 5.3 - *8* - 10.6 [N=2]	330 - *700* - 950/ 10.6 - *14.6* - 16/ 80 - *120* - 150/ 53.2 - *67.2* - 79.8/ 10.6 - *12.6* - 13.3 [N=4]	74.5 - *87.7* - 103.7	15.1 - *21.8* - 33.5/ 5.4 - *9.5* - 16.2/ 2.2 - *3* - 4.3/ 1.1 - *1.1* - 1.1	n.o..	4.3 - *5.4* - 6.5

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A343	Belize	USNM 32872	970 - *1232.7* - 1530/ 13.3 - *25.2* - 39.9	194.2 - *223.6* - 268.7/ 2.7 - *6.3* – 8	750 - *1032.6* - 1300/ 21.3 - *36.6* - 50.5/ 320 - *437.4* - 640/ 146.3 - *237.7* - 353.8/ 18.6 - *28.4* - *34.9*	n.o.	1420 - *1771* - 1920/ 5.3 - *7.2* - 8/ 40 - *106* - 280/ 10.6 - *48.1* - 79.8/ 5.3 - *5.9* - 10.6 [N= 10]	240 - *412.5* - 670/ 8 - *14.6* - 23.9/ 50 - *95* - 200/ 21.3 - *48.5* - 101.1/ 5.3 - *7.2* - 8 [N=4]	58.5 - *70.4* - 85.1	14 - *19.9* - 24.8/ 5.4 - *8.4* - 10.8/ 2.2 - *2.9* - 5.4/ 1.1 - *1.2* - 3.2	10.8 - *12.7* - 16.2	4.3 - *5.8* - 7.6
A316*	Bocas del Toro, Panama	UFBA 3627	1050 - *1403.7* - 1510/ 18.6 - *31.6* - 39.9	159.6 - *207.7* - 263.3/ 2.7 - *6.4* – 8	770 - *1172* - 1400/ 26.6 - *43.2* - 53.2/ 220 - *463* - 600/ 111.7 - *244.2* - 319.2/ 21.3 - *35.1* - 45.2	134/ 4/ 5/ 7/ 3 [N=1]	1010 - *1307.5* - 1600/ 5.3 - *12* - 26.6/ 100 - *115* - 106.4/ 37.2 - *81.1* - 106.4/ 6.3 - *6.7* - 8 [N=4]	n.o..	66.5 - *86.4* - 95.8	16.2 - *22* - 34.6/ 7.6 - *10.4* - 14/ 2.2 - *3.4* - 4.3/ 1.1 - *1.2* - 2.2	4.3 - *11.9* - 16.2 [N=11]	4.3 - *5.4* - 6.5 [N=11]
A318*	Bocas del Toro, Panama	ZMBN 81780	959 - *1281.6* - 1586/ 14 - *26.2* – 39	147 - *191.6* - 234/ 2 - *4.3* – 7	650 - *1188.3* - 1550/ 13.3 - *39.9* - 55.9/ 150 - *405* - 600/ 95.8 - *205.1* - 300.6/ 13.6 - *34* - 45.2	n.o..	n.o..	n.o..	69 - *81* - 92	10.8 - *27.4* - 43.2/ 5.4 - *12.1* - 16.2/ 3.2 - *4.2* - 1.4/ 1.1 - *1.4* - 2.2	8.6 - *11.4* - 18.4 [N=16]	4.3 - *6.4* - 7.6
A320*	Bocas del Toro, Panama	ZMBN 77928	822 - *1131.5* - 1477/ 19 - *27* – 36	144 - *172.8* - 208/ 3 - *4.5* – 6	950 - *1315.6* - 1570/ 42.6 - *52.2* - 63.8/ 300 - *449.4* - 560/ 143.6 - *238.5* - 332.5/ 31.9 - *42* - 53.2 [N=18]	1110 - *1469.6* - 1832/ 2.7 - *10.5* - 17 [N=10]	1260 - *1305* - 1350/ 2.7 - *4* - 5.3/ 40 - *45* - 50/ 18.6 - *26.6* - 34.6/ 2.7 - *4* - 5.3 [N=2]	n.o..	70 - *77.5* - 87	16.2 - *20.1* - 27/ 5.4 - *8.7* - 13/ 2.2 - *3.5* - 4.3/ 1.1 - *1.2* - 2.2	10.8 - *12.4* - 14 [N=4]	5 - *6.6* - 9

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A300	Archipelago Sabana, Cuba	UFBA 3061	974 -*1246.6* - 1487/ 14 - *19* – 25	91 - *115.9* - 214/ 2 - *3.4* – 5	750 - *1058.7* - 1250/ 13.3 - *21.2* - 31.9/ 200 - *475* - 750/ 106.4 - *256.9* - 404.3/ 10.6 - *18* - 26.6	n.o..	n.o..	n.o..	30 - *39.6* - 54	11 - *17.3* - 22/ 4 - *7.8* - 11/ 2 - *2.8* - 4/ 1 - *1* – 1	9 - *9* - 9 [N=2]	4 - *5* – 6
A301	Archipelago Sabana, Cuba	UFBA 3062	980 - *1120* - 1308/ 14 - *21.1* – 30	213 - *277.6* - 340/ 3 - *4.6* - *7*	770 - *962.7* - 1280/ 21.3 - *26.2* - 37.2/ 300 - *415* - 640/ 133 - *220.4* - 324.5/ 13.3 - *20* - 23.9	n.o.	n.o.	n.o.	36 - *46.3* - 55	12 - *17.3* - 22/ 4 - 7.*8* - 11/ 2 - *2.8* - 4/ 1 - *1* – 1	n.o.	4 - *5.2* – 6
A308	Cayeria de San Felipe, Cuba	UFBA 3071	994 - *1224.7* - 1551/ 19 - *27.2* – 39	228 - *284* - 328/ 5 - *6.7* – 10	820 - *1138.8* - 1310/ 31.9 - *40.2* - 50.5/ 320 - *473.8* - 640/ 172.9 - *256.4* - 324.5/ 26.6 - *28.2* - 50.5 [N=16]	n.o.	n.o.	680/ 18.6/ 120/ 69.2/ 10.6 [N=1]	50 - *58.9* - 70	11 - *18.3* - 25/ 5 - *8.1* - 12/ 3 - *3.4* - 5/ 0.5 - *1* – 1	n.o.	4 - *5.8* – 8
A312*	Guadalupe (France)	UFBA 3622	910 - *1213.3* - 1450/ 13.3 - *22.4* - 37.2	133 - *203.8* - 242.1/ 2.7 - *2.9* - 5.3	770 -*1022.3* - 1390/ 12.5 - *25.9* - 54.6/ 150 - *305.3* - 460/ 80 - *160.1* - 288.6/ 10 - *18.1* - 31.2	n.o..	n.o..	n.o..	58.5 - *69.1* - 79.8	17 - *22* - 31/ 7 - *10.1* - 16/ 2 - *2.5* - 4/ 1 - *1* – 1	n.o.	4 - *5* – 7

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A313*	Guadalupe (France)	UFBA 3623	1090 - *1343* - 1610/ 12.5 - *25.3* – 35	186.2 - *231* - 271.3/ 2.7 - *4.4* - 5.3	970 - *1286.3* - 1780/ 12.5 - *23.7* - 32.5/ 220 - *420* - 680/ 117.5 - *219.1* - 350/ 7.5 - *16.3* - 22.5	n.o..	n.o..	n.o..	55.9 - *66.9* - 82.5	12 -*18.8* - 22/ 6 - *8.8* - 11/ 2 - *2.6* - 4/ 1 - *1* – 1	n.o.	3 - *4.3* - 5
A311*	Guadalupe (France)	UFBA 3624	880 - *1200* - 1450/ 10.6 - *27* - 45.2	172.9 - *225.7* - 351.1/ 2.7 - *5.4* – 8	580 - *1141.7* - 1570/ 18.6 - *38.4* - 61.2/ 240 - *941* - 2050/ 66.6 - *195.5* - 319.2/ 10.6 - *30.6* - *47.9*	n.o..	n.o..	n.o..	79.8 - *87.9* - 98.4	16.2 - *22.6* - 28.1/ 7.6 - *9.9* - 13/ 2.2 - 3.9 - 5.4/ 1.1 - *1.1* - 2.2	n.o.	5.6 - *8.1* - 9.8
A139	Praia dos Dois Coqueiros, Ceará, Brazil	UFBA 4040	1006 - *1371.2* - 1670/ 14 - *24.4* – 33	153 - *189.8* - 198/ 3 - *4.4* – 7	720 - *1171.7* - 1560/ 23.9 - *43.4* - 53.2/ 250 - *504.3* - 740/ 159.6 - *271.6* - 396.3/ 23.9 - *36.6* - 45.2	n.o..	- / 8/ 87/ 68/ 5 [N=1]	420 - *568.3* - 680/ 16 - *23.5* - 32.2/ 90 - *221.7* - 600/ 61.2 - *93.1* - 133/ 13.3 - *16.4* - 21.3 [N=6]	63 - *70* - 77	16.2 - *21.9* - 27/ 7.6 - *10.4* - 13/ 2.2 - *3.1* - 4.3/ 1.1 - 1.1 - 1.1	10.8 - *13.3* - 16.2 [N=7]	n.o..
A140*	Praia dos Dois Coqueiros, Ceará, Brazil	MNRJ 14755	821 - *1131* - 1386/ 12 - *21.9* – 35	134 - *167* - 207/ 2 - *3.3* – 5	650 - *916* - 1140/ 20 - *29.8* - 47.5/ 160 - *268.3* - 560/ 70 - *127.6* - 192.5/ 17.5 - *23.8* - 32.5	n.o..	n.o..	270 - *410* - 560/ 10 - *13.5* - 20/ 70 - *89.2* - 110/ 37.5 - *54* - 82.5/ 7.5 - *11.9* - 15 [N=12]	56 - *66.6* - 76	18.2 - *26* - 35/ 8.4 - *11.7* - 16.8/ 2.8 - *3.6* - 5.6 / 1.4 - *1.5* - 2.8	8.6 - *12.8* - 16.2 [N=7]	4.3 - *5.9* - 8.6

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A110	Maracajaú, Rio Grande do Norte, Brazil	MNRJ 9148	754 - *1092.4* - 1451/ 12 - *22.6* – 31	179 - *202.6* - 259/ 3 - *5.1* – 7	650 - *929* - 1140/ 18.6 - *34.4* - 47.9/ 230 - *342* - 500/ 106.4 - *180.2* - 252.7/ 18.6 - *28.9* - 39.9	n.o..	n.o..	530 - *580* - 620/ 13.3 - *20.3* - 23.9/ 70 - *114* - 150/ 58.5 - *64.4* - 79.8/ 10.6 - *15.4* - *18.6* [N=5]	57 - *63.1* - 72	17 - *21.2* - 33/ 7 - *9.4* - 15/ 2 - *3.4* - *5/ 1* - *1.1* – *2*	9 - *11.6* - 13	4 - *5.8* - 7 [N=7]
A111	Maracajaú, Rio Grande do Norte, Brazil	MNRJ 9149	906 - *1112.4* - 1391/ 12 - *20.8* – 31	148 - *186.4* - 222/ 2 - *4.5* – 7	760 - *1050.4* - 1400/ 18.6 - *28.3* - 50.5/ 230 - *325.4* - 500/ 119.7 - *182.8* - 258/ 13.3 - *23.2* - 42.6 [N=26]	n.o..	1870 /5.3 /240 /34.6 /5.3 [N=1]	320 - *366* - 420/ 5.3 - *6.2* - 8/ 40 - *46.7* - 50/ 23.9 - *28.4* - 34.6/ 5.3 - *5.3* - 5.3 [N=3]	50 - *60.6* - 70	16 - *22.3* - 28/ 8 - *10* - 13/ 2 - *3.5* - 5/ 1 - *1.1* – 2	n.o..	4 - *5.3* - 7
A112*	Maracajaú, Rio Grande do Norte, Brazil	MNRJ 9150	810 - *1104.5* - 1373/ 12 - *19* – 29	138 - *177.2* - 200/ 2 - *4.1* – 6	580 - *812.3* - 1290/ 17.5 - *25.3* - 32.5/ 220 - *322.7* - 480/ 112.5 - *156.7* - 225/15 - *20.5* - 27.5	n.o.	1620/ 2.5/ 40/ 25/ 2.5 [N=1]	370 - *395* - 420/ 12.5 - *15* - 17.5/ 80 - *110* - 140/ 42.5 - *61.3* - 80/ 10 - *13.8* - 17.5 [N=2]	53 - *61.7* - 70	16 - *20.9* - 28/ 7 - *9.5* - 13/ 2 - *3.5* - 4/ 1 - *1.1* – 2	12 - *14* - 16 [N=2]	4 - *5.8* - 7
A113*	Maracajaú, Rio Grande do Norte, Brazil	MNRJ 9151	658 - *872.7* – 1106	136 - *173.2* - 215/ 2 - *3.8* – 6	490 - *745* - 1000/ 22.5 - *22.8* - 32.5/ 120 - *276* - 400/ 75 - *140.8* - 200/ 12.5 - *19.9* - 30	n.o.	1440/ 7.5/ 60/ 32.5/ 10 [N=1]	290 - *387* - 530/ 10 - *16.5* - 25/ 60 - *92* - 140/ 37.5 - *53.8* - 75/ 7.5 - *12.3* - 17.5/ 7.5 - *12.3* - 17.5 [N=10]	53 - *60.1* - 72	23.8 - *31.2* - 43.4/9.8 - *13.3* - 21/ 2.8 - *3.5* - 4.2/ 1.4 - *1.5* - 2.8	11.2 - *15* - 21 [N=15]	7 - *7.7* - 9.8

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cortical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A114	Maracajaú, Rio Grande do Norte, Brazil	MNRJ 9154	683 - *938.5* - 1346/ 10 - *19.4* – 39	137 - *168.8* - 207/ 1 - *3.5* – 5	300 - *729* - 970/ 16 - *28.9* - 42.6/ 170 - *346* - 750/ 93.1 - *180.1* - 252.7/ 10.6 - *24.1* - 39.9	n.o.	n.o.	n.o.	56 - *67.1* - 82	18.4 - *25.5* - 44.3/ 8.6 - *11.5* - 21.6/ 1.1 - *2.9* - 5.4/ 1.1 - *1.2* - 2.2	15 [N=1]	4 - *5.7* - 8
A133*	Ponta do Cabo Branco, Paraíba, Brazil	UFBA 4041	1010 - *1223.3* - 1383/ 7 - *21.5* – 35	145 - *209.6* - 493/ 2.5 - *3.8* - 6	750 - *1049* - 1320/ 17.5 - *35.4* - 47.5/ 150 - *349* - 520/ 82.5 - *177.8* - 237.5/ 15 - *26.3* - 37.5	n.o.	n.o.	n.o.	61 - *71.2* - 79	26.6 - *34.2* - 42/ 9.8 - *15.7* - 25.2/ 2.8 - *3.9* - 5.6/ 1.4 - *1.5* - 2.8	16.8 - *17.5* - 18.2 [N=2]	7 - *8.4* - 9.8
A134*	Ponta do Cabo Branco, Paraíba, Brazil	UFBA 4042	1083 - 1339.8 - 1514/ 23 - *29.8* – 41	162.3 - *243.9* - 295.3/ 2.7 - *6.6* – 8	870 - *1240.5* - 1620/ 29.3 - *42.1* - 66.5/ 300 - *455.9* - 600/ 146.3 - *244.8* - 465.5/ 18.6 - *30.7* - 39.9 [N=22]	n.o.	n.o.	450 - *700* - 800/10.6 - *19.3* - 23.9/ 40 - *157.5* - 200/ 18.6 - *77.1* - 111.7/ 13.3 - *27.9* - 66.5 [N=4]	61 - *74.6* - 84	17.3 - *24.7* - 30.2/ 7.6 - *10.7* - 16.2/ 3.2 - *4.3* - 5.4/ 1.1 - *1.3* - 2.2	10.8 - *13.5* - 16.2 [N=6]	5.4 - *6.3* - 7.6
A135*	Ponta do Cabo Branco, Paraíba, Brazil	UFBA 4043	874 - *1030.1* - 1215/ 12 - *22.4* – 32	175 - *209.1* - 240/ 2.5 - *4.8* - 7.5	780 - *1152* - 1460/ 23.9 - *35.7* - 53.2/ 240 - *391.7* - 600/ 133 - *203.2* - 305.9/ 18.6 - *28.2* - 45.2	n.o.	n.o.	470 - *500* - 540/ 13.3 - *17.7* - 21.3/ 100 - *103.3* - 110/ 61.2 - *69.2* - 79.8/ 10.6 - *13.3* - 16 [N=3]	59 - *68.3* - 81	17 - *22.1* - 26/ 6 - *9.8* - 13/ 2 - *3.9* - 5/ 1 - *1.4* – 2	13 - *15* – 17	4 - *5.7* - 7

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A138*	Ponta do Cabo Branco, Paraíba, Brazil	UFBA 4044	940 - *1169.3* - 1900/ 18.6 - *29.6* - 42.6	940 - *252.3* - 292.6/ 2.7 - *5.3* – 8	750 - *1124* - 1600/ 26.6 - *38.7* - 53.2/ 249 - *368* - 500/ 93.1 - *199.4* - 279.3/ 18.6 - *31.2* - 50.5	n.o.	n.o.	790 /18.6 /110 /58.5 / 13.3 [N=1]	53.2 - *76.9* - 101.1	16.2 - *29.8* - 48.6/ 9.7 - *14.7* - 23.8/ 2.2 - *4.9* - 6.5/ 1.1 - *2* - 3.2	n.o.	4.3 - *5.9* - 7.6
A127	São Miguel dos Milagres, Alagoas, Brazil	UFBA 3273	886 -*1092*- 1428/ 8 - *21.7* – 34	157 - *221.3* - 398/ 3 - *5.4* – 8	900 - *1197.6* - 1430/ 26.6 - *37.1* - 55.9/ 300 - *444.8* - 660/ 149 - *243.2* - 337.8/ 13.3 - *30.8* - 47.9	n.o.	202/ 4/ 14/ 32/ 3 [N=1]	450 - *710* - 930/ 5.3 - *19.5* - 26.6/ 50 - *138.9* - 230/ 31.9 - *89* - 133/ 5.3 - *13.3* - 18.6	43 - *62.7* - 76	17.3 - *23.4* - 34.6/ 5.4 - *10.5* - 13/ 2.2 - *4* - 10.8/ 1.1 - *1.3* - 2.2	9.7 - *11.9* - 14 [N=7]	4.3 - *6* - 8.6
A142*	Maceió, Alagoas, Brazil	UFBA 4045	813 - *1059.9* - 1298/ 15 - *24.2 – 34*	155 - *190.8* - 233/ 3 - *5.1* – 8	600 - *902.2* - 1170/ 17 - *31.1* - 57.5/ 170 - *280.9* - 480/ 81 - *144.1* - 212.5/ 14 - *26.9* - 45	n.o.	1230 - *1340* - 1450/ 7.5 - *8.1* - 10/ 50 - *67.5* - 100/ 50 - *55.6* - 62.5/ 2.5 - *5.6* - 7.5 [N=4]	560 - *630* - 730/ 15 - *17.5* - 20/100 - *136.7* - 160/ 50 - *70.8* - 92.5/7.5 - *12.5* - 15 [N=3]	54- *69.6* - 81	17 - *34.2* - 58.8/ 7 - *16.3* - 25.2/ 2 - 4.3 - 7/ 1 - 1.9 - 2.8	n.o.	2 - *5.9* - 8.4
A143*	Pituba, Bahia, Brazil	UFBA 4046	783 - *953.1* - 1189/ 7 - *15.1* – 24	132 - *162.2* - 198/ 3 - *4.2* – 6	500 - *877.7* - 1150/ 13.3 - *17.8* - 26.6/ 170 - *386* - 560/ 9.8 - *200.9* - 292.6/ 10.6 - *16.2* - 23.9	n.o.	n.o.	157 - *368* - 457/ 7 - *9.3* - 14/ 60 - *134* - 280/ 28 - *74.5* - 159/ 5 - *9.5* - 15	42 - *46* - 51	10.8 - *16.5* - 22.7/ 4.3 - *7.7* - 10.8/ 1.1 - *2.1* - 4.3/ 1.1 - *1.1* - 1.1	7.6 - *9.1* - 10.8	3.2 - *5.3* - 7.6

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A144*	Pituba, Bahia, Brazil	UFBA 4047	700 - *1038* - 1150/ 10.6 - 16.4 - 23.9	133 - *177.2* - 218.1/ 2.7 - *3.8* - 5.3	730 - *955.3* - 1270/ 13.3 - *22.1* - 34.6/ 140 - *386* - 540/ 119.7 - *208.3* - 292.6/ 13.3 - *20.1* - 39.9	n.o.	1180 - *1165* - 1200/ 2.7 - *4.3* - 8/ 60 - *90* - 120/ 37.2 - *54.5* - 71.2/ 2.7 - *4* - 5.3 [N=2]	300 - *390* - 650/ 2.7 - *5.9* - 10.6/ 50 - *70* - 120/ 21.3 - *39.4* - 66.4/ 1.7 - *7.3* - 8 [N=5]	37.2 - *50.4* - 61.2	14 - *18.4* - *23.8*/ *5.4* - *7.6* - *10.8*/ 2.2 - *2.4* - 3.2/ 1.1 - *1.1* - 1.1	9.7 - *10.2* - 10.8 [N=5]	4.3 - *6* - 7.6
A147	Pituba, Bahia, Brazil	UFBA 4048	630 - *758* - 1300/ 8 - *17* - 26.6	121 - *145.5* - 175/ 2 - *4* - 6	680 - *944* -1100 / 16 - *22.3* - 29.3/ 200 - *319* - 440/ 95.8 - *168.1* - 247.4/ 10.6 - *19.2* - 26.6 [N=10]	n.o.	n.o.	256 - *376.8* - 470/ 5 - *6.7* - 8/ 38 - *65.7* - 100/ 18 - *37.5* - 53.2/ 3 - *6.4* - 9 [N=9]	42 - *48.3* - 59	11.9 - *17.9* - 21.6/ 5.4 - *8.4* - 10.8/ 2.2 - *3.2* - 5.4/ 1.1 - *1.1* - 2.2 [N=21]	7 - *8.9* - 11 [N=14]	4 - *5.8* - 8
A148*	Pituba, Bahia, Brazil	UFBA 4049	650 - *942.3* - 1450/ 8 - *12.2* - 21.3	138.3 - *195.2* - 152.7/ 2.7 - *3.4* - 5.3	400 - *722.3* - 970/ 10.6 - *16* - 26.6/ 150 - *318.7* - 850/ 93.1 - *174* - 271.3/ 10.6 - *15.1* - 26.6	113/ 2/ 2/ 6/ 2 [N=1]	n.o.	200 - *460.8* - 660/ 5.3 - *8* - 10.6/ 50 - *84.6* - 120/ 26.6 - *53.2* - 69.2/ 5.3 - *7.2* - 10.6 [N=13]	42.6 - *58.3* - 66.5	10.8 - *17.6* - 27/ 5.4 - *7.3* - 10.8/ 1.1 - *2.9* - 3.2/ 1.1 - *1.1* - 1.1	6-5 - *9* - 10.8	4.3 - *5.8* - 7.6
A108*	Salvador, Bahia, Brazil	MNRJ 8396	770 - *1044* - 1370/ 16 - *26* - 37.2	159.6 - *262.4* - 385.7/ 2.7 - *5.9* - 10.6	790 - *1096.7* - 1970/ 16 - *32.7* - 50.5/ 200 - *365.7* - 560/ 130.3 - *199.6* - 300.6/ 10.6 - *30.4* - 45.2	n.o.	n.o.	370 - *623.3* - 800/ 18.6 - *21.3* - 23.9/ 50 - *123.3* - 170/ 26.6 - *70.9* - 98.4/ 8 - *12.4* – 16	32.2 - *52.1* - 61.2	18.4 - *24.5* - 33.5/ 6.5 - *10.6* - 16.2/ 2.2 - *4* - 5.4/ 1.1 - *1.2* - 2.2	n.o.	4.3 - *6* - 7.6

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A129*	Pituba, Bahia, Brazil	UFBA 3629	700 - *833.3* - 960/ 10.6 - *14.5* - 26.6	133 - *180.8* - 260.7/ 1.7 - *3.7* - 5.3	670 - *860* - 1020/ 13.3 - *23.1* - 37.2/ 170 - *306.7* - 460/ 93.1 - *167.5* - 239.4/ 10.6 - *20.3* - 31.9	n.o.	n.o.	250 - *437.5* - 540/ 8 - *11.3* - 13.3/ 50 - *122.5* - 160/ 5.3 - *10* - 13.3/ 5.3 - *10* - 13.3/ 8.6 - *9.7* - 10.8 [N=4]	47.9 - *55.3* - 85.1	13 - *20.9* - 28.1/ 5.4 - *9.9* - 14/ 2.2 - *3.2* - 4.3/ 1.1 - *1.1* - 1.1 [N=16]	8.6 - *9.7* - 10.8 [N=2]	4.3 - *6.2* - 7.6
A118*	Capit. dos Portos, Bahia, Brazil	UFBA 964	1050 - *1294.3* - 1470/ 17.5 - *25.5* - 32.5	150 - *196.5* - 232.5/ 2.5 - *3.2* – 5	730 - *1106.7* - 1400/ 10 - *26.4* - 39.9/ 180 - *356.3* - 520/ 60 - *185.5* - 452.2/ 7.5 - *20.3* – 25	n.o.	n.o.	n.o..	50 - *64.8* - 77.5	15 - *19* - 24/ 6 - *8.5* - 11/ 2 - *3* - 4/ 1 - *1* - 1	9 - *10* - 11/ 4 - *4.5* - 5/ 2 - *2.3* - 3 [N=4]	4 - *5.2* - 7
A141*	Veracruz, Bahia, Brazil	UFBA 4150	870 - *1092.9* - 1295/ 11 - *17.9* – 28	144 - *214.2* - 276/ 2 - *4.1* – 6	780 - *1007.3* - 1370/ 20 - *28.8* - 45/ 180 - *319.3* - 600/ 75 - *159* - 267.5/ 12.5 - *23.8* - 37.5	n.o..	n.o..	250 - *410.8* - 640/ 7.5 - *13.3* - 20/ 40 - *81.7* - 150/ 17.5 - *39.3* - 62.5/ 5 - *11* - 17.5 [N=12]	48 - *60.3* - 70	14 - *24.3* - 37.8/ 6 - *11* - 16.8/ 2 - *3.6* - 5.6/ 1 - *1.1* - 2	15.4 [N=1]	3 - *5* - 7
A120*	Pedra Pequena, Veracruz, Bahia, Brazil	UFBA 1817	1152 - *1351.5* - 1559/ 10 - *30.7* – 41	143 - *196.9* - 264/ 2 - *4* - 6	740 - *1115* - 1500/ 15 - *41.5* - 62.5/ 200 - *405.7* - 500/ 130 - *211.9* - 275/ 12.5 - *31.4* – 50	n.o..	n.o..	n.o..	64 - *87.1* - 94	17 - *27.5* - 37/ 7 - *12.8* - 19/ 2 - *4.1* - 6/ 1 - *1.7* - 5	11 - *11.5* - 12 [N=2]	5 - *5.8* - 7

Table 1. (cont.)

Code	Locality	Examined materials	Choanosomal Oxea	Cotical Strongyloxea	Orthotriaene	Anatriaene	Protriaene	Plagiotriaene	Sterraster	Acanthoxy.	Acantho. I	Acantho. II
A100*	São Sebastião, São Paulo, Brazil	MNRJ 287	760 -*1056* - 1520/ 10 - *22.7* – 40	180 - *216.8* - 250/ 2.5 - *4.1* – 5	630 - *880.3* - 1180/ 22.5 - *33.3* - 62.5/ 150 - *321.3* - 520/ 80 - *162.3* - 270 / 15 - *20.2* - 44	n.o..	n.o..	n.o..	62.5 - *73.5* - 97.5	15 - *20.2* - 44/ 5 - *8.5* - 15/ 2 - *2.7* - 4/ 1 - *1.1* - 2	n.o.	3 - *4.9* - 6

Table 2. Mean genetic divergences (uncorrected *p*-distances) between groups (bold), and among individuals within groups (italic) for COI and ATP6 markers. The clades are presented in Figures 5 and 6.

COI	Clade I	Clade II	Clade III	Clade IV	314- Seq	*G. vosmaeri*	*P. johnstonia*
Clade I	*0.001*						
Clade II	**0.049**	*0.006*					
Clade III	**0.042**	**0.036**	*0.000*				
Clade IV	**0.040**	**0.038**	**0.049**	*0.007*			
314- Seq	**0.056**	**0.049**	**0.033**	**0.064**	-		
G. vosmaeri	0.055	0.074	0.068	0.065	0.081	-	
P. johnstonia	0.082	0.107	0.103	0.098	0.109	0.073	-

ATP6	Clade I	Clade II	Clade III	*P. johnstonia*
Clade I	*0.003*			
Clade II	**0.058**	*0.000*		
Clade III	**0.053**	**0.028**	*0.000*	
P. johnstonia	0.073	0.085	0.073	-

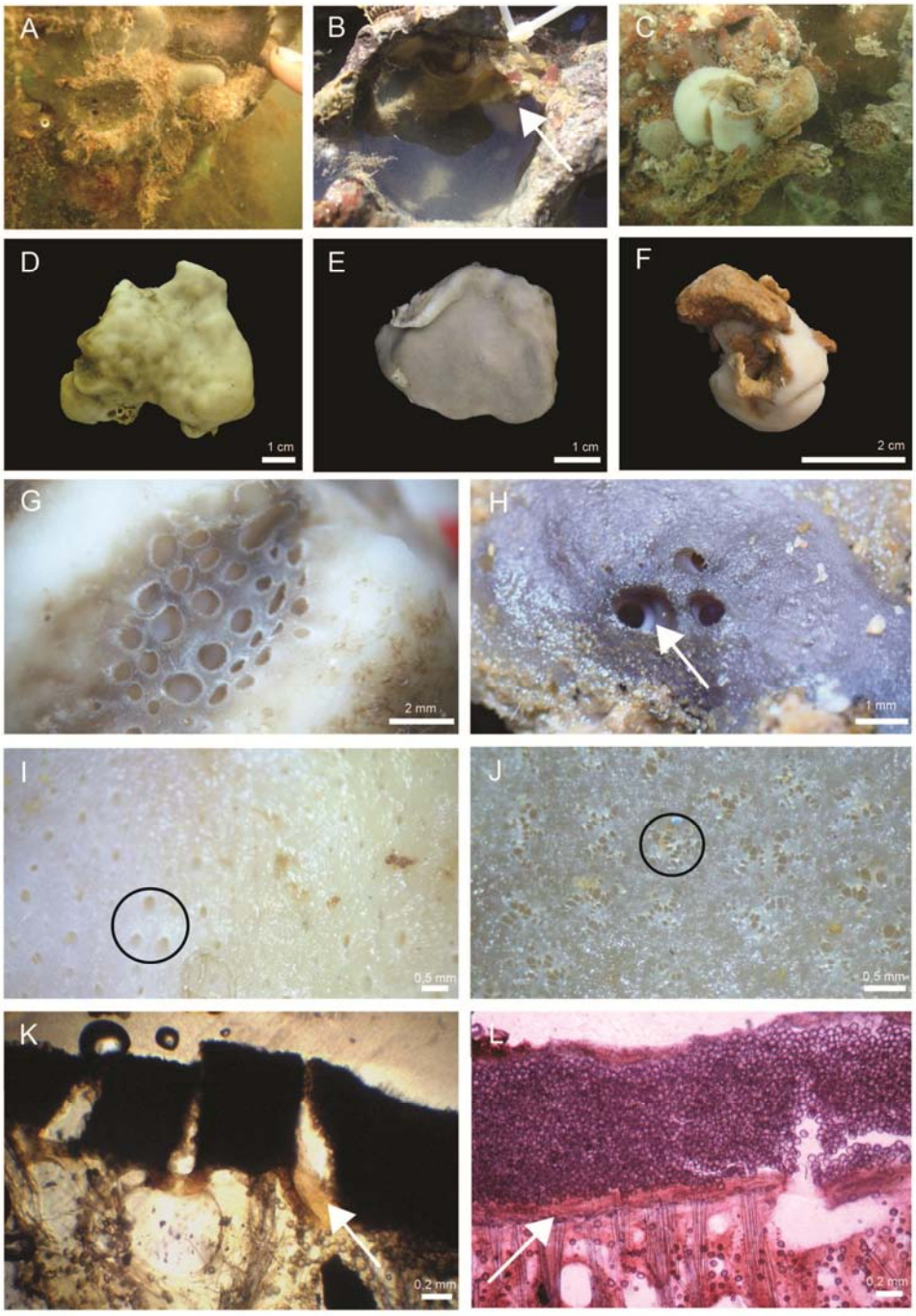

Figure 1. Gross morphology of *Geodia gibberosa* Lamarck, 1815. A,D. Massive shape. B,D. Encrusting shape. C,F. Globose shape. G. Clusters of uniporal oscules. H. Uniporal ocules with sphincter. I. Uniporal pores. J. Cribriporal pores. K. Skeleton arrangement with uniporal oscules. L. Three layered cortex with inner layer of fibers.

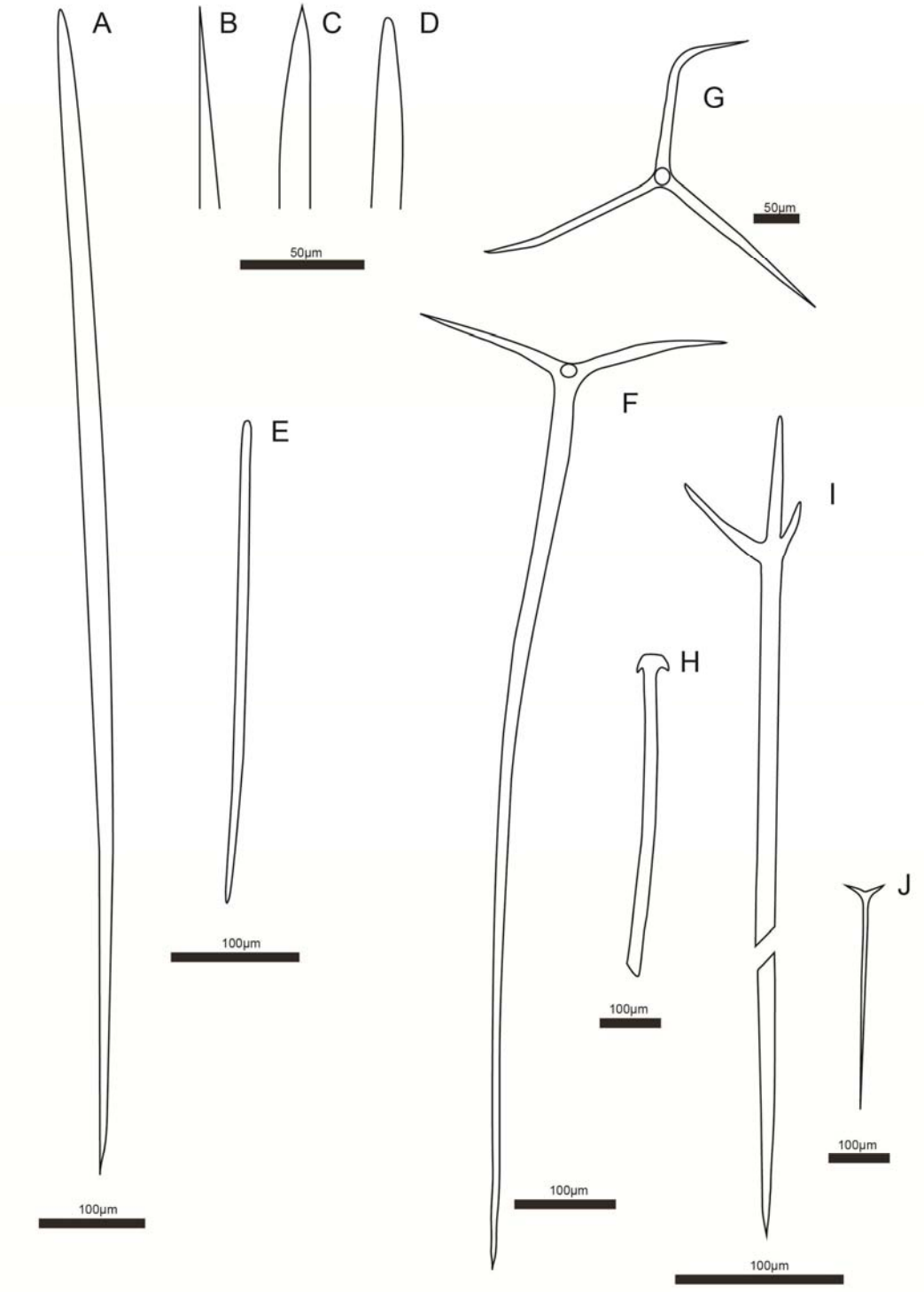

Figure 2. Megascleres of *Geodia gibberosa* Lamarck, 1815. A. Choanosomal oxea. B-D. Tips of oxea (B. Hastate. C. Acerate. D. Blunt). E. Cortical strongyloxea. F. Orthotriaene. G. Cladome of orthotriaene with bent clad. H. Anatriaene (with broken rhabdome). I. Protriaene. J. Plagiotriaene.

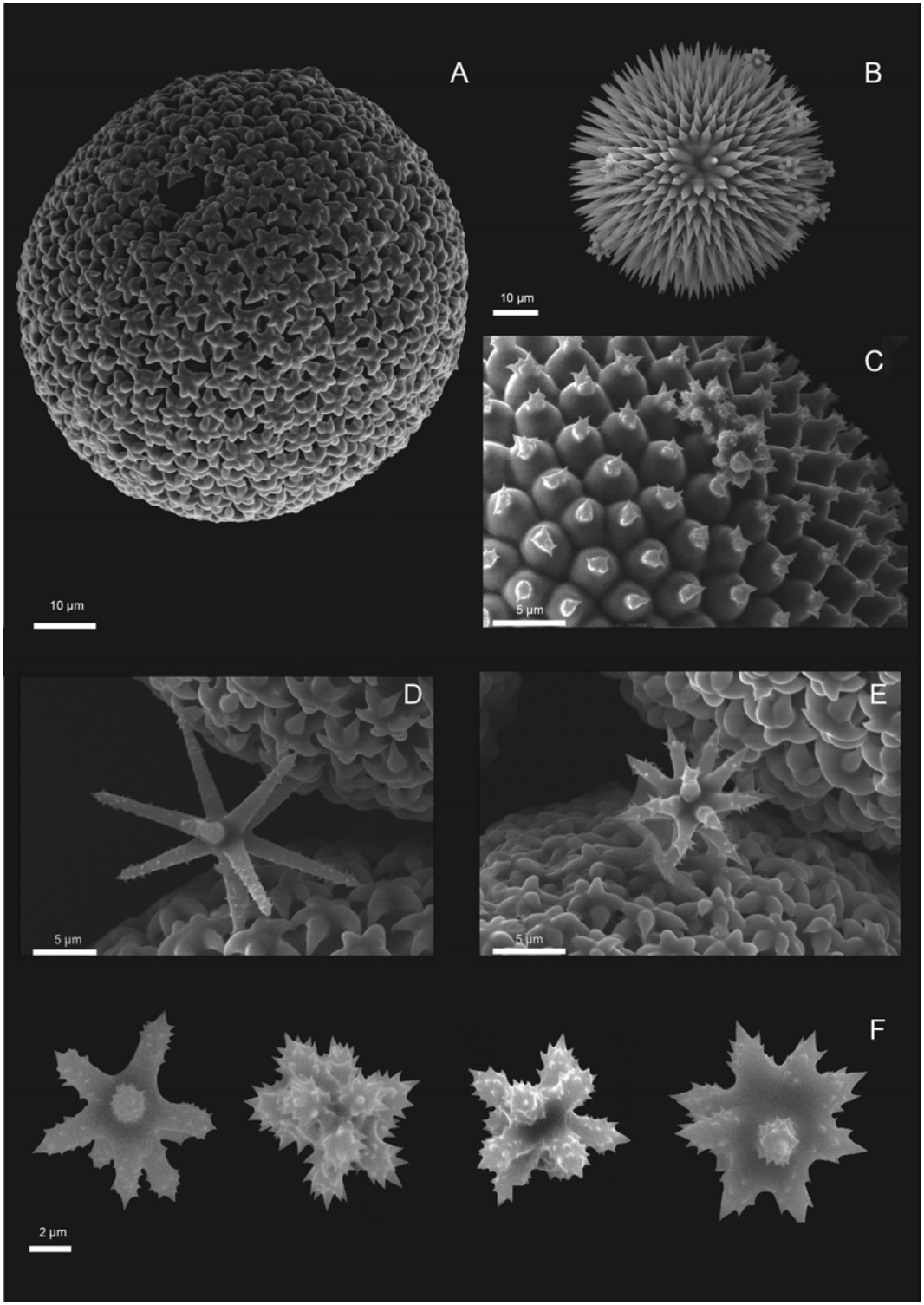

Figure 3. Microscleres of *Geodia gibberosa* Lamarck, 1815. Sterraster A-C (A. Mature sterraster. C. Sterraster in an intermediate stage of development. B. Young sterraster). D. Acanthoxyaster. E. Acanthospheroxyaster I. F. Variation of acanthospheroxyasters II.

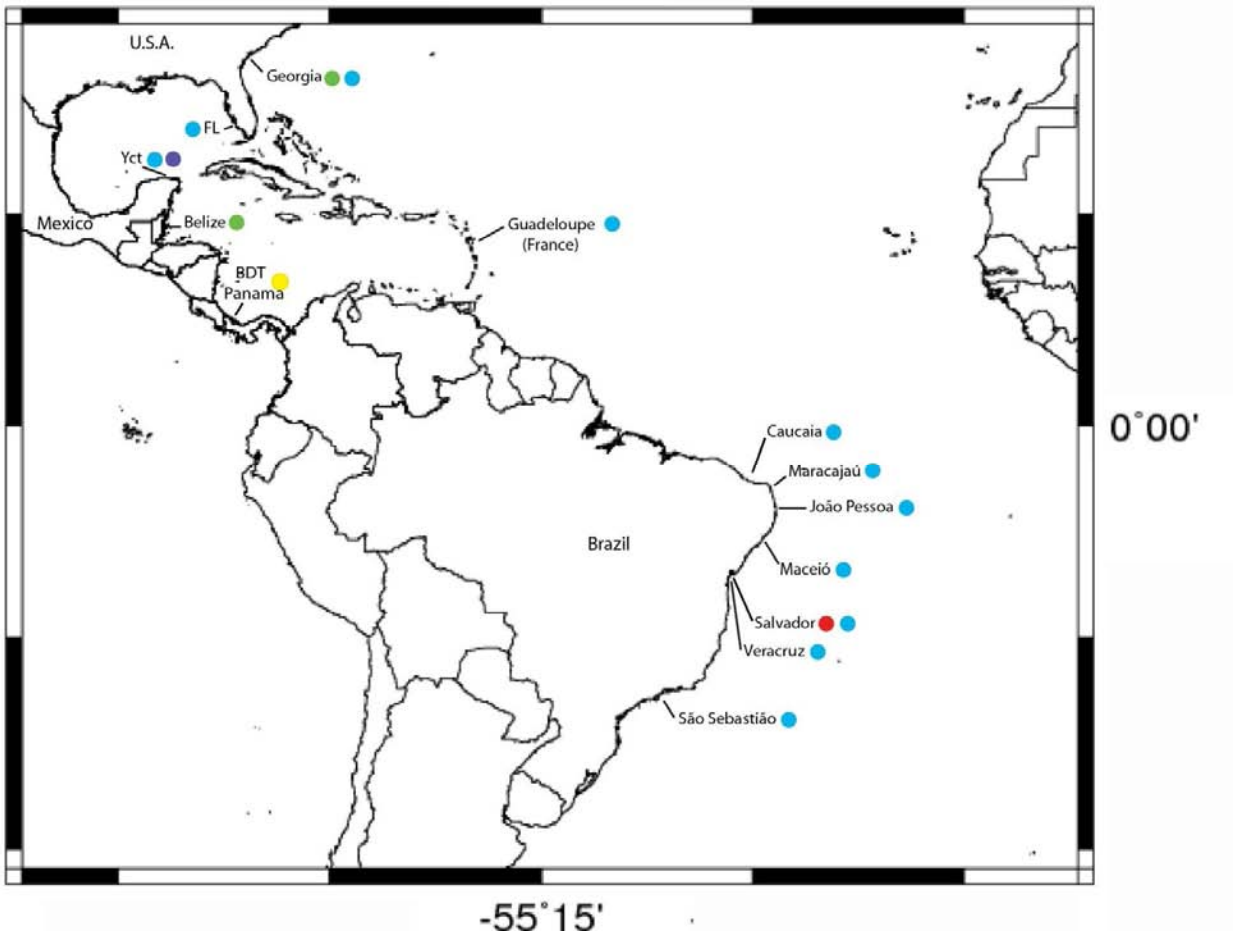

Figure 4. Map of the sampling localities of the amplified specimens through the Western Atlantic Ocean. Colors correspond to the clades found in the phylogenetic analysises: Clade I (red); Clade II (blued); Clade III (yellow); Clade IV (green) and Seq-314 (purple). FL= Florida. Yct= Yucatán. BDT= Bocas del Toro.

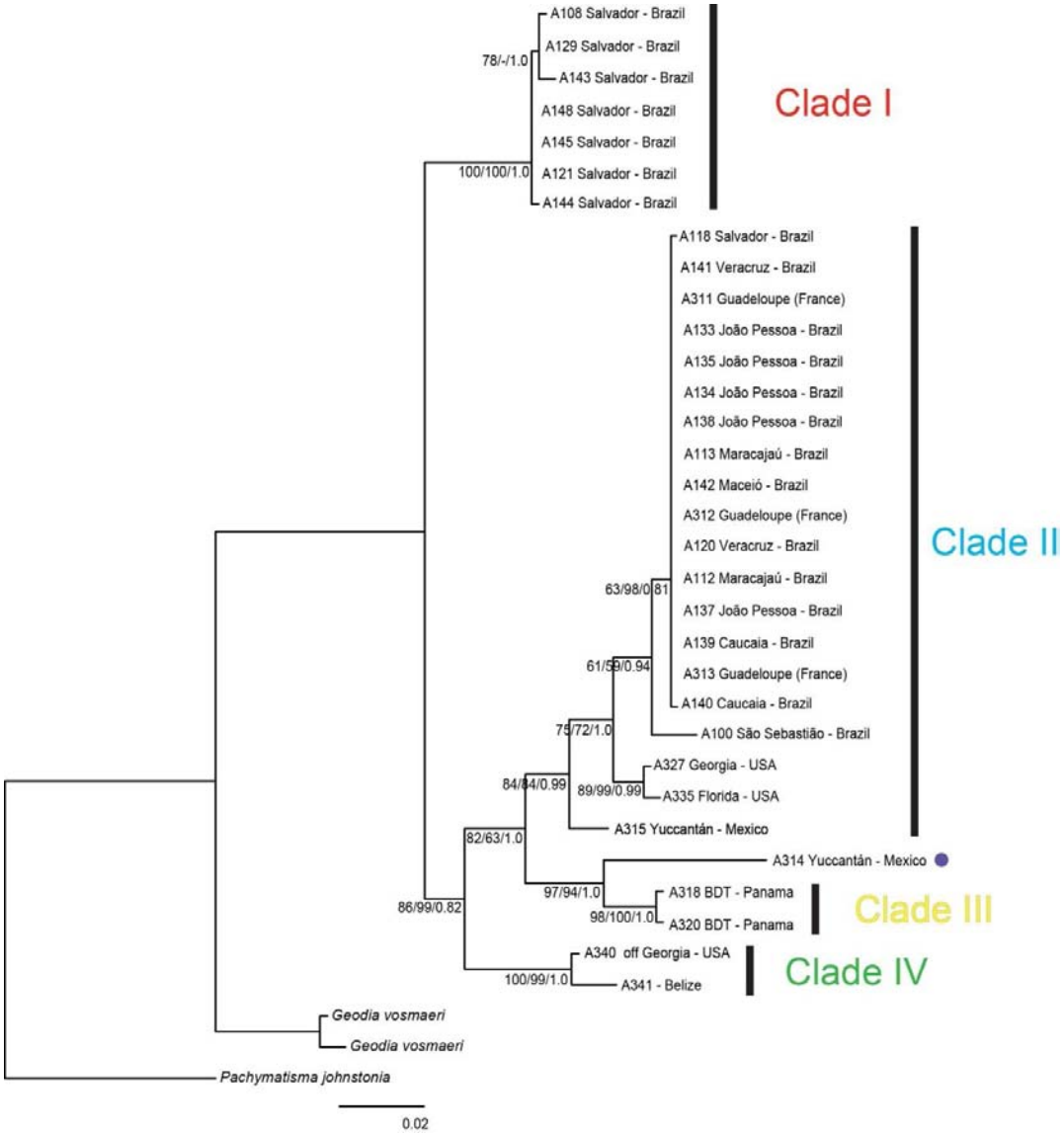

Figure 5. Four distinc clades (I-IV) are detected in the COI cladogram plus a divergent sequece (Seq – 314). The tree topology is based on the Bayesian Inference and the support values (superior to 50%) are written above each branch as follows: ML/NJ/BI. Each terminal branch has a code and it is followed by its sampling locality.

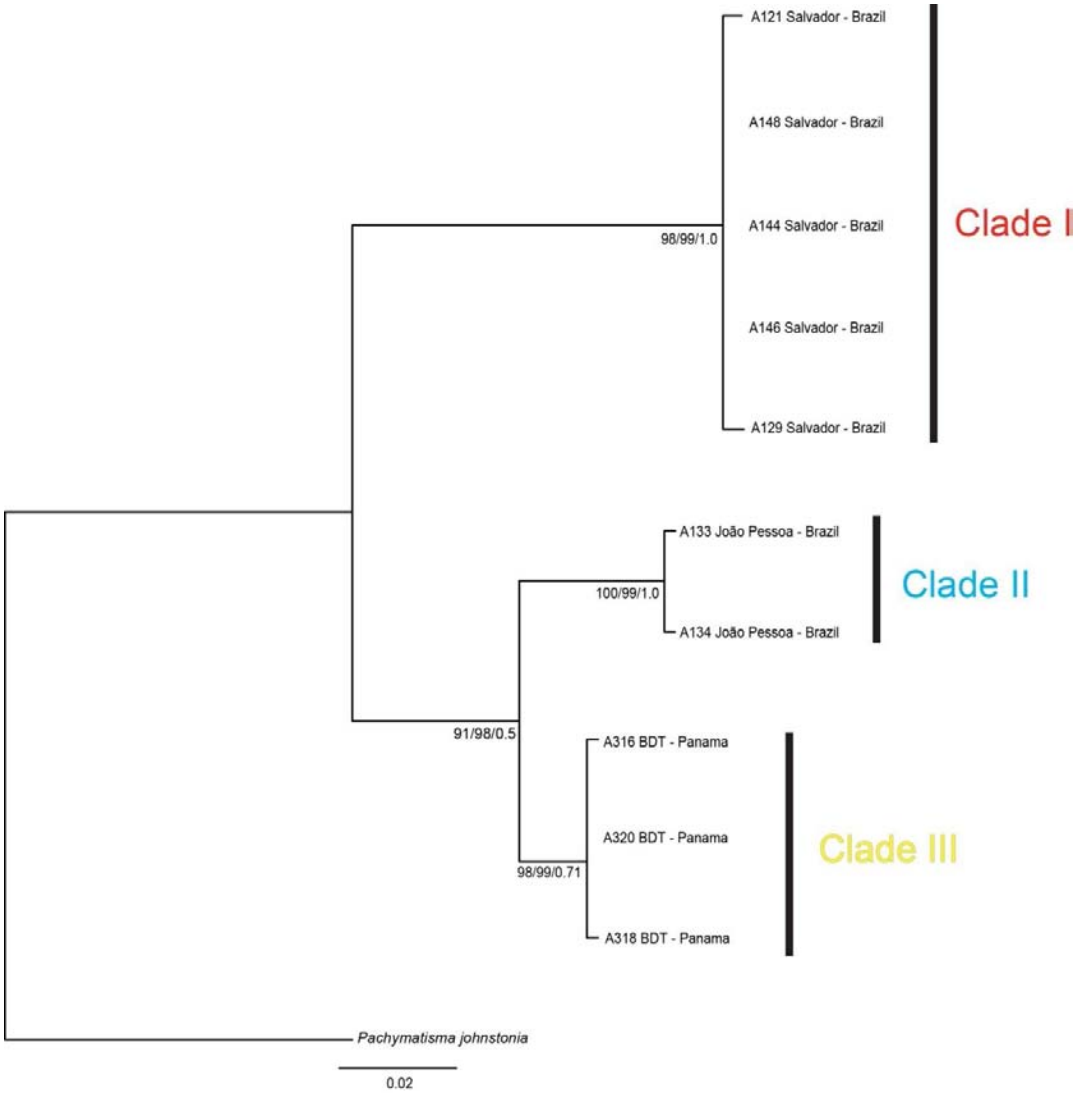

Figure 6. Three distinc clades (I-III) are detected in the ATP6 cladogram. The tree topology is based on the Bayesian Inference and the support values (superior to 50%) are written above each branch as follows: ML/NJ/BI. Each terminal branch has a code and it is followed by its sampling locality.

Appendix A. Genetic distances between mean group within the genus *Geodia* for the COI partition gene. Used sequences were both obtained from the present study (2 sequences of *G. vosmaeri* with no accession number) and from GenBank. Accession numbers are the following: *G. vosmaeri* (HM592711), *G. megastrella* (HM592741, HM592731, HM592721), *G. cochilega* (HM592739, HM592742), *G. cydonium* (HM592715, HM592693, HM592738, EU442199), *G. macandrewi* (HM592689, HM592696, EU442198), *G. corticostylifera* (HM592681), *G. hentscheli* (HM592671, EU442197), *G. pachydermata* (EU442197), *G. barretti* (HM592720, HM592684, EU442194), *G. californica* (EU442200), *G. angulata* (EU442203), *G. cf. atlantica* (EU442195, HM592695, HM592679), *G. papyracea* (AY561961), *G. vaubani* (EU442202).

G._vosmaeri		0.009	0.010	0.009	0.009	0.007	0.009	0.011	0.009	0.007	0.009	0.009	0.008	0.007
G._megastrella	0.063		0.010	0.008	0.008	0.009	0.004	0.010	0.004	0.010	0.008	0.009	0.010	0.007
G._cochilega	0.068	0.053		0.007	0.006	0.010	0.009	0.011	0.009	0.011	0.010	0.010	0.010	0.009
G._cydonium	0.068	0.046	0.035		0.005	0.009	0.008	0.010	0.008	0.010	0.008	0.010	0.008	0.007
G._macandrewi	0.058	0.037	0.024	0.020		0.009	0.008	0.011	0.008	0.010	0.008	0.010	0.009	0.008
G._corticostylifera	0.044	0.049	0.060	0.061	0.052		0.009	0.010	0.009	0.006	0.008	0.010	0.010	0.007
G._hentscheli	0.062	0.011	0.052	0.046	0.040	0.050		0.010	0.005	0.010	0.008	0.009	0.009	0.007
G._pachydermata	0.082	0.063	0.076	0.069	0.074	0.062	0.062		0.010	0.011	0.010	0.010	0.011	0.009
G._barretti	0.062	0.011	0.052	0.050	0.040	0.046	0.014	0.058		0.009	0.008	0.010	0.010	0.007
G._californica	0.045	0.057	0.068	0.070	0.062	0.018	0.058	0.070	0.054		0.009	0.010	0.011	0.008
G._angulata	0.061	0.041	0.058	0.047	0.038	0.048	0.040	0.060	0.044	0.058		0.009	0.010	0.006
G._cf._atlantica	0.064	0.051	0.064	0.062	0.060	0.054	0.052	0.064	0.052	0.062	0.050		0.010	0.008
G._papyracea	0.056	0.063	0.056	0.050	0.048	0.068	0.062	0.082	0.064	0.078	0.058	0.070		0.009
G._vaubani	0.044	0.030	0.042	0.039	0.034	0.032	0.030	0.050	0.032	0.040	0.024	0.034	0.050	

Capítulo 2

A NEW DESCRIPTION AND RECORD OF GEODIIDAE GRAY, 1867 (ASTROPHORIDA, DEMOSPONGIAE) SPECIES FROM THE CARIBBEAN ZONE

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Artigo a ser submetido no periódico Zootaxa

Description of a new species of *Geodia* and new record of *Pachymatisma* (Astrophorida, Demospongiae) from the Caribbean zone

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Abstract

In the present paper, we describe a new species of *Geodia* from the Gulf of Paria, Venezuela, and the new record of the genus *Pachymatisma* from Curaçao. *Geodia leispheroxyastera* **sp. nov.** is distinguished from other species of the genus by having a remarkable category of spheroxyaster. *Pachymatisma johnstonia* represents the first occurrence of the genus in the Caribbean waters. Additionally to the new species described here, we also provided an identification key to species of *Geodia* occurring in the Western Atlantic Ocean.

Key words: *Geodia*, *Pachymatisma*, taxonomy, morphology, DNA barcode, Western Atlantic ocean.

Résumé

Dans ce travail, nous décrivons une nouvelle espèce de *Geodia* du Golfe de Paria, au Venezuela, et nous enregistrons la présence du genre *Pachymatisma* de Curaçao. *Geodia leispheroxyastera* **sp. nov.** se difere des autres espèces du genre par une remarquable catégorie de spheroxyaster. *Pachymatisma johnstonia* représente un registre nouveaux de ce genre dans les eaux des Caraïbes. En plus de décrire la nouvelle espèce, nous fournissons une clé d'identification pour les espèces de *Geodia* qui se trouvent présentes dans l'ouest de l'océan Atlantique.

Mots-clés: *Geodia, Pachymatisma,* la taxonomie, la morphologie, code-barres moléculaires, l'Ouest Atlantique.

1. Introduction

With approximately 220 extant species, Geodiidae is one of the largest families within the Order Astrophorida Sollas, 1888 and one of the oldest in the Class Demospongiae Sollas, 1885, with fossil records going back to the Early Cambrian (Gruber & Reitner, 1991).

The family Geodiidae Gray, 1867 is generally characterized by massive or thickly encrusting poriferans. The skeleton is radially arranged at the periphery, and pores and oscules may be organized in a sieve. Sterrasters and different types of triaenes are the main spicules, but microrhabds and euasters are also present (Uriz, 2002).

Recently, a study based on molecular markers suggested the resurrection of the subdivision Erylinae/ Geodinae sensu Sollas (1888) and the synonimization of the genera *Isops* Sollas 1880 and *Sidonops* Sollas, 1889 (Cárdenas *et al*., 2010). The subfamily Erilinae would be composed of the genera *Erylus* Gray 1867, *Caminus* Schmidt 1862, *Penares* Gray 1867 and *Pachymatisma* Bowerbank in Johnston, 1842 and the second one would be then only consisted of *Depressiogeodia* Cárdenas, Rapp, Schander & Tendal, 2010, *Geodia* Lamarck, 1815 and *Cydonium* Fleming, 1828 (Cárdenas *et al*., 2010). The genus *Geodia* is hitherto the most representative with 145 species and geographically and bathymetrically widely distributed. *Pachymatisma* is composed of the species *Pachymatisma* johnstonia Bowerbank in Johnston, 1842, *Pachymatisma normani* Sollas, 1888 (restricted to the Northeastern Atlantic Ocean), *Pachymatisma bifida* Burton, 1959 and *Pachymatisma aerolata* Bowerbank 1972 (occurring in the Indian Ocean) (Silva, 2002; van Soest *et al*., 2012).

The specimens analyzed in this paper were previously identified as *Geodia gibberosa* Lamarck 1815 and were formerly destined to a morphologic and molecular study of this species (unpublished data). After morphologic analysis we could, then, confirm that these samples actually represented a new species of *Geodia* and the first record of the genus *Pachymatisma* for the Western Atlantic.

2. Materials and methods

2.1. Sampling

Geodiidae specimens were collected during different scientific expeditions. The sample of *Geodia* was obtained by L. J. K. Klein from the Gulf of Paria, Venezuela (10°22'30"N, 62°20'38.4"W) at 72 m depth within the "Calamar Expedition" between 1968 and 1969, under "The Caribbean Fisheries Development Project" developed by UNDP/FAO. The sample of *Pachymatisma* was collected under a biodiversity project in Curaçao (12°7'26.4" N, 68° 58'37.2"W) by N. van der Hal in 2005, at 20 m depth by scuba-diving. The sampling localities are shown in the Figure 1. Fragments of these samples were donated by NCB Naturalis (then Zoölogisch Museum van Amsterdam) for morphologic and molecular analysis and they were kept in ethanol 96%. The entire specimens are deposited at the same institution.

2.2. Morphologic analysis

A small fragment of the sponges (previously cut perpendicularly to their surface) was kept out of the ethanol until getting slightly dried. The tissue was then deposited in a small Petri dish and entirely embedded with distilled water. The cortex was held facing the base dish and kept at -20 °C for 2-3 hours. The ice was removed from the dish and very thin slices were promptly made (from the cortex to the choanosome) before the ice melting. The slices were transferred to a slide and dried in a hotplate. For clarifying, few drops of xylene (2-3) covered each cut and the slide rested in a laminar flow until the evaporation. Balsam of Canada was used to cover the slide. Dissociated spicules mounts and SEM preparation followed Silva (2002). Thirty spicules per spicule type were measured unless stated otherwise (N= number of spicules measured). Spicule measurements were made with a Zeiss Axioplan 2 Imaging and Zeiss Axiovision 4.5 software, and are given in μm as follows: minimum – *mean* – maximum. The measures of triaenes' are given in the ensuing order: rhabdome length/ rhabdome width; cladome length/clad length/clad width. The base of both rhabdome and clad were used to obtain width measures. The microscleres' measures are: total diameter/center diameter/rays length/ rays width. For better observing the details of these types of spicules, they were coated with gold in the Denton Vacuum LLC Desk IV and later photographed with JEOL-JSM

6390LV scanning electronic microscopy at Centro de Pesquisas Gonçalo Muniz (Fundação Oswaldo Cruz, Salvador). Photos of microscleres were treated with Adobe® Photoshop® CS and draws of megascleres and plates were made using Adobe® Illustrator® CS5 software.

2.3. DNA extraction, PCR amplification and sequencing

The choanosomal region of the sponges was separated to perform the DNA extraction, which followed a modified Sambrook *et al*., protocol (1989) using p*henol:*c*hloroform:isoa*myl alcohol (25:24:1). It was used 50 - 100 ng of DNA template, 1 unit of Platinum® *Taq* polymerase (Invitrogen), 2,5µl of 10x PCR buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl), 2 mM of $MgCl_2$, 200 µM of dNTP, 0.5µM, and 15 µl of sterile distilled water for a 25µl PCR reaction. The universal primers described by Folmer *et al.*(1994) (LCO 1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'; HCO 2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') were used to amplify a 650 bp partial region of COI under the PCR thermal condition: 1 cycle [94 °C/5 min], 5 cycles [94 °C/30 bios, 46 °C/45 s, 72 °C/1 min], 35 cycles [94 °C/30 s, 50 °C/45 s, 72 °C/1 min], 1 cycle [72 °C/10 min]. Two internal primers designed by Lopes *et al.* (in preparation) were used to amplify short fragments of approximately 350 pb each of COI. The primers and thermal regimes are the following: PorF1 (5'-CGG GTA TGA TAG GTA CAG GGT T-3') and PorR1 (5'-TGA ATG TGC TTG TAC GCT CG-3'), 1 cycle [94 °C/5 min]; 35 cycles [94 °C/1 min, 60 °C/45 s, 72 °C/30 s]; 1 cycle [72 °C/10 min]. Second pair: PorF2 (5'-TCA GCT TTT GTT GAA CAA GG-3') and PorR2 (5`-CTT CTG GGT GTC CAA ARA AYC A-3'), 1 cycle [94 °C/5 min]; 35 cycles [94 °C/1 min, 48 °C/45 s, 72 °C/30 s]; 1 cycle [72 °C/10 min]. All PCR thermal regimes were ran in an Applied Biosystems Veriti® Thermal Cycler and unincorporated primers and excess dNTPS were removed from the PCR products with PEG, based on the protocol described by Lis & Schleif (1975). Sequencing was performed by *Macrogen, Inc.*, in South Korea (www.macrogen.com), and the sequences generated were submitted to BLAST at NCBI (http:// www.ncbi.nlm.nih.gov/BLAST/).

2.4. Sequence analysis

The edition of the sequences was performed using BioEdit v. 7.0.9.0. (Hall, 1999) and posteriorly compared to additional ones obtained from the GenBank (www.ncbi.nlm.nih.gov). Uncorrected *p*-distance was estimated by 1000 bootstrap replicates

with MEGA5 (Tamura *et al.*, 2011). The sequences used for this purpose are the following: *Pachymatisma normani* (EF 564329, EF564327, EF564325, EF564323, EF564328, EF564326, EF564324, EF564322); *Pachymatisma johnstonia* (EF654340, EF654338, EF654335, EF654333, EF654331, EF654341, EF654339, EF654337, EF654334, EF654332, EF654330).

3. Results

3.1. Systematic description and spicules nomenclature

The classification and the spicules nomenclature adopted in this work follows, respectively, the *Systema Porifera* (Hooper & van Soest, 2002) and the *Thesaurus of Sponge Morphology* (Boury-Esnault & Rützler, 1997).

Class: Demospongiae Sollas, 1885

Order: Astrophorida Sollas, 1888

Family: Geodiidae Gray, 1867

Genus: *Geodia* Lamarck, 1815

***Geodia leispheroxyastera* sp. nov.**

(Figure 2 A-L)

Holotype. ZMA-POR 03558, Gulf of Paria, Venezuela, 10°22’30”N, 62°20’38.4”W. *Additional materials examined. Geodia gibberosa* Lamarck, 1815. Guiana. Schizoholotype MNHN-DT 608 (slide MCN 3639): collecting data not referred; Yucatán, CNPGG 1203: J. Sierra coll., 25.IV.1985. *Geodia pachydermata* (Sollas, 1886). Bermuda. Schizoholotype of BMNH 1889.1.1.91 (slide MCN 4050): off Bermuda, 32°8’45”N/64°59’35”W (Sta. 56), H. M. S. “Challenger” coll., 29.V.1873, 1.935 m. *Geodia cumulus* Schmidt, 1870. Florida. Schizotype BMNH 1870:5:3:85 (slide of spicular set): no collecting data (Schmidt, 1870 as *G. cumulus*); Schizotype ZMB 6663 (slide of spicules identified by Oscar Schmidt): no collecting data (Schimidt, 1870 como *G. cumulus*). *Geodia apiarium* (Schmidt, 1870). Florida. Schizotype BMNH 1870.5.3.84: 275.4 m (slide of spicules). *Geodia tuberculosa* Bowerbank, 1872. Mexico. Schizoholotype BMNH without number (slides of spicules and

skeletal section). *Geodia neptuni* (Sollas, 1888). Brazil. Schizoholotype of BMNH 1889:1:1:88 (fragment deposited at UFRJPOR 3728; slide deposited at MCN 2543): Off Pernambuco/Alagoas States, 09°09'S/ 34°53'W, H. M. S. "Challenger" coll., 10.IX.1873, 58.5 m (Sollas, 1886 as *Synops neptuni*). *Geodia thomsoni* Schmidt, 1870. Cuba. Schizotype of BMNH 1870.5.8.82 (slide of spicules): Cozera, 450 m. *Geodia spherastrea* Lévi, 1964. Puerto Rico. Schizoholotype of MNHN D.C.L. 1394 (slide of spicules): Puerto Rico Trench , Sta. 758, 1950-52, "Galathea" Expedition coll., 2.840 m. *Geodia papyracea* (Hechtel, 1965). Jamaica. Holotype YPM 5045 (slide MCN 4044), paratype YPM 5310 (slide MCN 3152): Mangrove boat channel, Port Royal, G. J. Hechtel coll., 13.VII.1961, 1m. *Geodia corticostylifera* Hajdu, Muricy, Custódio, Russo & Peixinho, 1992. Brazil. Schizoholotype of UFRJ-POR 3098 (slide MCN 3884): Rio de Janeiro, Arraial do Cabo, 22°58'48"S/42°00'36"W, E. Hajdu coll., 07.XII.1986, 8 m. *Geodia megastrella* Carter, 1876. Portugal. Schizotype of BMNH 1882:7:28:128 (slide MCN 4049): Off Portugal, near to St. Vincent Cape, Sta. 25, H.M.S. "Porcupine" coll., 1869, 374 fathoms [684 m]; Barbados. POR 05272 (slide MCN POR 4299): Barbados, off Paynes Bay, 11.VIII.1978, 144-153 m.

Diagnosis. Geodia leispheroxyastera **sp. nov.** is the only species of the genus from the Western Atlantic Ocean that has a smooth spheroxyasters, which represents the second largest microsclere (second only to the sterraster).

Description. Outer morphology: massive small fragment (size up to 1,5 cm) with shortly compressive consistency and micro-hispid surface. The color of the specimen was not registered in the field; after fixation, external coloration is whitish and the choanosome is brownish. Oscules and pores were not visualized given the dimension of the specimen (Figure 2 A).

Skeleton: The cortex (ca. 500 μm thick) is subdivided into an ectocortex of acanthospheroxyaster II and a remarkable endocortex with sterrasters. Under this level, notorious bundles of choanosomal oxeas and orthotriaenes are radially distributed, and the cladome of the latter is positioned right bellow the endocortex. Rare strongyloxeas are found in the choanosome, but most of them are placed in the cortex. Both mature and developing sterrasters are found scattered throughout the choanosome. Spheroxyaster is more abundant than the acanthospheroxyaster I, and both are placed in the choanosome (Figure 2B).

Spicules. *Megascleres*: (a) choanosomal oxea (Figure 2D), stout, straight or slightly curved or bent with acerate tips, length: 970 - *1305* - 1571 μm; width: 22 - *34* - 50 μm. (b) cortical strongyloxea (Figure 2E), fusiform, slender, usually straight, length: 163 - *205.3* - 257 μm; width: 2 - *3.5* - 7 μm. (c) orthotriaene (Figure 2 C), rhabdome is sout, straight or slightly curved with acerate tip, shaft length: 1000 - *1066* - 1120 μm; shaft width: 40 - *46* - 52.2 μm; cladome length:440 - 520 - 580 μm; clad length: 273.5 - *262.5* - 275 μm; clad width: 30 - *39* - 52.5 μm.

Microscleres: (a) sterraster (Figure 2 F-G), slightly ovoid with smooth 4-8 branched star-like tubercles at the surface (ca. 8 μm), diameter: 76 - *87.1* – 99 μm. (b) spheroxyaster (Figure 2H) robust with 13 - 17 smooth rays and large center, total diameter: 32 - *39.3* - 47 μm; center diameter:11 - *13.4* - 17 μm; rays length:11 - *13.8* - 17 μm; rays width: 3 - *3.8* - 5 μm. (c) acanthoxyaster (Figure 2I), discreet center, 11 - 13 thin rays with thorns in the distal portion and directed to the center, total diameter: 32 - *39.3* - 47 μm; center diameter: 3 - *3.3* - 4 μm; rays length: 6 - *7.3* - 8 μm; rays width 1 - *1* - 1 μm. (d) acanthospheroxyaster I (Figure 2J) 13 - 16 rays with spines opposite directed to the center, diameter: 5μm [N=1]. (e) acanthospheroxyaster II (Figure 2L), diameter: 4 - *6* - 7 μm.

Distribution: Gulf of Paria, Venezuela.

Etymology: the epithet *leispheroxyastera* refers to the characteristic and unique presence of stout spheroxyaster with smooth rays.

Remarks: Geodia leispheroxyastera **sp. nov.** differs from *Geodia papyraceae* Hetchtel, 1965 by lack of small anatriaenes, and from *Geodia vosmaeri* Sollas, 1886 by presenting smooth sterrasters. The acanthospheroxyaster I of *G. gibberosa* is similar to the acanthospheroxyaster I of the new species described here, and in both species they are rare. *Geodia leispheroxyastera* **sp. nov**. is quite similar to the type-species of the genus, but can be distinguished by possessing a remarkable category of spheroxyaster which was not seen in any other *Geodia* from the Western Atlantic hitherto, according to the latest morphologic revision of the genus (Silva, 2002).

Key to the Geodia species occurring in the Caribbean

1. With one to four categories of triaenes 2

1'. Without triaenes recorded up to now (schyzoholotype). Megascleres are strongyles and microscleres are sterrasters and oxyasters ……..... *G. apiarium*

2. Dichotriaenes are the only triaenes present or are the main triaenes …..... 3

2'. Orthotriaenes or plagiotriaenes are the only triaenes present or are the main triaenes …..... 4

3. Triaenes are only dichotriaenes. Other megascleres are choanosomal oxeas and rare cortical strongyles. Only one category of microsclere (sterraster) reported to the schizoholotype ……..…… ..... *G. thomsoni*

4. 3'. Triaenes are dichotriaenes (principal), rare promesotriaenes and anatriaenes. Other megascleres are styles and oxeas I (choanosomal and II (cortical). Three categories of microcleres in the schizoholotype (sterrasters, spherasters and acanthostrongylasters) ..... *G. spherastrea*

4. Cortical megascleres are regular oxeas (with conical tips). Choanosomal megascleres are oxeas and orthotriaenes. Three categories of oxyasters and spherasters ..... *G. pachydermata*

4'. Cortical megascleres are strongyloxeas, styles or rarely strongyles ……..... 5

5. Cerebriform or barrel sponges with remarkable grooves, depressions and hollows on the outer and/or inner surface, sometimes massive developing specimens 6

5'. Massive or spherical sponges with regular surface 7

6. Barrel or inverted tan cones, with furrows in the inner surface and scattered holes in the outer surface. Cortical megascleres are strongyloxeas. Choanosomal megascleres are oxeas, orthotriaenes transitional to plagiotriaenes, rare protriaenes and anatriaenes. Microscleres are sterrasters, acanthoxyasters, acanthospheroxyasters and acanthostrongylasters ..... *G. neptuni*

6'. Cerebriform, massive to globular sponges, with surface marked by grooves containing various oscules. Cortical megascleres are long styles. Choanosomal megascleres are oxeas and orthotriaenes. Microscleres are sterrasters with rosette like spines, acanthoxyasters and acanthostrongylasters …… ……..……..... *G. corticostylifera*

7. The biggest euaster is a conspicuous smooth spheroxyaster. Other spicules are cortical strongyloxea, choanosomal oxea and orthotriaene, sterraster, acanthoxyaster and acanthospheroxyasters I and II …..….. ……..... *G. leispheroxyastera* **sp. nov.**

7'. The biggest euaster is a microspined, acanthoxyaster or acanthospheroxyaster ……..…… 8

8. Main triaenes are typical orthotriaenes 9

8'. Main triaenes are typical plagiotriaenes. Other spicules are minute and slender anatriaenes, sterrasters, acanthoxyasters I with 6 to 12 slender and sparsely spined rays, acanthoxyasters II with 10 to 20 stout rays strongly spined and acanthospherostrongylasters ……..... *G. papyracea*

9. Mesotriaenes present (promesotriaenes). Megascleres are cortical oxeas usually transitional to strongyloxeas and styles and choanosomal orthotriaenes, plagiotriaenes and anatriaenes. Microscleres are sterrasters, acanthoxyasters, acanthospheroxyasters and acanthostrongylasters ……..……. *G. megastrella*

9'. Mesotriaenes absent 10

10. Triaenes with rhabdome exhibiting typical round tips. Cortical sterrasters and strongyloxeas rarely transitional to oxeas. Choanosomal strongyloxeas, strongyles, oxeas, ortho-/ plagiotriaenes, anatriaenes, acanthoxyasters and acanthospherostrongylasters …..... *G. cumulus*

10'. Triaenes with rhabdome exhibiting conical tips ……..…… 11

11. Massive sponges with irregular surface, with deep depressions and nodular projections. Cortical sterrasters and strongyloxeas. Choanosomal oxeas (some transitional to styles), ortho-, ana- and protriaenes, spherasters, acanthoxyasters and acanthostrongylasters …….……... *G. tuberculosa*

11'. Massive or spherical sponges with regular surface. Cortical spicules are sterrasters and strongyloxeas. Choanosomal spicules are oxeas, long and stout orthotriaenes frequently transitional to plagiotriaenes, additional rare triaenes (anatriaenes, protriaenes and remarkable minute slender plagiotriaenes), acanthoxyasters, acanthospheroxyaster I and acanthospheroxyaster II ..... *G. gibberosa.*

Genus: *Pachymatisma*

***Pachymatisma johnstonia* Bowerbank, 1842**

(Figure 3 A-L)

Examined material. ZMAPOR19064, Curaçao, 12°7'26.4" N, 68° 58'37.2"W.

Description. Outer morphology: Massive specimen (up to 3 cm in size), hardly compressive, with smooth surface. Dark brown externally and ranging from brownish to whitish in the choanosome, after fixation. Both oscules (ca. 2 mm in length) and pores (0.1 – 0.3 mm) are uniporal.

Skeleton: Slender ectocortex is made of microhabds. Sterrasters form a quite developed endocortex, and are spread throughout the choanosome, as well as some microhabds. Oxeas and orthotriaenes are somewhat organized radially under the layer of sterrasters. Acanthoxyaster II is much less abundant then the acanthoxyaster I, and both seem not have a specific position in the choanosome.

Spicules. Megascleres:(a) choanosomal oxea (Figure 3C), stout, straight or slightly curved with acerate tips, length: 560 - *814.*3 - 970 µm; width: 7.5 - *14* - 17.5 µm. (b) orthotriaene (Figure 3D-E), cladome with clades perfectly directed at right angle to the rhabdome or bent and slightly straight robust rhabdome with acerate tip, rhabdome length: 340 - *498* - 650 µm; rhabdome width: 10 - *15.8* - 22.5 µm; cladome length: 200 - *417.7* - 680 µm; clad length: 87.5 - *214.8* - 375 µm; clad width 7.5 - *13.9* - 20 µm.

Microscleres: (c) sterraster (Figure 3F-G-H), large, mostly ovoid, diameter: 65 - *82.5* - 97.5 µm. (d) acanthoxyaster I (Figure 3I), 10 - 12 rays with up to 30 spines, total diameter: 29.4 - *53.5* - 72.8 µm; rays length: 8.4 - *24.9* - 36.4 µm; rays width: 1.4 - *2.6* - 4.2 µm; center diameter: 2.8 -*5.7* - 8.4 µm. (e) acanthoxyaster II (Figure 3J), up to 15 rays with few robust spined on the distal, diameter: 14 - *21* - 28 µm. (f) microrhabd (Figure 3L), lentgh: 39.2 - *59.3* - 85.4 µm; width 2.8 - *3.4* - 5.6µm.

Habitat: information about the site of collection and/or ecology remains unknown.

Distribution: Ireland, United Kingdom, France, Spain, Portugal, Italy, Adriatic Sea (Cárdenas *et al*., 2007), Canaries Economic Zone, Azores and Madeira islands (van Soest *et al*., 2012) and Curaçao (present work).

Remarks: This is the first record of the genus for the Caribbean zone and represents a significant enlargement of the *P. johnstonia* distribution, which was restricted to the southern part of the north-east Atlantic coasts. This species differs from *Pachymatisma areolata* Bowerbank, 1872 by the lack of anatriaene and strongylasters (Burton, 1926), and from *Pachymatisma normani* Sollas, 1888, based on length/width/thickness measures of the sterraster (which are smaller than the first one's) (Cárdenas *et al*., 2007). No dichotriaenes were seen. An interesting finding is the hollow inward of the broken developed sterraster (Figure 3H), which is different from that found in *Geodia* (Figure 3K) and might suggest at least two different patterns of development for the sterraster category.

3.2. Molecular results

It was not possible to amplify the DNA of *Geodia leispheroxyastera* **sp. nov.** with any primer tested, probably due to the age of the sample (1969) and /or to the fixative used after the collection. Even though *Pachymatisma johnstonia* was recently collected, mtDNA was apparently much degraded to amplify the COI with the primers described by Folmer *et al*. (1994). The internal primers described by Lopes *et al*. (in preparation) were then used to amplify two small fragments of the same region. The alignment made among the sequences of *P. johnstonia* and those obtained from the GenBank, revealed 42 polymorphic sites (Table 1). All these sites were monomorphic between all sequences obtained from the database. Genetic distances values were discreetly higher between *P. johnstonia* from Caribbean and *P. normani* (*p*-distance = 0.077) than between the former and *P. johnstonia* from the Northeastern Atlantic (*p*-distance = 0.075).

4. Discussion and considerations

Given the well documented morphological plasticity of poriferans in the literature (Hill & Hill, 2002; López-Legentil *et al.*, 2010) we encourage and usually prioritize the description of new species based on a larger type-series. However, we chose to bring this to light based on (1) the difficulty of obtaining more samples from the collection sites and (2) the remarkable diagnostic characteristic of *Geodia leispheroxyastera* **sp. nov.**, which does not occur in other species of *Geodia* described so far and does not seem to be a variation of any other spicule. Regarding the description of this new species, we enhance the number *Geodia* from the Caribbean zone to 12 (and 18 from the entire Western Atlantic), and also enlarge the distribution of *P. johnstonia*.

Molecular results showed higher values of genetic distance between the sample from Caribbean and Europe than those found between the *P. normani* and *P. johnstonia* from Northeastern Atlantic (which presented just one polymorphic site). Since all the morphological characteristics were in accordance with the typical *P. johnstonia* (Cárdenas *et al.*, 2007), and we did not have more samples from the same place to compare, we chose to keep its identification this way. Nevertheless, given the low capability of the sponge larvae to disperse, it is also known and highly debated in the academy that species with such huge distribution could be a reflex of an overconservative taxonomy (Klautau *et al.*, 1999; Plotkin & Boury-Esnault, 2004). It cannot be affirmed or denied in the present work because of our small sampling, but gives the suggestion that the distribution of this species must be at least further investigated.

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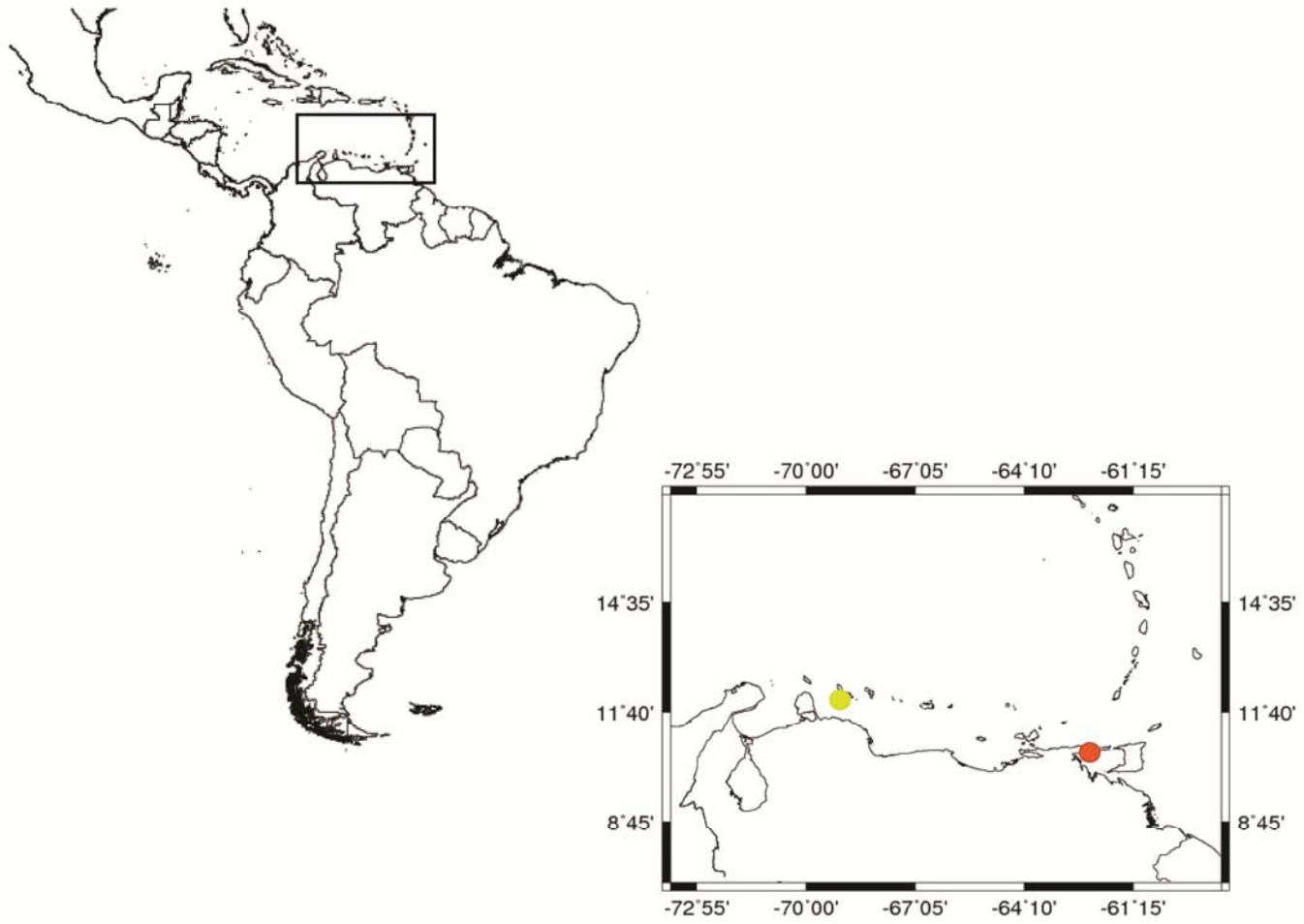

Figure 1. Map of part of Central America, showing the collection sites of the new species of *Geodia* (red mark: Gulf of Paria, Venezuela) and *Pachymatisma johnstonia* (mustard mark: Curaçao).

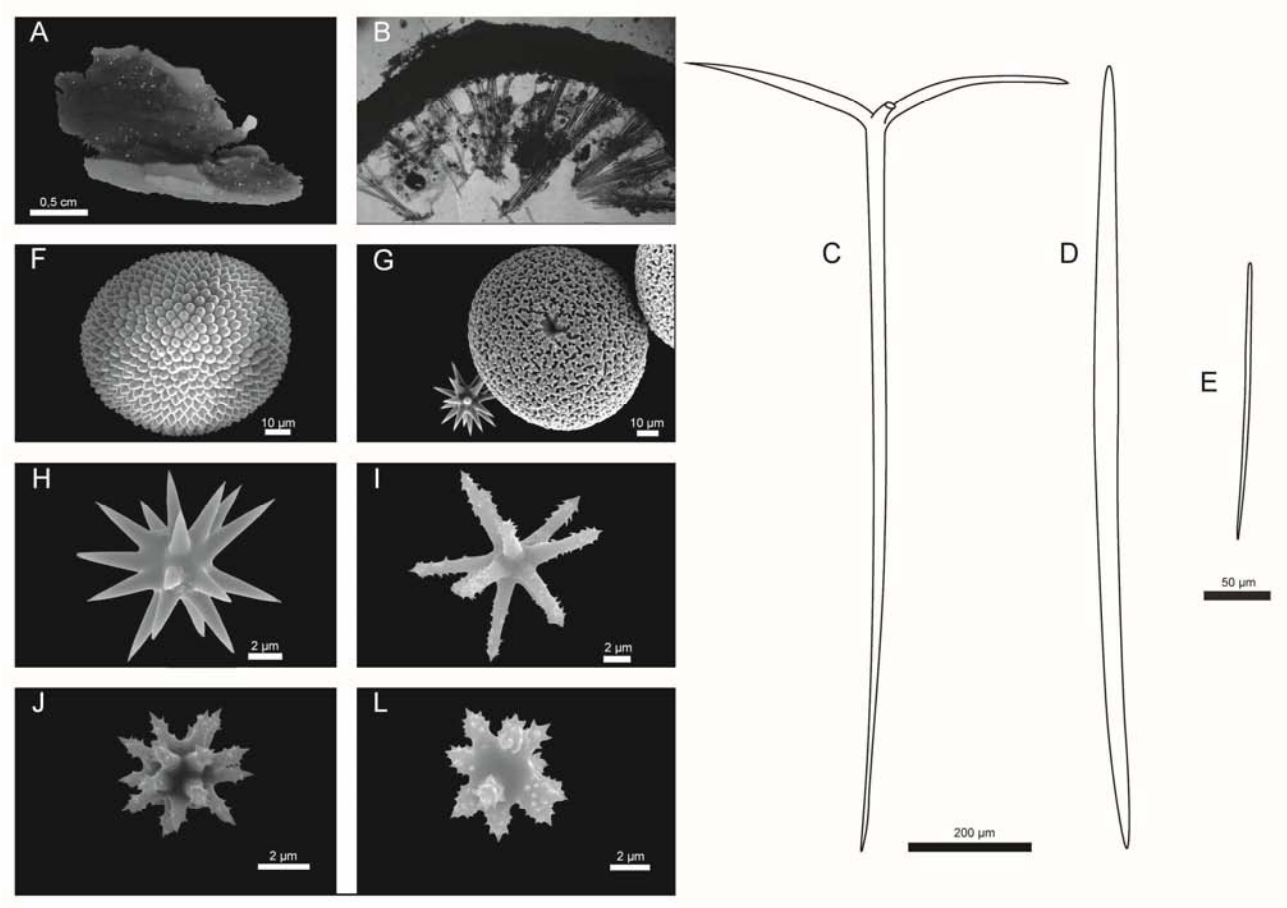

Figure 2. A. Holotype of *Geodia leispheroxyastera* **sp. nov.** (ZMAPOR 03558) . B. Transverse section (from the surface to the centrer) showing the radial arrangement of the spicules in the skeleton. C. Orthotriaene. D. Choanosomal oxea. E. Cortical strongyloxea. F-G. Developing and mature sterrasters, respectively. H. Spheroxyaster. I. Acanthoxyaster. J. Acanthospheroxyaster I. L. Acanthospheroxyaster II.

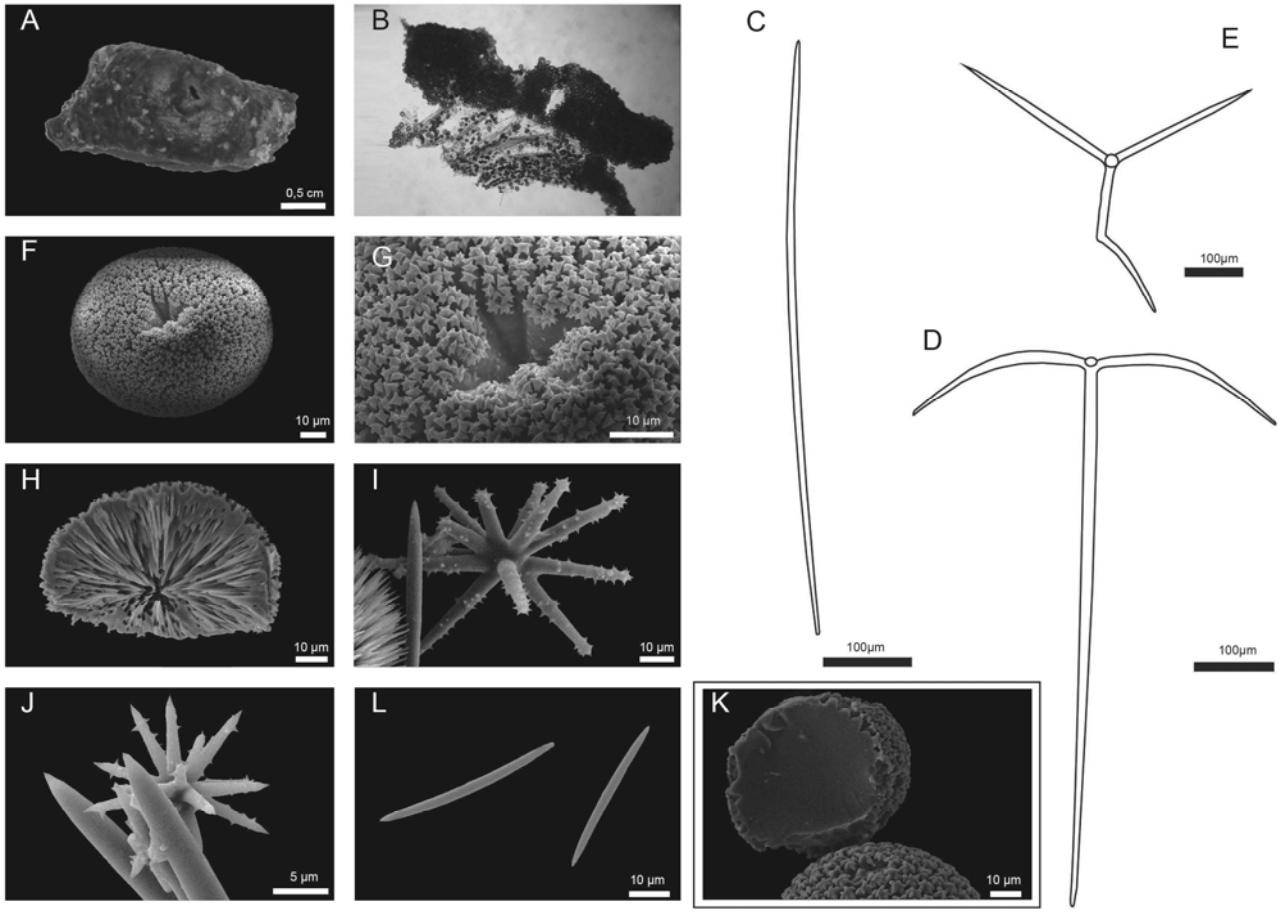

Figure 3. A. Specimen of *Pachymatisma johnstonia* (ZMAPOR 19064). B. Skeleton showing the thick endocortex of sterrasters and the radial organization of the megascleres. C. Oxea. D. Orthotriaene. E. Cladome with a bent clad. F. Sterraster. G. Hilo. H. Broken sterraster. I. Acanthoxyaster I. J. Acanthoxyaster II. L. Microrhabds. K. Broken sterraster (CNPGG 1203, *Geodia gibberosa* Lamarck, 1815).

Table 1. Polymorphic sites found in the COI of *P. johnstonia* and *P. normani*.

	Nitrogenous bases			
	A	C	T	G
Positions of mutation	195, 276, 288, 300, 420, 525, 579, 642	189, 219, 298, 354, 366, 396, 468, 474, 505, 510, 553, 621	126, 162, 336, 426, 435, 511, 597, 606, 651	180, 228, 330, 346, 393, 423, 462, 465, 490, 573, 585, 639

Considerações finais

Embora possuam significativa riqueza em termos de conjunto espicular, muitas espécies do gênero *Geodia* não tem uma taxonomia fácil, dada à variação da morfologia externa, à infrequência de determinadas categorias de espículas, ou ainda, à variação de formas que as mesmas apresentam. Tais fatores levam taxonomistas à identificações errôneas e acabam por provocar implicações em outros tipos de estudos.

Além de corroborar o polimorfismo (outrora mencionado na literatura) para a morfologia externa de *Geodia gibberosa*, o presente estudo também detectou categorias raras de triênios (ana- e plagiotriênios) que jamais haviam sido vistas no holótipo, e propõe algumas hipóteses para explicar a infrequência de algumas espículas.

Os marcadores mitocondriais COI e ATP6, mesmo mostrando-se relativamente conservados, foram eficientes em revelar a existência três de espécies crípticas dentro de *G. gibberosa*. Contudo, mais amostras precisariam ter sido amplificadas para o segundo gene, em especial aquelas que constituem o Clado IV (evidenciado apenas com o COI). Por meio dos métodos de taxonomia clássica, no entanto, não foi possível detectar padrões que permitissem separar espécies morfologicamente, e talvez outras análises (e.g. análises estatísticas aplicadas à morfometria, estudos de desenvolvimento, etc.) devessem ser empregadas para esse fim.

O presente estudo, assim como diversas pesquisas já realizadas com poríferos considerados cosmopolitas ou com ampla distribuição (KLAUTAU *et al.*, 1999; DE PAULA *et al.*, 2012), conseguiu detectar a ocorrência de espécies crípticas, e é o primeiro de sua natureza com um representante da família Geodiidae (que é uma das maiores e mais antigas dentro de sua Ordem). Seus resultados só vem reforçar quão subestimado é o conhecimento acerca da biodiversidade em esponjas, e reforçam a necessidade da utilização de diferentes fontes de informação/ferramentas em estudos de taxonomia, com o intuito de se verificar o status de espécies cujos limites não são muito claros. Nesses termos, a taxonomia integrativa tem se mostrado muito eficiente e vem se consolidando cada vez mais como uma prática comum na academia.

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Apêndice 1. Protocolos de extração de DNA genômico total

1.1. Fenol:Clorofórmio:Álcool Isoamílico (Sambrook *et al.*, 1989 – adaptado)

1. Colocar em microtubos (1,5ml) as amostras (0,05g) e adicionar 550 µL de tampão de lise (50mM de Tris HCl, 50mM de EDTA, 100mM de NaCl).
2. Adicionar 40 µL de SDS 20% (dodecil sulfato de sódio) e 15 µL de proteinase K (20mg/mL).
3. Vortexar as amostras e em seguida incubar em termo bloco a 55°C *overnight* (ou por 2hs na mesma temperatura).
4. Retirar o tubo do Termo bloco e adicionar 550 µL de fenol/clorofórmio/álcool isoamilico (ou somente 275 µL de fenol e 275 µL de clorofórmio).
5. Fechar muito bem os tubos e misturar as fases por inversão de tubos por 10 minutos.
6. Centrifugar por 30 minutos a 14000 rpm (enquanto isso nomear novos tubos de 2 mL)
7. Transferir o sobrenadante para outro tubo (muito cuidado para não encostar a ponteira na fase abaixo ao sobrenadante).
8. Repetir os passos 4 a 7.
9. O mesmo volume dado de sobrenadante adicionar de Clorofórmio.
10 Misturar novamente por inversão de tubos por 10 minutos.
11. Centrifugar por 10 minutos a 14000rpm (enquanto isso nomear novos tubos).
12. Transferir o sobrenadante para um novo tubo.
13. Adicionar 10% de Acetato de Sódio 3M (pH 7,0) e 2,5x do volume do sobrenadante de etanol absoluto gelado.
14. Misturar as fases por inversão de tubos cuidadosamente por 10 minutos.
15. Incubar as amostras em freezer a -20°C por 2 horas ou *overnight.*
16. Centrifugar as amostras por 15 minutos a 14000 rpm.
17. Reverter cuidadosamente para retirar o etanol.
18. Adicionar 600 µL de etanol 70%, agitar cuidadosamente o tubo e centrifugar por mais 10 minutos a 14000 rpm, depois reverter o tubo para descartar o etanol.
19. Adicionar 600 µL de etanol 70% (ou 100%), agitar cuidadosamente o tubo e centrifugar por mais 10 minutos a 14000 rpm, depois reverter o tubo para descartar o etanol.

20. Secar o DNA em temperatura ambiente ou em estufa a 37°C.

21. Com o DNA sem resíduo algum de etanol adicionar TE (10mM de Tris pH 8,0 e 1mM de EDTA) ou água MilliQ de acordo com o tamanho do *pellet*.

22. Adicionar 30 µL de RNAse (5 µL).

23. Deixar o DNA em termo bloco a 37°C por 40 minutos e depois conservar a -20°C até a utilização.

1.2. Tampão Salino (Aljanabi & Martinez, 1997* - adaptado)

1. Homogeneizar o tecido (50 – 100 mg ou 1 cm2) em 400 µl de tampão salino (NaCl 0,4 M; Tris HCl 10 mM pH 8,0 e EDTA pH 8,0 a 0,2 mM).

2. Adicionar 40 µl de SDS 20% e 8,0 µl de Proteinase K 20 mg/ml e misturar bem.

3. Incubar as amostras a 55 - 65°C por pelo menos 2h ou *overnight*.

4. Adicionar 300 µl de NaCl 6 M.

5. Vortexar as amostras por 30 segundos a velocidade máxima e centrifugar por 30 min. a 10000 rpm.

6. Transferir o sobrenadante para outro tubo (2,0 ml).

7. Adicionar igual volume de isopropanol e misturar bem.

8. Incubar a -20°C por 1h.

9. Centrifugar por 20 min. a 10000 rpm.

10. Lavar o pellet com 300 µl de etanol 70% e centrifugar por 5 minutos.

11. Lavar o pellet novamente com 300 µl de etanol 100% e centrifugar por mais 5 min.

12. Secar o DNA em estufa e ressuspender em 50 µl de H2O. Caso haja necessidade, colocar RNAse (2,0 µl a uma concentração de 10 ng/µl).

* Referência

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Apêndice 2. *Primers* adicionais utilizados no trabalho

Tabela A. Relação de *primers* adicionais utilizados no presente trabalho, mas que não funcionaram.

Região	*Primers*	Sequência	Referência*
COI	*cox1*-D2	AATACTGCTTTTTTTGATCCTGTTGG	Rot *et al*., 2006
	cox1-R1	TGTTGRGGGAAAAARGTTAAATT	
COI	Uni-minibarF1	TCCACTAATCACAARGATATTGGTAC'	Meusnier *et al*., 2008
	Uni-minibarR1	GAAAATCATAATGAAGGCATGAGC	
28S	C2	GAAAAGAACTTTGRARAGAGAGT	Chombard *et al*., 1998
	D2	TCCGTGTTTCAAGACGGG	
ITS	18S	TCATTTAGAGGAAGTAAAAGTCG	Lobo-Hajdu *et al*., 2004
	28S	GTTAGTTTCTTTTCCTCCGCTT	

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Apêndice 3. Purificação com PEG, para um volume final de 25µl de produto de PCR (Lis & Schleif, 1975 - adaptado)

1. Adicionar 0,4 µl de glicogênio por amostra.
2. Adicionar igual volume de solução de PEG (25µl) ao produto de PCR .
3. Homogeneizar.
4. Incubar em banho seco. A 37 °C por 20 min.
5. Centrifugar por 15 min. a 14.500 rpm.
6. Descartar o sobrenadante.
7. Adicionar levemente 125 µl de etanol gelado 80% .
8. Centrifugar por 4 min a 14.500 rpm.
9. Repetir os passos 6 e 7.
10. Centrifugar por 10 min.
11. Verter o álcool e manter os tubos a 37 °C por 40 min.
12. Adicionar 12-15µl de água deionizada autoclava ou TE.

Anexo. Normas de submissão (Zootaxa)

Information for authors

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Zootaxa is a peer-reviewed international journal for rapid publication of high quality papers on any aspect of systematic zoology, with a preference for large taxonomic works such as monographs and revisions. *Zootaxa* considers papers on all animal taxa, both living and fossil, and especially encourages descriptions of new taxa. All types of taxonomic papers are considered, including theories and methods of systematics and phylogeny, taxonomic monographs, revisions and reviews, catalogues/checklists, biographies and bibliographies, identification guides, analysis of characters, phylogenetic relationships and zoogeographical patterns of distribution, descriptions of taxa, and nomenclature. Open access publishing option is strongly encouraged for authors with research grants and other funds. For those without grants/funds, all accepted manuscripts will be published but access is secured for subscribers only. All manuscripts will be subjected to peer review before acceptance. *Zootaxa* aims to publish each paper within one month after the acceptance by editors.

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providing more information (e.g. checklist of or key to species of the genus, biological information.....). Short papers of 4 or 5 pages accepted for publication may be shortened for publication in the Correspondence section.

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- taxonomic/nomenclatural notes of importance
- book reviews meant to introduce readers to new or rare taxonomic monographs (interested authors/publishers must write to subject editors before submitting books for review; editors then prepare the book review or invite colleagues to prepare the review; unsolicited reviews are not published)
- and short papers converted from manuscripts submitted as research articles but are too short to qualify as formal research articles.

These short contributions should have no more than **20 references** and its **total length should not exceed four printed pages (except editorials).** Neither an abstract nor a list of key words is needed; major headings (Introduction, Material and methods...) should NOT be used, except for new taxon heading and references. A typical correspondence should consist of (1) a short and concise title, (2) author name and address (email address), (3) a series of paragraphs of the main text,and (4) a list of references if any. For correspondence of 3 or 4 pages, the first or last paragraph may be a summary.

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2) The **title** should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

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Smith, A. (1999) Title of the paper. *Title of the journal in full*, volume number, page range.

B) **Book chapter**:
Smith, A. & Smith, B. (2000) Title of the Chapter. *In*: Smith, A, Smith, B. & Smith, C. (Eds), *Title of Book*. Publisher name and location, pp. x–y.

C) **Book**:
Smith, A., Smith, B. & Smith, C. (2001) *Title of Book*. Publisher name and location, xyz pp.

C) **Internet resources**
Author (2002) *Title of website, database or other resources*, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

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