UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-graduação em Zoologia

Letícia Curvello Franco

ANTILLESOMA ANTILLARUM (Grübe & Oersted, 1858): COSMOPOLITAN SPECIES OR SPECIES COMPLEX

Belo Horizonte
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ANTILLESOMA ANTILLARUM (Grübe & Oersted, 1858): COSMOPOLITAN SPECIES OR SPECIES COMPLEX

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Orientadora: Profa. Dra. Gisele Yukimi Kawauchi

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PÓS-GRADUAÇÃO EM ZOOLOGIA

FOLHA DE APROVAÇÃO

***Antillesoma antillarum* (Grübe & Oersted, 1858): cosmopolitan species or species complex?**

LETÍCIA CURVELLO FRANCO

Esta dissertação foi apresentada em sessão pública e submetida a avaliação em 26 de outubro de 2023, tendo sido aprovada pela Banca Examinadora composta pelos seguintes membros:

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"No name, no information, wrong name, wrong information."

- ***Meredith Lane (Botanist)***

RESUMO

Os Sipuncula, anelídeos marinhos, têm sido amplamente negligenciados em estudos de taxonomia e sistemática a nível global. Esta dissertação visa preencher essa lacuna histórica ao documentar a presença da espécie supostamente cosmopolita *Antillesoma antillarum* (Grübe & Oersted, 1858) em dezenove localidades brasileiras, com novos registros em três estados, fornecendo considerações sobre sua distribuição no Brasil e no mundo. Detalhes morfológicos para as espécies do litoral brasileiro são descritos. Ademais, por meio de uma abordagem integrativa que combina dados morfológicos e moleculares, foi investigada e refutada a hipótese do cosmopolitismo de *A. antillarum*. Identificamos três espécies putativas dentro da família Antillesomatidae (Kawauchi, Sharma, & Giribet, 2012)): uma linhagem correspondente a populações do Atlântico Ocidental de, abrangendo da Flórida, nos EUA, até o Espírito Santo, Brasil; uma segunda linhagem correspondente a indivíduos da Tailândia; e uma terceira linhagem correspondente a espécie *A. mexicanum* no Pacífico Oriental. Este estudo marca o início de investigações futuras, abrindo perspectivas para uma compreensão mais profunda da biodiversidade e taxonomia neste grupo.

Palavras-chave: Annelida; Antillesomatidae; delimitação de espécies; distribuição geográfica; espécies pseudo-crípticas.

ABSTRACT

Sipuncula are marine annelids that have been widely overlooked in global taxonomic and systematic studies. This dissertation aims to fill this historical gap by documenting the presence of the supposedly cosmopolitan species *Antillesoma antillarum* (Grübe & Oersted, 1858) in nineteen Brazilian localities, with new records in three states, shedding light on its distribution in Brazil and worldwide. Morphological details for coastal Brazilian species are delineated. Furthermore, through an integrative approach that combines morphological and molecular data, the hypothesis of *A. antillarum*'s cosmopolitanism was investigated and debunked. We identified three putative species within the Antillesomatidae (Kawauchi, Sharma, & Giribet, 2012) family: one lineage corresponding to populations of the Western Atlantic, spanning from Florida, the USA, to Espírito Santo, Brazil; a second lineage corresponding to individuals from Thailand; and a third lineage corresponding to *A. mexicanum* in the Eastern Pacific. This study marks the inception of future investigations, providing insights for a deeper understanding of biodiversity and taxonomy within this group.

Keywords: Annelida; Antillesomatidae; species delimitation; geographic distribution; pseudo-cryptic species.

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1.1 Sipuncula

Sipuncula é um grupo de invertebrados exclusivamente marinhos (Cutler, 1994). São protostômios celomados com simetria bilateral e um corpo vermiforme (Murina, 1984; Cutler, 1994). Esses animais podem ser encontrados em uma ampla variedade de habitats marinhos, incluindo sedimentos consolidados e não consolidados; dentro de conchas de moluscos vazias, esqueletos de corais (Stephen & Edmonds, 1972) entre outros. Estão distribuídos por todos os oceanos, desde regiões entre-marés a grandes profundidades (Cutler, 1994; Jaeckle & Rice, 2002). No entanto, é em águas tropicais e subtropicais que a maior diversidade desses animais é observada (Rice, 1975).

Os sipúnculos na fase adulta apresentam um plano corpóreo relativamente simples, com características únicas que são conservadas dentro do grupo (Rice, 1985; Schulze *et al*., 2012). O corpo, que pode variar de alguns milímetros até 30 centímetros de comprimento, é dividido em duas regiões principais: um tronco posterior e um introverte retrátil anterior (Fig. 1A) (Hyman, 1959; Jaeckle & Rice, 2002). Na região proximal, os tentáculos podem circundar a boca, como na família Sipunculidae, ou estarem dispostos ao redor do órgão nucal, como em Phascolosomatidae (Cutler, 1994; Kawauchi *et al*., 2012). Possuem uma cavidade celômica espaçosa sem segmentação aparente, um intestino recurvado, em formato de U espiralado sobre si mesmo, um ânus que se abre dorsalmente na porção anterior do tronco e um cordão nervoso ventral mediano (Fig. 1B) (Schulze & Kawauchi, 2021).

A taxonomia desse grupo se baseia em poucos caracteres morfológicos externos disponíveis, que incluem o arranjo e o número de tentáculos, a distribuição e a estrutura dos ganchos do introverte (quando presentes), o formato e a posição dos escudos (quando presentes), além da distribuição e da forma das papilas epidérmicas (Cutler, 1994; Kawauchi & Giribet, 2010; Schulze *et al*., 2007, 2012). No final do século XIX, naturalistas europeus como Keferstein (1862, 1865, 1866, 1867) e Selenka *et al*. (1883) voltaram sua atenção para a anatomia interna dos Sipuncula, fornecendo a base para o desenvolvimento das técnicas de dissecação fundamentais para o estudo desse grupo (Stephen & Edmonds, 1972; Cutler, 1994). A identificação adequada de muitas espécies requer a dissecação dos indivíduos adultos para análise de caracteres morfológicos internos (Figura 3B), tais como o número e o

grau de fusão dos músculos retratores do introverte, seu ponto de inserção na parede do tronco, o arranjo da musculatura da parede do tronco, o formato e a posição dos nefrídios, e a presença ou ausência de vilosidades dos vasos contráteis, entre outros (Stephen & Edmonds, 1972; Cutler, 1994; Schulze *et al*., 2012).

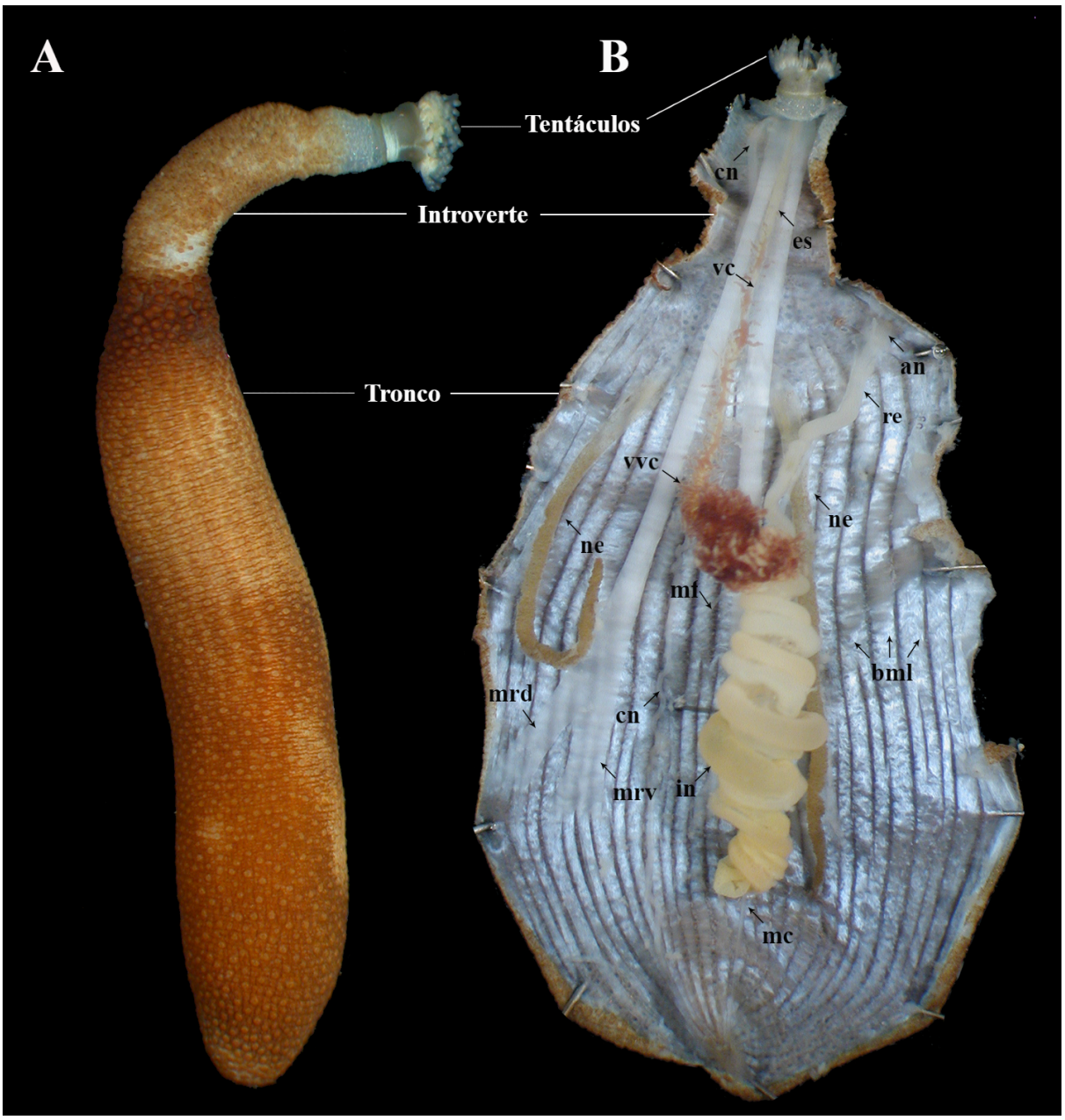

Figura 1. Anatomia externa e interna de Sipuncula representada pela espécie *Antillesoma antillarum*. (A) Morfologia externa. (B) Morfologia interna. Abreviações: an = ânus, bml = banda muscular longitudinal, cv = cordão nervoso ventral, es = esôfago, , mc = músculo columelar, mf = músculo de fixação, mrd = músculo retrator dorsal. mrv = músculo retrator ventral, ne = nefrídio, re = reto vc = vaso contrátil, vvc = vilosidade do vaso contrátil. Modificado de Schulze & Kawauchi (2021).

A posição filogenética dos Sipuncula entre os metazoários foi controversa durante muitos anos (Rice, 1985; Saíz Salinas, 2018), tendo sido classificado como família, ordem,

classe e filo em diferentes momentos (Stephen & Edmonds, 1972; Cutler, 1994). Na tentativa de solucionar este problema, estudos utilizando dados moleculares foram conduzidos e, atualmente, Sipuncula é considerado uma linhagem dentro do clado Annelida. (Tzetlin & Purschke, 2006; Dunn *et al.*, 2008, 2014; Zrzavy *et al.*, 2009; Dordel *et al.*, 2010; Struck *et al.*, 2011; Andrade *et al*., 2015; Lemer *et al*., 2015; Weigert & Bleidorn, 2016).

A primeira compilação sistemática do grupo foi feita por Stephen & Edmonds (1972), contando com cerca de 320 espécies descritas. Posteriormente, Cutler e Gibbs (1985) deram início a uma revisão taxonômica de Sipuncula que estabeleceu a base para taxonomia moderna do grupo. O grupo foi dividido em 17 gêneros, seis famílias, quatro ordens, duas classes e o número de espécies foi reduzido de 320 para 147 espécies (Cutler, 1994). Atualmente, o número de espécies reconhecidas aumentou para 160, devido a descrição de 12 novas espécies e o restabelecimento de uma subspécie previamente sinonimizada (Kawauchi & Rice, 2009; Hylleberg, 2013; Saiz Salinas *et al*., 2015; Silva-Morales *et al*., 2019; Schulze & Kawauchi, 2021).

Diversos estudos utilizando abordagens moleculares (Maxmen et al., 2003; Staton, 2005; Kawauchi et al., 2012) e morfológicas (Schulze et al., 2005; 2007) foram realizados para esclarecer a sistemática interna de Sipuncula, gerando questionamentos acerca da clássica classificação de Cutler (1994). Entretanto, apesar de algumas divergências nas hipóteses filogenéticas, o monofiletismo do grupo é bem suportado (Schulze & Kawauchi, 2021). Kawauchi *et al.* (2012) propuseram uma nova classificação para Sipuncula (Fig. 2), baseada na análise conjunta de seis marcadores genéticos (18S, 28S, 16S, COI, H3 e H4). Foram criadas duas novas famílias, Siphonosomatidae e Antillesomatidae, para solucionar agrupamentos parafiléticos presentes na classificação de Cutler & Gibbs (1985). Diante disso, os 16 gêneros existentes foram reorganizados em seis famílias, sem classificações taxonômicas superiores (Kawauchi *et al.,* 2012).

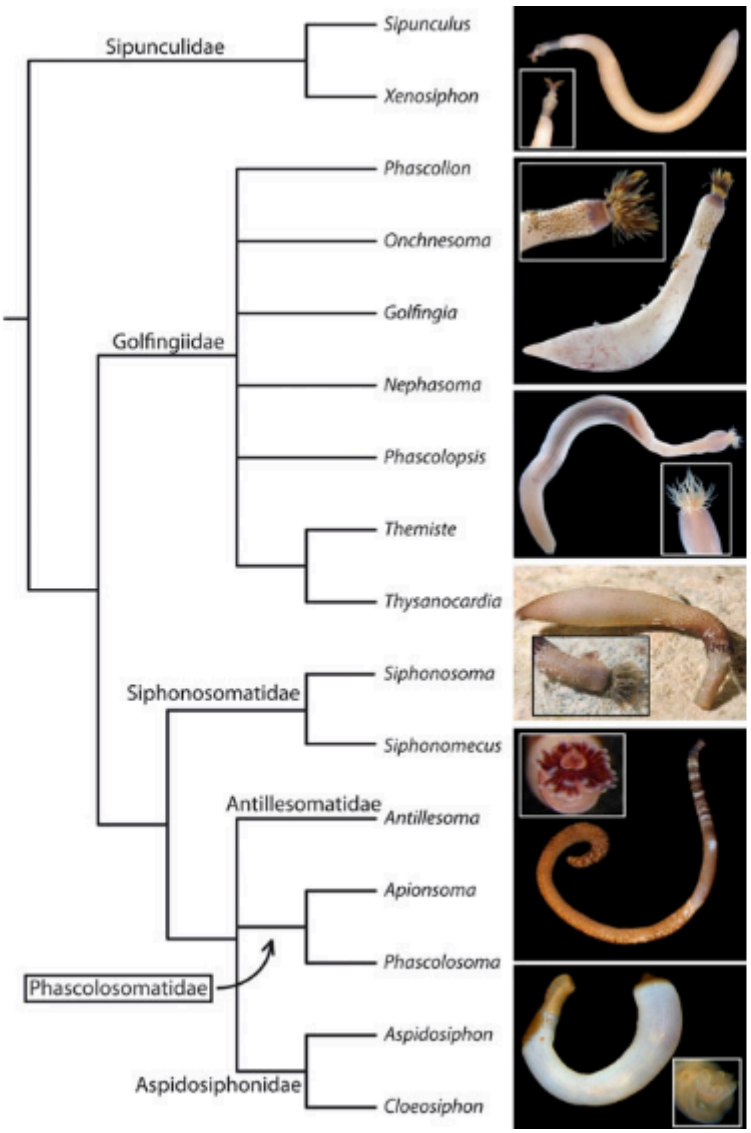

Figura 2. Filogenia atual de Sipuncula. À direita, estão representados um exemplar de cada família. Associado a cada uma destas imagens, temos um quadrado em destaque ilustrando os tentáculos de cada espécie ilustrada. De cima para baixo: *Sipunculus phalloides, Themiste alutacea, Siphonosoma cumanense*, *Antillesoma antillarum*, *Phascolosoma nigrescens*, *Aspidosiphon fischeri* (Kawauchi, Sharma & Giribet, 2012).

1.2 Cosmopolitismo em Sipuncula

Os trabalhos de revisão realizados foram eficazes em reduzir grande parte das confusões existentes em diversas espécies de Sipuncula (Cutler, 1979; Cutler & Cutler, 1981; 1982; 1983; 1985a; 1985b; 1987; 1988; 1989; Cutler *et al*., 1983; Cutler & Jurczak, 1975; Cutler & Murina, 1977; Cutler, N.J. & Cutler, 1986; 1990). No entanto, resultou em extensas listas de sinonímias, refletindo um considerável número de espécies classificadas como cosmopolitas.

Estima-se que essas espécies correspondam a cerca de 20% do total de espécies conhecidas (Kawauchi *et al*., 2010).

Entre os invertebrados marinhos, muitos grupos são considerados cosmopolitas devido à sua ampla distribuição em todos os oceanos (Spellerberg & Sawyer, 1999). Por muito tempo, a dispersão transatlântica de algumas espécies marinhas foi atribuída às larvas pelágicas planctotróficas, o que explicaria tal status cosmopolita (Scheltema, 1968; Rice, 1978, 1981). Acreditava-se que a larva pelagosfera planctotrófica de Sipuncula, que em laboratório foram mantidas por até sete meses sem se metamorfosear, seria responsável pela dispersão e conexão de populações geograficamente distantes (Rice, 1976: 1978; 1981). No entanto, a relação entre a duração das larvas pelágicas e a conectividade das populações tem sido objeto de contestação em diferentes táxons, demonstrando que uma possui pouca influência sobre a outra (Cowen & Sponaugle, 2009; Shanks, 2009; Schulze *et al.,* 2012).

Estudos têm demonstrado que a distribuição geográfica ampla em conjunto com a morfologia simples do organismo e a falta de especialistas capazes de identificar corretamente as espécies, pode resultar em um agrupamento de espécies morfologicamente semelhantes, mas evolutivamente distintas (Aron & Sole-cava, 1991; Knowlton, 1993; Klautau *et al.*, 1999; Lee & Foighil, 2004). Essas espécies podem ser denominadas crípticas, quando duas ou mais espécies são classificadas sob um mesmo nome devido à aparente indistinguibilidade morfológica (Mayr, 1948), ou pseudocrípticas, quando as espécies podem ser diferenciadas após uma análise detalhada de caracteres morfológicos e não morfológicos, assim como de características de outros estágios de vida (Sáez & Lozano, 2005). A identificação de espécies que se enquadram como espécies crípticas/pseudocripticas é relevante para conservação, manejo e estimativas da biodiversidade, sendo o não reconhecimento destas prejudiciais à compreensão evolutiva e ecológica do ambiente (Knowlton, 1993; Sáez & Lozano, 2005 Bickford *et al.,* 2007; Trontelj & Fier, 2009; Struck *et al*., 2019)

As descrições das espécies de Sipuncula são muitas vezes precárias devido aos limitados caracteres morfológicos internos e externos (Kawauchi *et al*., 2010; Schulze *et al*., 2012). Dessa forma, algumas espécies do grupo se enquadram no cenário descrito acima, sendo na verdade complexos de espécies agrupadas sob um mesmo nome (p. ex. Schulze *et al*., 2012; Kawauchi *et al.,* 2014; Schulze & Kawauchi, 2021).

As primeiras evidências da existência de espécies crípticas em Sipuncula foram encontradas por Staton & Rice (1999). Eles investigaram diferenças no desenvolvimento de

indivíduos de *Apiosoma misakanum* coletados em diferentes localidades na Flórida (EUA). Apesar dos adultos serem morfologicamente indistinguíveis (Rice, 1981), divergências genéticas indicaram isolamento reprodutivo, sugerindo a ocorrência de especiação críptica (Staton & Rice, 1999). Análises filogenéticas subsequentes, que envolveram diversos representantes de espécies de Sipuncula supostamente cosmopolitas de diferentes localidades ao redor do mundo, revelaram o amplo potencial das espécies de Sipuncula representarem espécies crípticas ou pseudocrípticas (Maxmen *et al*., 2003; Schulze *et al*., 2005, 2007; Kawauchi *et al*., 2012). Com base nisso, o cosmopolitismo de outras espécies como *Phascolosoma perlucens* (Kawauchi & Giribet, 2010); *Phascolosoma agassizii*, *Thysanocardia nigra*, *Themiste pyroides* (Schulze *et al*., 2012) e *Sipunculus nudus* (Kawauchi *et al*., 2014), já foram contestados.

1.3 *Antillesoma antillarum*

O questionamento da existência de tantas espécies de Sipuncula serem consideradas cosmopolitas, recebeu destaque no trabalho de Schulze & Kawauchi (2021), e *Antillesoma antillarum* (Grube Öersted, 1858) é uma delas (Silva-Morales *et al*., 2019; Schulze & Kawauchi, 2021). Essa espécie foi originalmente descrita como *Phascolosoma antillarum*, com base em exemplares-tipo coletados em Puntarenas, Costa Rica (Pacífico) e Saint Croix, Ilhas Virgens (Atlântico) (Grübe & Oersted, 1858). O gênero *Antillesoma* foi considerado um dos quatro subgêneros de *Phascolosoma* por Stephen & Edmonds (1972), e era originalmente composto por seis espécies [*P. antillarum* (Grube Öersted, 1858)*, P. asser* (Selenka & Man, 1883)*, P. pelmum* (Selenka & Man, 1883)*, P. microdentigerum* (ten Broeke, 1925)*, P. minutum* (ten Broeke, 1925) *e P. horsti* (ten Broeke, 1925)]. Cutler & Cutler (1983) revisaram o subgênero *Antillesoma*, considerando as quatro espécies sem gancho que o compunham (*P. antillarum, P. asser, P. pelmum* e *P. schmidti*), como sinônimos juniores de *Phascolosoma (Antillesoma) antillarum*, junto com quatro espécie do antigo subgênero *Rueppellisoma* [*P. gaudens* (Lanchester, 1905)*, P. onomichianum* (Ikeda, 1904)*, P. simile* (Chen & Yeh, 1958) e *P. weldoni* (Shipley, 1802)]. Posteriormente, *Antillesoma* foi elevado ao status de gênero por Cutler & Gibbs (1985), sendo composto por apenas uma espécie cosmopolita, *A. antillarum*.

A espécie *A. antillarum* foi caracterizada por Cutler & Cutler (1983) por possuir um tronco de até 85 mm, de coloração amarelada e recoberto por papilas escuras e proeminentes. O introverte, sem ganchos, apresenta papilas diminutas, um colarinho distinto e numerosos

tentáculos arroxeados que rodeiam o órgão nucal, estando dispostos dorsalmente a boca (Cutler & Cutler, 1983). Internamente, destaca-se a presença de dois pares de retratores do introverte e o vaso contrátil recoberto por vilosidades digitiformes (Stephen & Edmonds, 1972; Rice, 1970).

Em Sipuncula, o desenvolvimento embrionário é dividido em quatro padrões: (I) Direto; (II) Indireto, com apenas um estágio larval (larva trocófora); (III) Indireto, com dois estágios larvais, larva trocófora seguido de uma larva pelagosfera lecitotrófica de curta duração; (IV) Indireto, com dois estágios larvais, larva trocófora seguido de larva pelagosfera planctotrófica de longa duração (Rice, 1978; 1981). Sendo o padrão IV o mais comum dentro do grupo (Schulze & Kawauchi, 2021) e o observado em *A. antillarum* (Rice, 1975).

A espécie *A. antillarum* foi representante única da família Antillesomatidea criada por Kawauchi *et al*. (2012) até Silva-Morales (2019) descrever uma nova espécie desse gênero, *A. mexicanum*, para a costa oeste do México. Com base em análises moleculares de dois indivíduos de *A. mexicanum* e três exemplares de *A. antillarum* (Caribe, Flórida e da Tailândia), utilizando o gene mitocondrial citocromo oxidase subunidade I (COI), e de observações morfológicas em relação a pigmentação e tamanho corporal, *A. mexicanum* foi separado da espécie congenérica (Silva-Morales *et al*., 2019). Assim como a redescrição de *A. antillarum* de Cutler & Cutler (1983), a descrição da nova espécie, *A. mexicanum*, apresenta limitações a respeito de informações morfológicas, o que torna desafiadora a tarefa de inferir os limites entre essas espécies.

Segundo Saiz Salinas (2018), a tarefa de como caracterizar as espécies em Sipuncula permanece sem solução, tornando-se necessário dedicar esforços para estabelecer uma base sólida para estudos futuros. A integração de dados morfológicos e moleculares se apresenta como um caminho promissor a ser explorado, empregando como ferramenta métodos de delimitação de espécies com base na coalescência (Queiroz, 2007; Carstens et al., 2013; Mason et al., 2020; Mirarab et al., 2021; Chan et al., 2022). Portanto, partindo da premissa destacada por Saiz Salinas (2018) e adotando uma abordagem integrativa que combina dados morfológicos e moleculares, este estudo tem como principal objetivo investigar os limites da espécie *A. antillarum*, definindo seu real status taxonômico. Diante disso, pretende-se gerar novas perspectivas sobre como diferenciar e delimitar espécies crípticas e pseudo crípticas no contexto envolvendo espécies de Sipuncula.

2 OBJETIVOS

O objetivo geral desta dissertação é estabelecer o real status taxonômico de *A. antillarum*. Para cumprir este objetivo elencamos tres questões norteadoras: 1. Qual a distribuição de *A. antillarum* no Brasil e no mundo? 2. *Antillesoma antillarum* é uma espécie cosmopolita?; 3. *Antillesoma antilarum* não sendo cosmopolita, seria possível distinguir as espécies de diversas localidades através de características morfológicas?

3 BIBLIOGRAFIA

Andrade, S. C. S., Novo, M., Kawauchi, G. Y., Worsaae, K., Pleijel, F., Giribet, G., & Rouse, G. W. (2015). Articulating "Archiannelids": Phylogenomics and Annelid Relationships, with Emphasis on Meiofaunal Taxa. *Molecular Biology and Evolution*, 32(11), 2860–2875. https://doi.org/10.1093/molbev/msv157

Aron, S. & Sole-cava, A. (1991). Genetic evaluation of the taxonomic status of two varieties of the cosmopolitan ascidian *Botryllus niger* (Ascidiaceae: Botryllidae). *Biochemical Systematics and Ecology*, 19, 271–276.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*(3), 148–155.

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, *22*(17), 4369–4383.

Chan, K. O., Hertwig, S. T., Neokleous, D. N., Flury, J. M., & Brown, R. M. (2022). Widely used, short 16S rRNA mitochondrial gene fragments yield poor and erratic results in phylogenetic estimation and species delimitation of amphibians. *BMC Ecology and Evolution*, *22*(1), 37.

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, *1*(1), 443–466.

Cutler, E. B. (1979). A reconsideration of the sipunculan taxa *Fisherana* Stephen, *Mitosiphon* Fisher and *Apionsoma* Sluiter. *Zoological Journal of the Linnean Society*, London, 65: 367-384.

Cutler, E. (1994). *The Sipuncula: Their Systematics, Biology, and Evolution*. Cornell University Press.

Cutler, E .B. & Cutler, N. J. (1981). A reconsideration of Sipuncula named by I. Ikeda and H. Sato. *Publications of the Seto Marine Biological Laboratory*, Kyoto, XXVI(1/3): 51-93.

Cutler, E. B. & Cutler, N. J. (1982). A revision of the genus *Siphonosoma* (Sipuncula). *Proceedings of the Biological Society of Washington*, Washington, 95(4): 748- 762.

Cutler, E., & Cutler, N. J. (1983). An examination of the *Phascolosoma* subgenera *Antillesoma*, *Rueppellisoma* and *Satonus* (Sipuncula). *Zoological Journal of the Linnean Society*, 77(2), 175–187.

Cutler, E. B. & Cutler, N .J. (1985a). A revision of the genera *Phascolion* Théel, and *Onchnesoma* Koren and Danielssen (Sipuncula). *Proceedings of the Biological Society of Washington*, Washington, 98(4): 809-850.

Cutler, E.B. & Cutler, N.J. (1985b). A revision of the genera *Sipunculus* and *Xenosiphon* (Sipuncula). *Zoological Journal of the Linnean Society, London*, 85: 219-246.

Cutler, E. B. & Cutler, N. J. (1987). Revision of the genus *Golfingia* (Sipuncula: Golfingiidae). *Proceedings of the Biological Society of Washington*, Washington, 100(4): 735-761.

Cutler, E. B. & Cutler, N. J. (1988). A revision of the genus *Themiste* (Sipuncula). *Proceedings of the Biological Society of Washington,* Washington, 101(4): 741- 766.

Cutler, E. B. & Cutler, N. J. (1989). A revision of the genus *Aspidosiphon* (Sipuncula: Aspidosiphonidae). *Proceedings of the Biological Society of Washington*,Washington, 102(4): 826-865.

Cutler, E. B.; Cutler, N. J. & Gibbs, P. E. (1983). A revision of the *Golfingia* subgenera *Golfingiella*, Stephen, 1964, and *Siphonoides*, Murina, 1967 (Sipuncula). *Proceedings of the Biological Society of Washington*, Washington, 96(4): 669-674.

Cutler, E. B., & Gibbs, P. E. (1985). A Phylogenetic Analysis of Higher Taxa In the Phylum Sipuncula. *Systematic Zoology*, *34*(2), 162.

Cutler, E. B., & Jurczak, E. (1975). The sipunculan genus *Lithacrosiphon* Shipley: A taxonomic review. *Zoological Journal of the Linnean Society*, London, 56(3): 235-248.

Cutler, E.B . & Murina, V. V. (1977). On the sipunculan genus *Golfingia* Lankester, 1885. *Zoological Journal of the Linnean Society*, London, 60(2): 173-187.

Cutler, N .J. & Cutler, E. B. (1986). A revision of the genus *Nephasoma* (Sipuncula: Golfingiidae). P*roceedings of the Biological Society of Washington*, Washington, 99(4): 547-573.

Cutler, N. J. & Cutler, E. B. (1990). A revision of the subgenus *Phascolosoma* (Sipuncula: *Phascolosoma*). *Proceedings of the Biological Society of Washington*, Washington, 103(3): 691-730.

De Queiroz, K. (2007). Species Concepts and Species Delimitation. *Systematic Biology*, *56*(6), 879–886.

Dordel, J., Fisse, F., Purschke, G., & Struck, T. H. (2010). Phylogenetic position of Sipuncula derived from multi-gene and phylogenomic data and its implication for the evolution of segmentation. *Journal of Zoological Systematics and Evolutionary Research*.

Dunn, C. W., Giribet, G., Edgecombe, G. D., & Hejnol, A. (2014). Animal Phylogeny and Its Evolutionary Implications. *Annual Review of Ecology, Evolution, and Systematics*, *45*(1), 371–395.

Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., Seaver, E., Rouse, G. W., Obst, M., Edgecombe, G. D., Sørensen, M. V., Haddock, S. H. D., Schmidt-Rhaesa, A., Okusu, A., Kristensen, R. M., Wheeler, W. C., Martindale, M. Q., & Giribet, G. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*, *452*(7188), 745–749.

Hylleberg, J. (2013). Classification and Identification of Sipunculans from Thailand, with description of new species and a new subgenus. *Phuket Marine Biological Center Special Publication*, *32*, 53–82.

Hyman, L. H. (1959) “The Protostomatous Coelomates – Phylum Sipunculida.” In *The Invertebrates*. Volume 5: Smaller Coelomate Groups, Chaetognatha, Hemichordata, Pogonophora, Phoronida, Ectoprocta, Brachiopoda, Sipunculida, the Coelomate Bilateria, pp. 610–696. New York: McGraw Hill.

Jaeckle, W., & Rice, M. E. (2002). Phylum Sipuncula. Em *Atlas of Marine Invertebrate larvae* (p. 375–396). Academic Press.

Johnson, N. D., & Schulze, A. (2016). Genetic structure in two *Phascolosoma* species in the Pacific Ocean. *Marine Biology Research*, 12(7), 739–747. https://doi.org/10.1080/17451000.2016.1196819.

Kawauchi, G. Y., & Giribet, G. (2010). Are there true cosmopolitan sipunculan worms? A genetic variation study within *Phascolosoma perlucens* (Sipuncula, Phascolosomatidae). *Marine Biology*, *157*(7), 1417–1431. https://doi.org/10.1007/s00227-010-1402-z.

Kawauchi, G. Y., & Giribet, G. (2014). *Sipunculus nudus* Linnaeus, 1766 (Sipuncula): Cosmopolitan or a group of pseudo-cryptic species? An integrated molecular and morphological approach. *Marine Ecology*, *35*(4), 478–491.

Kawauchi, G. Y., & Rice, M. E. (2009). Two New Species of *Nephasoma* (Sipuncula: Golfingiidae) from the Western Atlantic Ocean. *Proceedings of the Biological Society of Washington*, *122*(1), 1–13.

Kawauchi, G. Y., Sharma, P. P., & Giribet, G. (2012). Sipunculan phylogeny based on six genes, with a new classification and the descriptions of two new families: Sipunculan phylogeny. *Zoologica Scripta*, *41*(2), 186–210.

Keferstein, W. (1862) Uber die Gattung Phascolosoma. *Nachrichten von der Koniglichen Gesellschaft der Wissenschaften und der Georg-Augusts Universitat zu Gottingen,* 65-67.

Keferstein, W. (1865) Beitrage zur anatomischen und systematischen Kenntnis der Sipunculiden. *Nachrichten von der Koniglichen Gesellschaft der Wissenschaften und der Georg-Augusts Universitat zu Gottingen*, 189-209.

Keferstein, W. (1866) Untersuchungen iiber einige amerikanische Sipunculiden. *Nachrichten von der Koniglichen Gesellschaft der Wissenschaften und der Georg-Augusts Universitat zu Gottigen*, 14, 215-228.

Keferstein, W. (1867) Untersuchungen iiber einige amerikanische Sipunculiden. *Zeitschrift fiir Wissenschaftliche Zoologie*, 17, 44-55.

Klautau, M., Russo, C. A. M., Lazoski, C., Boury-Esnault, N., Thorpe, J. P., & Solé-Cava, A. M. (1999). Does Cosmopolitanism Result From Overconservative Systematics? A Case Study Using The Marine Sponge *Chondrilla Nucula*. *Evolution*, *53*(5), 1414–1422.

Knowlton, N. (1983). Sibling Species in the Sea. *Annual Review of Ecology and Systematics*, *24*, 189–216.

Knowlton, N. (2000). Molecular genetic analyses of species boundaries in the sea, *Marine Genetics,* Springer Netherlands*,* p. 73–90..

Lee, T., & Ó Foighil, D. (2004). Hidden Floridian biodiversity: Mitochondrial and nuclear gene trees reveal four cryptic species within the scorched mussel, *Brachidontes exustus*, species complex: Multiple Genetic Disjunctions In Marine Mussel Morphospecies. *Molecular Ecology*, *13*(11), 3527–3542.

Lemer, S., Kawauchi, G. Y., Andrade, S. C. S., González, V. L., Boyle, M. J., & Giribet, G. (2015). Re-evaluating the phylogeny of Sipuncula through transcriptomics. *Molecular Phylogenetics and Evolution*, 83, 174–183. https://doi.org/10.1016/j.ympev.2014.10.019

Mason, N. A., Fletcher, N. K., Gill, B. A., Funk, W. C., & Zamudio, K. R. (2020). Coalescent-based species delimitation is sensitive to geographic sampling and isolation by distance. *Systematics and Biodiversity*, *18*(3), 269–280.

Maxmen, A. B., King, B. F., Cutler, E. B., & Giribet, G. (2003). Evolutionary relationships within the protostome phylum Sipuncula: A molecular analysis of ribosomal genes and histone H3 sequence data. *Molecular Phylogenetics and Evolution*, *27*(3), 489–503.

Mirarab, S., Nakhleh, L., & Warnow, T. (2021). Multispecies Coalescent: Theory and Applications in Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, *52*(1), 247–268.

Murina, G. V., Pancucci-Papadopoulou, M. A., & Zenetos, A. (1999). *Phylum Sipuncula in the Mediterranean Sea*. Hellenic Centre for Marine Research (HCMR).

Murina, G.-V. (1984). Ecology of Sipuncula. *Marine Ecology Progress Series*, *17*, 1–7.

Rice, M. E. (1970). Survey of the Sipuncula of the Coral and Beach Rock Communities of the Caribbean Sea. *Proceding of the International of Symposium on the Biology of the Sipuncula and Echiura*, *1*, 35–49.

Rice, M. E. (1975) Observations on the development of six species of Caribbean Sipuncula with a review of development in the phylum. In: Rice ME, Todorovic M, editors. *Proceedings of the International Symposium of Sipuncula and Echiura.* Belgrade: Naucno Delo Press, p 141–60.

Rice, M. E. (1976) Larval development and metamorphosis in Sipuncula. *American Zoologist,* 16:563–71.doi:10.1093/ icb/16.3.563

Rice, M. E. (1978). Morphological And Behavioral Changes At Metamorphosis In The Sipuncula. In: *Proceedings of the Symposium on Settlement and Metamorphosis of Marine Invertebrate Larvae* (p. 83–102).

Rice, M. E. (1981). Larvae Adrift: Patterns and Problems in Life Histories of Sipunculans. *American Zoologist*, *21*(3), 605–619.

Rice, M. E. (1985). Description of a wood-dwelling sipunculan, *Phascolosoma turnera*e, new species. *Proceedings of the Biological Society of Washington.* 98 (1):54–60.

Sáez, A. G., & Lozano, E. (2005). Body doubles. Cryptic species: As we discover more examples of species that are morphologically indistinguishable, we need to ask why and how they exist. *Nature*, 433, 111.

Saiz Salinas, J. I., Bustamante, M., Tajadura, J., Vijapure, T., & Sukumaran, S. (2015). A new subspecies of *Phascolion Théel*, 1875 (Sipuncula: Golfingiidae) from Indian waters. *Zootaxa*, 3931(3), 433.

Scheltema, R. S. (1968). Dispersal of Larvae by Equatorial Ocean Currents and Its Importance to the Zoogeography of Shoal- Water Tropical Species. *Nature*, 217*,* 1159–1162.

Schulze, A., Cutler, E.B., Giribet, G., 2005. Reconstructing the phylogeny of the Sipuncula. *Hydrobiologia* 535/536, 277–29. 10.1007/1-4020-3240-4_15

Schulze, A., Cutler, E. B., & Giribet, G. (2007). Phylogeny of sipunculan worms: A combined analysis of four gene regions and morphology. *Molecular Phylogenetics and Evolution*, 42(1), 171–192.

Schulze, A., Hipes, J., Borda, E., & Rice, M. E. (2018). Who's Who in the Sipuncula: Matching Larvae and Adults Using DNA. S*mithsonian Contributions to the Marine Sciences*, 42, 83–93.

Schulze, A., & Kawauchi, G. Y. (2021). How Many Sipunculan Species Are Hiding in Our Oceans? *Diversity*, 13(2), 43.

Schulze, A., Maiorova, A., Timm, L. E., & Rice, M. E. (2012). Sipunculan Larvae and "Cosmopolitan" Species. *Integrative and Comparative Biology*, 52(4), 497–510.

Shanks, A. (2009). Pelagic Larval Duration and Dispersal Distance Revisited. *The Biological bulletin*, 216, 373–385.

Selenka, E., J. G. de Man & C. Biilow. (1883). Die Sipunculiden, eine systematische Monographic 2, vol. 4. *Reisen in Archipel Phillippinen von Dr. C. Semper Wiesbaden*, 131 pp.

Silva-Morales, I., López-Aquino, M. J., Islas-Villanueva, V., Ruiz-Escobar, F., & Bastida-Zavala, J. R. (2019). Morphological and molecular differences between the Amphiamerican populations of *Antillesoma* (Sipuncula: Antillesomatidae), with the description of a new species. *Revista de Biología Tropical*, 67(S5), S101–S109.

Spellerberg, I. F., & Sawyer, J. W. D. (1999). *An introduction to applied biogeography*. Cambridge University Press.

Staton, J. L. (2005). Phylogenetic analysis of the mitochondrial cytochrome c oxidase subunit 1 gene from 13 sipunculan genera: Intra and interphylum relationships. *Invertebrate Biology*, 122(3), 252–264.

Stephen, A. C., & Edmonds, S. J. (1972). *The phyla Sipuncula and Echiura*. London: Trustees of the British Museum (Natural History), 528.

Struck, T. H., & Cerca, J. (2019). Cryptic Species and Their Evolutionary Significance. *Encyclopedia of life sciences,* 1–9.

Struck, T. H., Paul, C., Hill, N., Hartmann, S., Hösel, C., Kube, M., Lieb, B., Meyer, A., Tiedemann, R., Purschke, G., & Bleidorn, C. (2011). Phylogenomic analyses unravel annelid evolution. *Nature*, 471(7336), 95–98.

Trontelj, P., & Fišer, C. (2009). Perspectives: Cryptic species diversity should not be trivialised. *Systematics and Biodiversity*, *7*(1), 1–3.

Tzetlin, A. B., & Purschke, G. (2006). Fine structure of the pharyngeal apparatus of the pelagosphera larva in *Phascolosoma agassizii* (Sipuncula) and its phylogenetic significance. *Zoomorphology*, 125(3), 109–117.

Weigert, A., & Bleidorn, C. (2016). Current status of annelid phylogeny. *Organisms Diversity & Evolution*, *16*(2), 345–362.

Zrzavý, J., Říha, P., Piálek, L., & Janouškovec, J. (2009). Phylogeny of Annelida (Lophotrochozoa): Total-evidence analysis of morphology and six genes. *BMC Evolutionary Biology*, *9*(1), 189.

4 CHAPTER 1:

Worldwide distribution of Antillesomatidae (Kawauchi, Sharma, & Giribet, 2012) (Annelida: Sipuncula), and new records of *Antillesoma antillarum* (Grübe & Oersted, 1858) along Brazilian Coast

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4.1 ABSTRACT

Sipuncula has historically received limited attention in Brazil, particularly along its northern coasts. This study highlights the occurrence of the Sipuncula species *Antillesoma antillarum* (Grübe & Oersted, 1858) in nineteen Brazilian localities, including new records for Alagoas, Bahia, and Espírito Santo states. *Antillesoma antillarum*, known for its cosmopolitan distribution, thrives in shallow intertidal zones, often found under loose rocks and inside sandstone reefs, where it can attain high population densities. This compilation presents recent discoveries regarding its distribution and presence in Brazil, providing a comprehensive species description, including detailed insights into its external and internal anatomy.

Keywords: Peanut worm; geographical distribution; marine environment; Western Atlantic.

4.2 INTRODUCTION

Sipunculans, commonly known as peanut worms, are annelids characterized by their apparent lack of segmentation (Cutler, 1994; Struck *et al*., 2007; Dordel *et al*., 2010; Carrillo‐Baltodano, 2019). They possess unique characteristics, with their body divided into

two distinct regions: a retractable introvert and a trunk, with the anus opening dorsally-anteriorly (Ditadi & Migotto, 1982).

Within the Sipuncula, species with extensive distributions are observed. Approximately 20% of recognized Sipuncula species are considered cosmopolitan (Kawauchi *et al*., 2010). The wide distribution of several species is a result of Cutler's work, as he conducted the most recent comprehensive systematic revision of the group, reducing the previously recognized number of species from 320 (Stephen & Edmonds, 1972) to 147 (Cutler, 1994). Despite these advancements, our understanding of Sipuncula distribution remains incomplete and uncertain due to limited and biased sampling conducted by oceanographic expeditions and marine biologists, particularly concerning smaller, soft-bodied infaunal taxa (Amor, 1975; Cutler, 1994). This limited sampling has hindered the comprehensive assessment of spatial patterns and the visualization of the true extent of species distribution. Moreover, recent discoveries of cryptic and pseudo-cryptic species complexes within the Sipuncula group have challenged the cosmopolitanism hypothesis (Staton & Rice, 1999; Kawauchi et al., 2010; 2014; Schulze et al., 2012; Johnson et al., 2016; Silva-Morales et al., 2019), making it crucial to establish a robust understanding of the global distribution of these species for further investigations.

Antillesoma antillarum (Grube & Öersted, 1858), considered one of the cosmopolitan species of Sipuncula, is commonly found in tropical and subtropical intertidal and shallow waters (Cutler, 1994). It displays a preference for inhabiting crevices and coral/soft rock habitats. Its presence was documented in several regions (Fig. 1): in the Western Atlantic and Caribbean (Grube & Oersted, 1858; Diesing, 1859; Keferstein, 1865; Baird, 1868; Shipley, 1892; Gerould, 1913; Fischer, 1922; Leroy, 1936; Fisher, 1952; Rice, 1970; 1976; Cutler & Cutler, 1981; 1983; Young, 1986; Cutler *et al.,* 1992; Dean, 2001; Schulze & Rice, 2004; Collin *et al.*, 2005; Schulze, 2005; Matthewes-Cascon & Lotufo, 2006; Dean *et al.,* 2007; Garcia *et al*., 2010; Díaz-Díaz, 2011; Gomez *et al.,* 2013; Quirós-Rodríguez *et al.*, 2021); the Eastern Atlantic (Lanchester, 1905; Wesenberg-Lund, 1959; Cutler & Cutler, 1983), and various locations in the Indo-West Pacific (Selenka *et al*., 1883; Augener, 1903; Ikeda, 1904; Leroy, 1936; Cutler & Cutler, 1981; 1983; Haldar, 1991; Hsueh & Kuo, 2009; Pan-Wen & Siang, 2016), as well as in the Eastern Pacific (Grube & Oersted, 1858; Fischer, 1922; Fisher, 1952; Cutler *et al.,* 1992; Cutler & Cutler, 1983; Dean, 2001; Cortés, 2017).

The species was initially described based on specimens collected from Puntarenas, Costa Rica (Pacific Ocean), and Saint Croix, Virgin Islands (Atlantic Ocean) (Grübe & Oersted, 1858). Subsequently, it was reexamined by Cutler and Cutler (1983), who classified it as a subgenus of *Phascolosoma* and later elevated it to the rank of genus by Cutler and Gibbs (1985). The taxonomic status of *A. antillarum* has undergone significant synonymy. While currently recognized as the sole representative of the Antillesomatidae family (Kawauchi, Sharma, & Giribet, 2012), recent taxonomic research conducted by Silva-Morales *et al*. (2019) reported a new species, *A. mexicanum*, along the Pacific coast of Mexico and pointed to a possible third species in Thailand (Fig. 1).

The occurrence records of *A. antillarum* in Brazil are limited in number. The initial record dates back to 1913, when Gerould documented its presence in Pernambuco without providing further specifics. Subsequently, Young (1986) recorded another occurrence of the species in Tambaíu, João Pessoa, Paraíba. Up until 1994, this remained the sole documented occurrence within the country. In a distribution modeling study, Amor (1975) extended its potential range to southeast Brazil, despite the absence of official records. Additional sightings of the species were later recorded in Maracajaú, Rio Grande do Norte (Garcia *et al*., 2010), and Praia Flecheiras, Trairi, Ceará (Matthewes-Cascon & Lotufo, 2006) during studies investigating the associated coral and sponge fauna.

The primary objective of this study is to compile recent findings on the distribution and occurrence of *A. antillarum* in Brazil. By examining the extent of its occurrence in the country, this research aims to provide valuable insights and establish a solid foundation for future investigations, contributing to our comprehensive understanding of the global distribution patterns of this species.

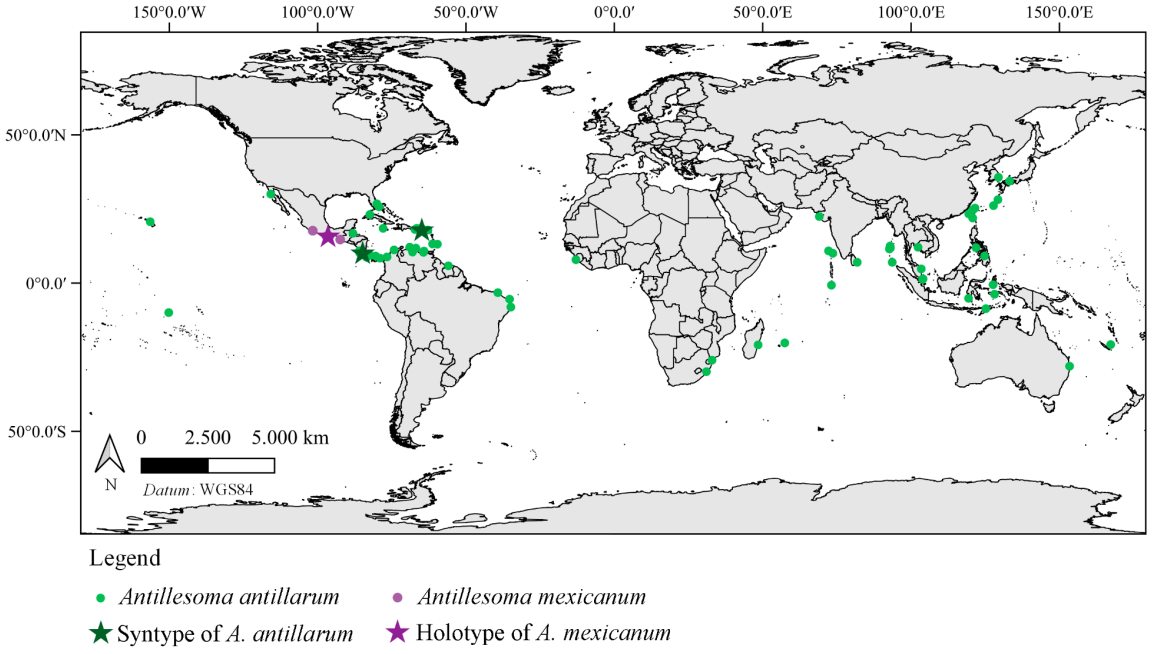

Figure 1. Worldwide Distribution of Antillesomatidae species. Data sourced from bibliographic records.

4.3 MATERIAL AND METHODS

Specimens of *A. antillarum* were collected in Salvador, Bahia, Brazil, and Cabo de Santo Agostinho, Pernambuco, Brazil. To collect the animals, rocks were overturned, or sandstone reefs were broken open with a chisel and hammer. Each fragment was broken into smaller pieces until the animals were exposed, allowing them to be removed without harm. The animals were anesthetized using methodologies described in the specialized literature (Stephen & Edmonds, 1972; Ditadi & Migotto, 1982). After anesthesia, specimens were fixed in 70% ethanol.

Specimens from three invertebrate collections (Invertebrate Collection at the Museu de Zoologia da Universidade de São Paulo - MZUSP, Paulo Young Invertebrate Collection from Universidade Federal da Paraíba - UFPB-SIP, and Centro de Coleções Taxonômicas at Universidade Federal de Minas Gerais - CCT-UFMG;) were used in this study. Additionally, specimens that have not yet undergone formal deposit procedures in collection repositories and are at the Laboratório de Sistemática de Biologia de Annelida (LabSBAnn) - UFMG, we designated the acronym "GYK" (initial letters from the second author name of this study) to identify those samples in this study (Tab. 1).

Specimens of *A. antillarum* were borrowed from the Invertebrate Collection at the Museu de Zoologia da Universidade de São Paulo (acronym used to identify those samples MZUSP) and from Paulo Young Invertebrate Collection from Universidade Federal da Paraíba (acronym used to identify those samples UFPB-SIP**)**. Regarding specimens collected in Salvador, BA, voucher specimens were deposited at the Centro de Coleções Taxonômicas at Universidade Federal de Minas Gerais (acronym used to identify those samples CCT-UFMG). Additionally, for specimens that have not yet undergone formal deposit procedures in collection repositories and are at the Laboratório de Sistemática de Biología de Annelida (LabSBAnn) - UFMG, we designated the acronym "GYK" (initial letters from the second author name of this study) to identify those samples in this study. According to Ditadi (1982), we documented the following measurements. For external anatomy: trunk length ($\overline{x} \pm$ SD), diameter at anal aperture; introvert length and diameter at the posterior end of the introvert; anal opening distance from trunk anterior region. For the internal anatomy, we took the following measurements: number of Longitudinal Muscle Bands (LMBs) at anterior, medium (at the base of the introvert retractors), and posterior end of the trunk; position of nephridiopores relative to LMB; nephridial length and their length attachment to the trunk; introvert retractor attachment position relative to LMBs and the distance from the posterior end of the trunk. All length measurements were taken in millimeters (mm).

Table 1. Relationship between collection numbers, collect year, locality, latitude and longitude.

Collection number	Collect year	Locality	Latitude	Longitude
MZUSP5366	2010	Coroa Vermelha, Porto Segura, BA, Brazil	-16.4456	-39.0656
MZUSP5369	1978	Praia Peracanga,Guarapari, ES, Brazil	-20.6792	-40.5011
MZUSP5370	2012	Foz do rio Cairu, Boipeba, BA, Brazil	-13.3845	-39.0358
MZUSP5371	2011	Tassimirim, Boipeba, Brazil	-13.3986	-38.9356
MZUSP5372	1972	Maceio, AL, Brazil	-9.6486	-35.7056
MZUSP5373	1979	João Pessoa, PB, Brazil	-7.1483	-34.7978
MZUSP5374	1994	Coroa Vermelha, Porto Seguro, BA, Brazil	-16.3372	-39.0080
MZUSP5375	2012	Praia de Bainema, Boipeba, BA, Brazil	-13.6258	-38.9035
MZUSP5376	2013	Praia dos Castelhanos, Boipeba, BA, Brazil	-13.6587	-38.8997
MZUSP5377	2012	Praia dos Castelhanos, Boipeba, BA, Brazil	-13.6588	-38.8997
MZUSP5378	2010	Ponta Grande, Porto Seguro, BA, Brazil	-16.3758	-39.0095
MZUSP5379	2011	Bainema, Boipeba, BA, Brazil	-13.6257	-38.9035
MZUSP5380	2011	Bainema, Boipeba, BA, Brazil	-13.6257	-38.9035

MZUSP54766	2012	Ilha de Trindade, Vitória, ES, Brazil	-20.5227	-29.3311
MZUSP5478	2013	Ilha de Trindade, Vitória, ES, Brazil	-20.5072	-29.3466
MZUSP5479	2013	Ilha de Trindade, Vitória, ES, Brazil	-20.4977	-29.3208
MZUSP5481	2012	Ilha de Trindade, Vitória, ES, Brazil	-20.5227	-29.3311
MZUSP5484	2012	Ilha de Trindade, Vitória, ES, Brazil	-20.5227	-29.3311
MZUSP5491	2012	Ilha de Trindade, Vitória, ES, Brazil	-20.5152	-29.3391
UFMG-INV 2300024- 2300053	2020	Pituba, Salvador, BA, Brazil	-13.0054	-38.4819
UFMG-INV 2300021-2300023	2020	Itapuã, Salvador, BA, Brazil	-12.9499	-38.4065
GYK 174-180	2018	Cabo de Santo Agostinho, PE, Brazil	-8.3530	-34.9265
GYK 114	2013	Fortaleza, CE, Brazil	-3.809642	-38.4096
UFPB.SIP.88	1982	Nísia Floresta, RN, Brazil	-6.053138	-35.1066

4.4 RESULTS

The examination of the material revealed the occurrence of *A. antillarum* on nineteen Brazilian localities spanning from Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, and Espírito Santo (Tab. 1; Fig. 2). Importantly, this study represents the first formal record of *A. antillarum* occurrence in three of these states.

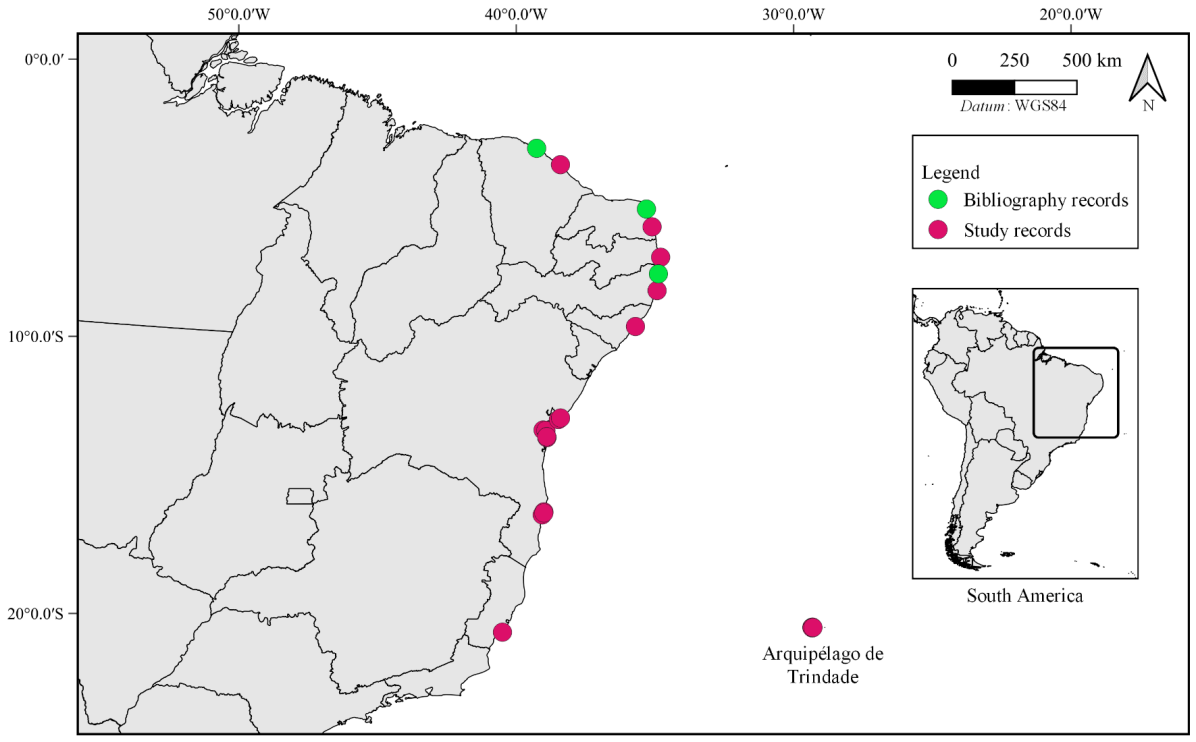

Figure 2. Map showing the current distribution of *A. antillarum* in Brazil.

Genus *Antillesoma* Stephen & Edmonds, 1972

Antillesoma antillarum (Grübe & Oersted, 1858)

Phascolosoma antillarum Grübe & Oersted, 1858:117–118; Fisher, 1952:434–436, plate 39, figs. 8–9; Rice & Macintyre, 1972:42.

Physcosoma antillarum Gerould, 1913:420–421, plate 62, figs. 19–20.

Phascolosoma (Antillesoma) antillarum Stephen & Edmonds, 1972:278–279, figs. 35D–F; Rice, M.E. 1970:42, fig. 5; Young, 1986:103; Cutler & Cutler, 1983:182–184.

Antillesoma antillarum Cutler N. *et al*., 1992:156, fig. 3B; Cutler, 1994:1868–189, fig. 52; Dean, 2001:87–88; Cutler & Schulze, 2004:226; Schulze & Rice, 2004:4; Collin *et al.*, 2005: 688; Matthewes-Cascon & Lotufo, 2006:69; Dean *et al*., 2007:50; Varela & Schulze, 2008:9; Garcia *et al*., 2010:10, fig. 3c; Díaz-Díaz, 2011:169; Gómez *et al*., 2013: 58–6; Quirós-Rodríguez *et al.*, 2021: 1201–1203, fig. 3.

Examined Material (Tab.1): Brazil: **MZUSP5366 (1 specimen)**: Porto Seguro, BA; November 2010. **MZUSP5369 (1 specipem)**: Guarapari, ES; January, 1978. **MZUSP5370 (1 specimen)**: Boipeba, BA; May, 2012. **MZUSP5371 (1 specimen):** Boipeba, BA; August, 2011. **MZUSP5372 (1 specimen)**: Maceió, AL; September, 1972. **MZUSP5373 (1 specimen)**: João Pessoa, PB; December, 1979. **MZUSP5374 (1 specimen):** Coroa Vermelha, BA; November, 2010. **MZUSP5375 (1 specimen):** Boipeba, BA; Abril, 2010. **MZUSP5376 (2 specipens)**: Boipeba, BA; April, 2012. **MZUSP5377 (1 specimenn)**: Boipeba, BA; March, 2013. **MZUSP5378 (1 specimen)**: Boipeba, BA; April , 2012. **MZUSP5379 (1 specimen):** Porto Seguro, BA; November, 2010. **MZUSP5380 (1 specimen):** Boipeba, BA; August, 2011. **MZUSP54766 (1 specimen)**: Trindade, ES; June, 2012. **MZUSP5478 (2 specimens)**: Trindade, ES; July, 2013. **MZUSP5479 (1 specimem)**: Trindade, ES; July, 2013. **MZUSP5481 (1 specimem)**: Trindade, ES; July, 2012. **MZUSP5484 (1 specimem)**:Trindade, ES; June, 2021. **MZUSP5491 (1 specimem)**: Trindade, ES; July, 24, 2012. **UFMG-INV 2300024-UFMG-INV 2300053 (30 specimens)**: Praia da Pituba, Salvador, BA; December, 2022. **UFMG-INV 2300021-UFMG-INV 2300023 (3 specimens)**: Praia de Itapuã, Salvador, BA; December, 2022. **GYK 114 (1 specimen)**: Praia de Sabiaguaba, Fortaleza, CE; 2013. **GYK 174-180 (7 specimens)**: Cabo

de Santo Agostinho; PE; Abril, 2018. **UFPB-SIP 88** (1 specimem): Pontal de Tabatinga, Nísia Floresta, RN; August, 1982.

Diagnosis:

External anatomy (Fig. 3A). Body measuring 34 ± 15 mm in length (N=63). Introvert: 8 ± 4 mm length (N=55), covered with conical papillae (some of them pointing to the posterior region), ratio between introvert diameter and introvert length 1:4 (1:38–1:7). Tentacles >40, digitiform purplish or greenish-brown. Trunk: 26 ± 11 mm length (N=63), pinkish or dark brown fixed specimens, and covered by papillae divided into three regions - anterior: rounded dark papillae; medial: small light papilla; posterior: rounded dark papilla. Ratio between trunk diameter and trunk length 1:7 (1:38–1:17). Anus opening at 2±1 mm (N=63) from the anterior region of the trunk

Internal anatomy (Fig. 3B). Internal trunk with longitudinal muscles in anastomosing bundles beginning 10±8 mm (N=24) from anterior region of the trunk; anterior region with 12–19 LMBs (N=34), 21–36 LBMs (N=34) at base of retractor muscles, and posterior end with 19–35 LBMs (N=34). Nephridia opening immediately posterior to anus, between 2–3, 3-4 LMBs (N= 33), measuring 17±9 mm (N=32) and 14±3 mm (N=25) in length attached to the trunk wall, brownish-yellow to orange in color in fixed specimens. Two pairs of introvert retractor muscles fixed to the trunk 17±4 mm (N=31) from the anterior region of trunk, ventral pair attached between 1–5, 1–6, 2–5, 2–6, 2–7, 3–6, 3–7 (N=22), dorsal pair attached between 5–8, 6–8, 6–9, 7–9, 8–10, 8–11 (N=22). Spindle muscle attached immediately anterior to anus. Fixing muscle originates from the spindle muscle at 2nd intestinal coil attached to left side of ventral nerve cord, 16±5 mm (N=13) from posterior end. Wing muscle extends from anus in both left and right directions, covering 3, 4, or 5 LMBs (N=31). Contractile vessel covered by digitiform villi, reddish or whitish in color.

Taxonomic Remarks: The external and internal anatomy of *A. antillarum* from Brazil closely adheres to the descriptions provided by Gerould (1913), Fisher (1952), and Cutler (1994) for specimens collected from various locations along the western Atlantic coast. In the color specimen account, our observations contrast with Cutler (1994), which described a brownish-green tentacle pattern only for fixed specimens. We have observed this collaboration pattern within fixed and live individuals. All specimens from Cabo de Santo Agostinho, Pernambuco, exhibit brownish-green tentacles and were found under loose rocks.

At Praia de Itapuã, Bahia, only three individuals were encountered under rocks, with two exhibiting greenish-brown tentacles and one displaying a purple hue. Conversely, at Praia da Pituba, Bahia, a substantial population of *A. antillarum* was found inhabiting the sandstone reefs' interiors, all exhibiting brownish-green tentacles. Variations in trunk coloration patterns were noted, with individuals displaying a pinkish-reddish (on museum specimens) or brownish (on live and fixed animals) pattern. The coloration of the contractile vessel villi also exhibited variability, with white villi observed in both fresh and long-preserved specimens, while the reddish pattern was limited to specific individuals within the museum specimens. The high variation in the total count of LMBs along the trunk was also observed due to the high degree of anastomosis, resulting in variations in the position of the introvert retractor along the LMBs.

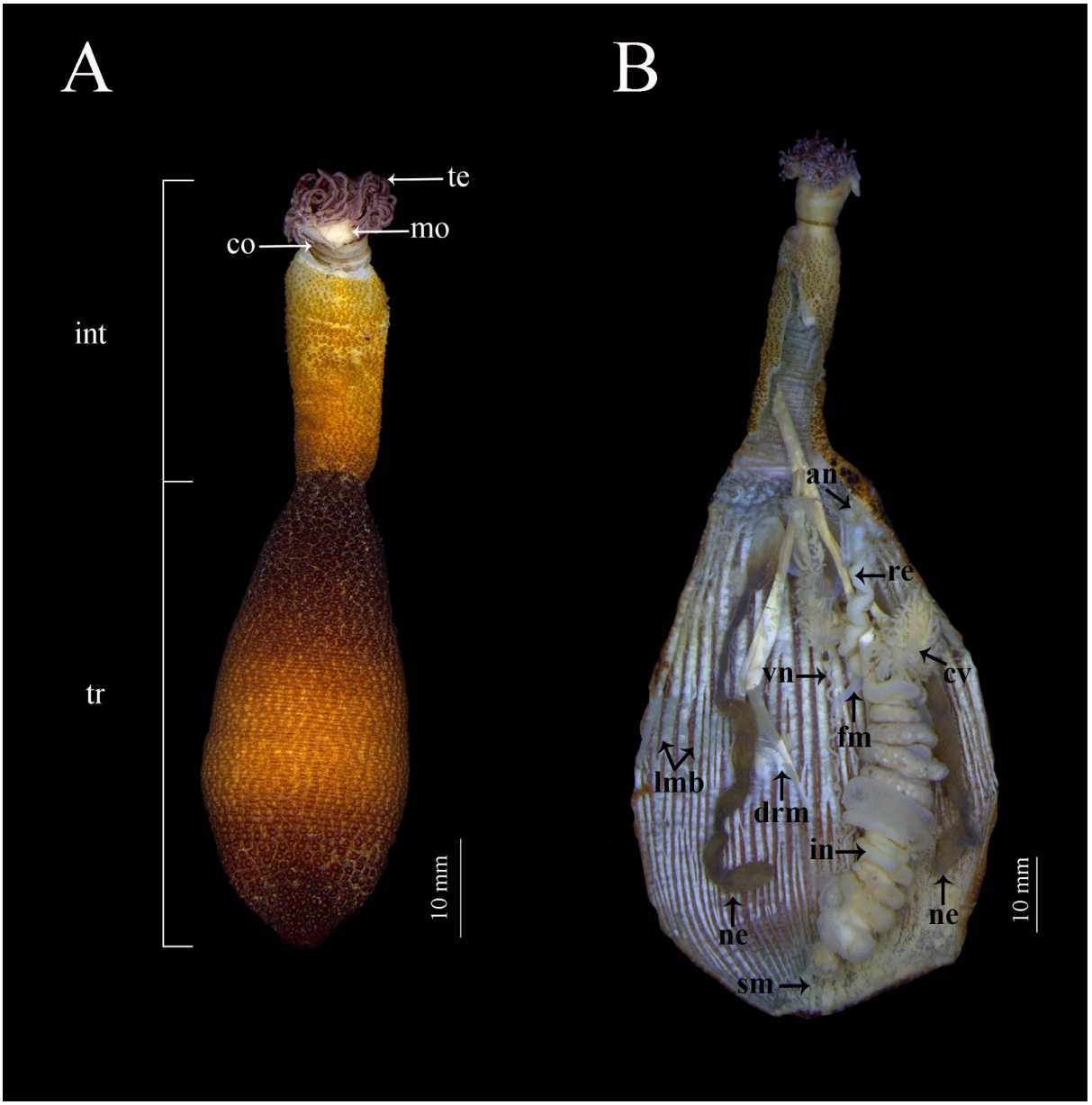

Figure 3. *Antillesoma antillarum* anatomy (fixed specimen). (A) External ventral view; (B) Internal view. Abbreviations: an = anus, co = collar, cv = contractile vessel, drm = dorsal retractor muscle, fm = fixing muscle; in = intestine, int = introvert, lmb = longitudinal muscle band, mo = mouth, ne = nephridia, re = rectum, sm = spindle muscle, tr = trunk, vn = ventral nerve cord, vrm = ventral retractor muscle.

Distribution (Tab.1; Fig. 2): *Antillesoma antillarum* has been documented on locations in the Indo-West Pacific, eastern Pacific on the Eastern and Western Atlantic (Cutler, 1994). In Brazil it ocorred on Praia de Flecheiras, Trairi, Ceará (Matthewes-Cascon & Lotufo 2006); Fortaleza, Ceará; Maracajaú, Rio Grande do Norte (Garcia et al. 2010); Nísia Floresta, Rio Grande do Norte; João Pessoa, Paraíba (Young 1986); Pernambuco (Gerould 1913); Cabo de

Santo Agostinho, Pernambuco; Maceió, Alagoas; Salvador, Bahia; Boipeba, Bahia; Porto Seguro, Bahia; Coroa Vermelha, Bahia; Guarapari, Espírito Santo; Arquipélago Trindade e Martim Vaz, Espírito Santo.

4.5 DISCUSSION

In this study, we provide a detailed description of the internal and external morphology of *Antillesoma antillarum* specimens from Brazil. Our description includes information on features such as the height of the anus, the number of LMBs in three regions of the trunk, and the measurement of the onset of anastomosis longitudinal muscles. These details, which had not been documented for the species previously (Gerould, 1913; Fisher, 1952; Stephen & Edmonds, 1972; Cutler & Gibbis, 1983; Cutler, 1994), have been added to enhance our understanding of species variations and facilitate more robust comparisons between different populations. Furthermore, our findings reveal significant variations in body size and trunk coloration, suggesting that these characteristics vary at an intrapopulational level, underscoring their ambiguous nature when attempting to describe new species. Additionally, our results expand the known range of *A. antillarum* in Brazil, aligning in part with the distribution modeling conducted by Amor (1975). According to this author, the distribution of *A. antillarum* may extend further south, reaching the state of São Paulo.

In the analyzed collections, no specimens were found from south Rio de Janeiro or São Paulo states, which are considered well-sampled regions within the given parameters of Sipuncula sampling. Considering that the collection of MZUSP belonged to Sergio Ditadi, the leading Brazilian researcher on Sipuncula for about 30 years, and a reference in Sipuncula in Brazil, any specimens from this group found by any marine biologist in the country may have been donated to Ditadi for identification. Based on that, it is difficult to believe that this absence of specimens for locations south of Espírito Santo state is solely due to sampling bias. Also, *A. antillarum* is not an inconspicuous species (measuring about 42 mm in total length), which makes it hard to be overseen in a collection of invertebrates by other researchers. The absence of *A. antillarum* records for locations south of Espírito Santo may suggest that this is the distribution limit of these species in Brazil. This apparent southern limit of *A. antillarum* in Brazil opens the door to discussions regarding potential factors, such as biogeographic breaks, that may be shaping the distribution of this species.

Traditionally, a biogeographic break has been recognized in the Cabo Frio (around 23°S) in Rio de Janeiro, being considered the limit for the tropical Southwestern Atlantic and the southeast limit for coral reefs (Spalding *et al*., 2007). It is considered a barrier for coastal benthic marine species due to the presence of the seasonal Cabo Frio upwelling system, which can act as a barrier to gene flow through temperature shifts and local selection (Spalding *et al*., 2007; Martins *et al*., 2022). Within the Cabo Frio region, the upwelling significantly influences the distribution of various organisms, as exemplified by the coral reef goby species complex, where population differentiation is influenced by the cold-water upwelling at Cabo Frio (Volk *et al.*, 2022). Recent research has expanded upon this biogeographic boundary, suggesting its northward extension from Cabo Frio, thereby establishing a novel demarcation for tropical fauna (Lotufo, 2002; Barroso *et al*., 2016; Pepato *et al*., 2019). This proposition finds support in instances where species composition differentiation extends further north of Cabo Frio, as evidenced in ascidian communities (Lotufo, 2002) and among Halacaridae mites (Pepato *et al.*, 2019).

Lotufo (2002) highlights that the influence of the Cabo Frio upwelling diminishes on north of Espírito Santo Espírito Santo, leading to warmer waters. Furthermore, along the Brazilian coastline, there is a shift in substrate composition starting from southern Bahia, marked by the transition from sandstone reefs to crystalline substrates (Lotufo, 2002). The species *A. antillarum* has been documented inhabiting rocks with diverse textures, ranging from soft and friable to hard and dense, displaying an apparent preference for calcareous rocks (Rice, 1970) and sandstone reefs. Moreover, Rice (1986) assessed factors influencing the metamorphosis of larvae in the Sipuncula species *Golfingia misakiana* (Ikeda, 1904) and pointed out the presence of a metamorphosis-inducing factor associated with the presence of substrates where adults are found. Consequently, these variations in substrate composition across the Brazilian coast (Lotufo, 2002) may potentially be linked to the observed occurrence limit of *A. antillarum*.

Nevertheless, it is essential to emphasize that the absence of evidence should not be interpreted as evidence of absence (Coro *et al.*, 2016; Fernandez *et al.*, 2022). To draw more comprehensive conclusions about the distribution limits of *A. antillarum* in Brazil, it is necessary to conduct field expeditions in Espirito Santo, Rio de Janeiro, and São Paulo states, as well as an investigation into formal and informal invertebrate collections, south of São Paulo state. Additionally, investigating environmental factors such as temperature, substrate

preference, and other variables, along with ecological niche modeling studies, may further enhance our understanding of the geographical boundaries of *A. antillarum*.

Like other sipunculans, *A. antillarum* displays a broad distribution spanning all oceans (Cutler, 1994). The idea of a species occurring in such distant and disparate locations challenges the notion of it being a single species (Kawauchi *et al.*, 2010; 2014; Schulze *et al.*, 2012). Based on previous studies such as those by Staton & Rice (1999), Kawauchi *et al.* (2010, 2014), Schulze *et al.* (2012), and Silva-Morales *et al*. (2019), there is a strong likelihood that *A. antillarum* compromise actually a species complex not only a single species (Silva-Morales *et al.* 2019; Schulze & Kawauchi, 2021). Furthermore, considering the recent description of the new species *A. mexicanum* along the Pacific coast of Mexico and the indicative of a third species in Thailand (Silva-Morales *et al.,* 2019), a reevaluation of *A. antillarum* records in the Pacific and Indian Ocean is necessary. Therefore, further investigations are warranted to delve deeper into its diversity and distribution.

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4.7 REFERENCES

Amor, A. (1975) On the zoogeography of the Sipuncula fauna in South America. En: Rice, M. & Todorovic, M. (Eds), *Proceedings of the International Symposium on the Biology of Sipuncula and Echiura. Naucno Delo, Vuka Karadzica 5, Beograd,* 1: 14–24.

Augener, H. (1903) Beitrage zur Kenntnis der Gephyreen nach Untersuchung der im Gottinger zoologischen Museum befindlichen Sipunculiden und Echiuriden Archiv fur Naturgeschichte, 69:297–371.

Baird, W. B. (1868) Monograph of the species of worms belonging to the subclass Gephyrea. *Proceedings of the Zoological Society of London 1868*, 77–114.

Barroso, C. X., Lotufo, T. M. da C., & Matthews-Cascon, H. (2016). Biogeography of Brazilian prosobranch gastropods and their Atlantic relationships. *Journal of Biogeography*, 43(12), 2477–2488. doi:10.1111/jbi.12821

Carrillo‐Baltodano, A. M., Boyle, M. J., Rice, M. E., & Meyer, N. P. (2019). Developmental architecture of the nervous system in Themiste lageniformis (Sipuncula): New evidence from confocal laser scanning microscopy and gene expression. *Journal of Morphology*, 280(11), 1628-1650. https://doi.org/10.1002/jmor.21054

Collin, R., Diaz, M. C., Norenburg, J. L., Rocha, R. M., Sanchez, J. A., Schulze, A., Schwartz, M. L., & Valdes, A. (2005) Photographic Identification Guide to Some Common Marine Invertebrates of Bocas Del Toro, Panamá. *Caribbean Journal of Science*, 41(3), 638–707.

Coro, G., Magliozzi, C., Vanden Berghe, E., Bailly, N., Ellenbroek, A. & Pagano, P. (2016). Estimating absence locations of marine species from data of scientific surveys in OBIS. *Ecological Modelling*, 323, 61–76. doi:10.1016/j.ecolmodel.2015.12.008

Cortés, J. (2017). Marine biodiversity baseline for Área de Conservación Guanacaste, Costa Rica: published records. *ZooKeys*, (652). https://doi.org/10.3897/zookeys.652.10427

Cutler, E. B. (1994) *The Sipuncula. Their systematics, biology and evolution*. Cornell University Press, Ithaca, N.Y., 453pp.

Cutler, E. B. & Cutler N. J. (1981) A reconsideration of Sipuncula named by I. Ikeda and H. Sato. *Publications of the Seto Marine Biological Laboratory*, 26:51–93.

Cutler, E. B., & Cutler, N. J. (1983) An examination of the *Phascolosoma* subgenera *Antillesoma*, *Rueppellisoma* and *Satonus* (Sipuncula). *Zoological Journal of the Linnean Society*, 77(2), 175–187. https://doi.org/10.1111/j.1096-3642.1983.tb00528.x

Cutler, E. B., & Schulze, A. (2004) Sipuncula from Barbados, including two new for the island plus *Siphonosoma vastum*; first record from the Atlantic Ocean. *Bulletin of Marine Science*, 74(1), 225–228.

Cutler, N.J., Cutler, E. B. & Vargas, J.A. (1992) Peanut worms (Phylum Sipuncula) from Costa Rica. *Revista de Biología Tropica*l, 40:153–158

Dean, H. K. (2001) Marine Biodiversity of Costa Rica: The phyla *Sipuncula* and *Echiura*. *Revista De Biología Tropical*, 49(S2), 85–90.

Dean, H. K., Hernandez-Avila, I., & Cutler, E. B. (2007) Sipunculans of the Caribbean coast of Venezuela and Curacao. *Zootaxa*, 1431(1), 45–54. https://doi.org/10.11646/zootaxa.1431.1.2

Díaz-Díaz, O. (2011) Estado del conocimiento de los sipuncúlidos (Sipunculida) del Golfo de Cariaco, Venezuela. *Boletín del IOV*, 50(2), 167–171. ISSN 0798-0639.

Diesing, K. M. (1859) Revision der Rhyngodeen. Sitzungsberichte der mathematisch-naturwissen-schaftliche Klasse, Akademie der Wissenschaften in Wien 37: 719–782.

Ditadi, A. S. F., & Migotto, A. E. (1982) *O Filo Sipuncula. Guia de coleta, Identificação e manutenção em laboratório*. Série Manuais de Identificação da Fauna Brasileira. CNPq, Brasília, 43p.

Dordel, J., Fisse, F., Purschke, G., & Struck, T. H. (2010). Phylogenetic position of Sipuncula derived from multi‐gene and phylogenomic data and its implication for the evolution of segmentation. *Journal of Zoological Systematics and Evolutionary Research*, 48(3), 197–207. https://doi.org/10.1111/j.1439-0469.2010.00567.x

Fernandez, M., Sillero, N., & Yesson, C. (2022). To be or not to be: The role of absences in niche modelling for highly mobile species in dynamic marine environments. *Ecological Modelling*, 471, 110040. https://doi.org/10.1016/j.ecolmodel.2022.110040

Fischer, W. (1922). Gephyreen des Reichsmuseums zu Stockholm. *Arkiv for Zoological,* 14 (19):1–39.

Fisher, W. K. (1952) The sipunculid worms of California and Baja California. *Proceedings of the U.S. National Museum*, 102, 371–450.

Floeter, S. R.; Soares-Gomes, A. (1999) Biogeographic and Species Richness Patterns Of Gastropoda On Southwestern Atlantic. *Revista Brasileira De Biologia*, 59 (4), 567–575.

Garcia, T. M., Matthews-Cascon, H., & Franklin-Junior, W. (2010) Sipuncula Associated with Branching Fire Coral (*Millepora Alcicornis*) in A Marine Protected Area In Northeastern Brazil. *Thalassas*, 1(26), 9–12.

Gerould, J. H. (1913) The sipunculids of the eastern coast of North America. *Proceedings of the United States National Museum*, 44, 373–437.

Gomez, C., Ardila, N., & Sanjuan-Muñoz, A. (2013) Sipunculans associated with dead coral skeletons in the Santa Marta region of Colombia, south-western Caribbean. *Journal of the Marine Biological Association of the United Kingdom*, 93(7), 1785–1793. https://doi.org/10.1017/S002531541300043X

Grube, E. & Oersted, A. S. (1858) Annulata *Oerstediana*. *Videnskabelige Meddelelser Dansk fra naturhistorisk Forening i Kjobenhavn*, 105–120.

Haldar, B. P. (1991)Sipunculans of the Indian coast. Memoirs of the Zoological Survey of India I7(4):1–169.

Hsueh, P. W. & Kuo, C. M. (2009). New records of sipunculan worms from Taiwan. *Zootaxa*, 2067(1), 51–61.

Ikeda, I. (1904) *The Gephyrea of Japan. Journal of the College of Science*, Imperial University of Tokyo 20: 1–87.

Johnson, N. D., Sanders, C., Maiorova, A., & Schulze, A. (2016). Cryptic species in Pacific sipunculans (Sipuncula: Phascolosomatidae): east‐west divergence between non‐sister taxa. *Zoologica Scripta*, 45(4), 455-463. https://doi.org/10.1111/zsc.12158.

Kawauchi, G. Y., & Giribet, G. (2010). Are there true cosmopolitan sipunculan worms? A genetic variation study within *Phascolosoma perlucens* (Sipuncula, Phascolosomatidae). *Marine Biology*, 157, 1417-1431. https://doi.org/10.1007/s00227-010-1402-z

Kawauchi, G. Y., & Giribet, G. (2014) *Sipunculus nudus* Linnaeus, 1766 (*Sipuncula*): cosmopolitan or a group of pseudo-cryptic species? An integrated molecular and morphological approach. *Marine Ecology*, 35(4), 478–491. https://doi.org/10.1111/maec.12104

Kawauchi, G. Y., Sharma, P. P., & Giribet, G. (2012) Sipunculan phylogeny based on six genes, with a new classification and the descriptions of two new families. *Zoologica Scripta*, *41*(2), 186–210. https://doi.org/10.1111/j.1463-6409.2011.00507.x

Keferstein, W. (1865) Beitrage zur anatomischen und systematischen Kenntniss der Sipunculiden. *Zeitschrift fiir Wissenschaftliche Zoologie* 15:404-445.

Leroy, P. (1936) Les sipunculiens du Museum National d'Histoire Naturelle de Paris. *Bulletin du Museum National d'Histoire Naturelle de Paris*, ser. 2, 8:423–426.

Longhurst, A.R. (1958) *An Ecological Survey of the West African Marine Benthos*. London: Colonial Office Fisheries Publication, 101pp.

Lotufo, T. M. C. (2002). Ascidiacea (Chordata: Tunicata) do litoral tropical brasileiro. São Paulo, Brazil: Universidade de São Paulo

Martins, N. T., Macagnan, L. B., Cassano, V., Gurgel, C. F. (2022). Brazilian Marine Phylogeography: A Literature Synthesis and Analysis Of Barriers. *Molecular Ecology*, 31(21), 5423–5439.

Matthewes-Cascon H., & Lotufo, T. M. C. (2006) *Biota marinha da costa oeste do Ceará* - Biodiversidade 24,. Ministério do Meio Ambiente, Brasília, 248 pp.

Pan-Wen, H., & Siang, T. K. (2016). New records of peanut worms (Sipuncula) from Singapore. *Raffles Bulletin of Zoology*, 40, 235–240.

Pepato, A. R., Vidigal, T. H. A., & Klimov, P. B. (2019). Evaluating the boundaries of marine biogeographic regions of the Southwestern Atlantic using halacarid mites (Halacaridae), meiobenthic organisms with a low dispersal potential. E*cology and Evolution*, 9,23,13359–13374, 2019.

Quirós-Rodríguez, J. A., Santafé-Patiño, G., & Nisperuza-Pérez, C. (2021) On the occurrence of *Antillesoma antillarum* (Grube, 1858) (Sipuncula, Antillesomatidae) on the south-central part of the Colombian Caribbean coast. *Check List*, 17(5), 1199–1204. https://doi.org/10.15560/17.5.1199.

Rice, M. E. (1970) Survey of the Sipuncula of the coral and beach-rock communities of the Caribbean Sea. *In:* Rice, M.E. & Todorović, M. (Eds), *Proceedings of the International Symposium on the Biology of Sipuncula and Echiura.* Naučno Delo Press, Belgrade, 35–49.

Rice, M. E. (1976). Larval development and metamorphosis in Sipuncula. *American Zoologist*, 16(3), 563–571.

Rice, M. E., and Macintyre I. G. (1972) A preliminary study of sipunculan burrows in rock thin sections. *Caribbean Journal of Science*, I2(I–2), 41–44.

Selenka, E., J. G. de Man & C. Biilow. (1883) *Die Sipunculiden, eine systematische Monographie. Reisen in Archipel Phillippinen von Dr. C. Semper. C.W. Kreidel,* Wiesbaden, 131 pp.

Schulze, A. & Rice, M. E. (2004) Sipunculan diversity at Twin Cays, Belize with a key to the species. *Atoll Research Bulletin*, 502, 1–9. https://doi.org/10.5479/si.00775630.521.1–9

Schulze, A., & Kawauchi, G. Y. (2021) How Many Sipunculan Species Are Hiding in Our Oceans? *Diversity*, 13(2), 43. https://doi.org/10.3390/d13020043.

Schulze, A., Maiorova, A., Timm, L. E., & Rice, M. E. (2012). Sipunculan larvae and "cosmopolitan" species. *Integrative and Comparative Biology,* 52(4):497-510. https://doi.org/10.1093/icb/ics082.

Shipley, A. E. (1903) Report on the *Gephyrea* collected by Prof. Herdman Ceylon in 1902. C*eylon Pearl Oyster Fisheries Supplementary Repository,* 3:169-176.

Silva-Morales, I., López-Aquino, M. J., Islas-Villanueva, V., Ruiz-Escobar, F., & Bastida-Zavala, J. (2019). Morphological and molecular differences between the Amphiamerican populations of *Antillesoma* (Sipuncula: Antillesomatidae), with the description of a new species. *Revista de Biología Tropical,* 67, 101–109. https://doi.org/ 10.15517/rbt.v67iS5.38934

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Robertson, J. (2007). Marine eco regions of the world: A bioregionalization of coastal and shelf areas. *BioScience*, 57, 573–583. https://doi.org/10.1641/B570707

Staton, J. L., & Rice, M. E. (1999). Genetic differentiation despite teleplanic larval dispersal: allozyme variation in sipunculans of the *Apionsoma misakianum* species-complex. *Bulletin of Marine Science*, 65(2), 467–480.

Stephen, A. C. & Edmonds, S. J. (1972) *The phyla Sipuncula and Echiura*. Trustees British Museum (Natural History), London, 528 pp.

Struck, T. H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., & Halanych, K. M. (2007). Annelid phylogeny and the status of Sipuncula and Echiura. *BMC evolutionary Biology*, 7, 1–11. https://doi.org/10.1186/1471-2148-7-57

Varela, C. & A. Schulze. (2008) An updated checklist of the sipunculans (Phylum Sipuncula) of Cuba. *Cocuyo*, 17, 9–11.

Volk, D. R.; Konvalina, J. D.; Floeter, S. R.; Ferreira, C. E. L.; Hoffman, E. A. (2021). Going Against The Flow: Barriers To Gene Flow Impact Patterns Of Connectivity In Cryptic Coral Reef Gobies Throughout The Western Atlantic. *Journal of Biogeography*, 48, 2, 427–439. 10.1111/jbi.14010

Wesenberg-Lund, E. (1959) Sipunculoidea and Echiuroidea from Mauritius. Viddenskabelige Meddelelser fra Dansk naturhistorisk Forening I København, 121, 53–73.

Young, P. S. (1986) Análise Qualitativa E Quantitativa Da Fauna Associada A Corais Hermatípicos (*Coelenterata*, *Scleractinia*) Nos Recifes De João Pessoa. *Revista Brasileira de Biologia*, 46(1), 99–126.

5. CHAPTER 2:

How many species are hidden in the Antillesomatidae (Kawauchi, Sharma, & Giribet, 2012) (Annelida: Sipuncula) family?

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5.1ABSTRACT

Sipuncula are marine annelids with a relatively simple morphology that presents few diagnostic characters for species differentiation. In recent decades, the supposed cosmopolitanism of some Sipuncula species has been questioned and contested, revealing hidden diversity within the group. This study investigated the taxonomic status of the cosmopolitan Sipuncula species *Antillesoma antillarum* (Grübe & Oersted, 1858). Using an integrative approach of morphological and molecular data, together with coalescent-based delimitation methods, we provide evidence for the identification of three putative species within the genus *Antillesoma*: the recently described species *A. mexicanum* (Silva-Morales *et a*l., 2019), a lineage in the Western Atlantic, and another lineage from Thailand. Our results provide molecular and morphological support for distinguishing these three lineages. Additionally, we discuss the use of some traditional morphological characters in Sipuncula, shedding light on the importance of body contraction state and the relationships of these characters to trunk size.

Keywords: Peanut worm; pseudo-cryptic species; species delimitation; Antillesomatidae; Eastern Pacific; Western Atlantic; Thailand.

5.2 INTRODUCTION

Sipuncula, a marine annelid group, succeeds in diverse marine habitats across the globe (Rice, 1975; Jaeckle & Rice, 2002; Weigert & Bleidorn, 2016). Their adult forms are characterized by a relatively simple body plan, typically divided into a retractable anterior introvert and a posterior trunk (Rice, 1985; Schulze *et al*., 2012). Approximately 160 species of Sipuncula have been recognized, with around 20% of them regarded as cosmopolitan, (Kawauchi & Rice, 2009; Kawauchi & Giribet, 2010; Hylleberg, 2013; Saiz Salinas *et al*., 2015; Silva-Morales *et al*., 2019; Schulze & Kawauchi, 2021). This notable prevalence of cosmopolitan species is not unique to sipunculans, and it is observed in various groups of marine invertebrates with limited morphological distinction (Klautau *et al.*, 1999; Kawauchi & Giribet, 2010; Schulze *et al*., 2012, 2018).

The Sipuncula species *Antillesoma antillarum* (Grübe & Oersted, 1858) stands out as a cosmopolitan, commonly found in tropical and subtropical intertidal and shallow waters, often residing in crevices and coral/soft rock habitats (Rice, 1970; Rice & Macintyre, 1972). Its presence has been documented in various regions, including the Western Atlantic (Grube & Oersted, 1858; Diesing, 1859; Keferstein, 1865; Baird, 1868; Shipley, 1892; Gerould, 1913; Fischer, 1922; Leroy, 1936; Fisher, 1952; Rice, 1975; 1976; Cutler & Cutler, 1981; 1983; Young, 1986; Cutler *et al.,* 1992; Dean, 2001; Schulze & Rice, 2004; Collin *et al.*, 2005; Schulze, 2005; Matthewes-Cascon & Lotufo, 2006; Dean *et al.,* 2007; Garcia *et al*., 2010; Díaz-Díaz, 2011; Gomez *et al.,* 2013; Quirós-Rodríguez *et al.*, 2021); the eastern Atlantic (Lanchester, 1905; Wesenberg-Lund, 1959; Cutler & Cutler, 1983), and he Indo-West Pacific (Selenka *et al*., 1883; Augener, 1903; Ikeda, 1904; Leroy, 1936; Cutler & Cutler, 1981; 1983; Haldar, 1991; Hsueh & Kuo, 2009; Pan-Wen & Siang, 2016), as well as in the eastern Pacific (Grube & Oersted, 1858; Fischer, 1922; Fisher, 1952; Cutler *et al.,* 1992; Cutler & Cutler, 1983; Dean, 2001; Cortés, 2017).

Antillesoma antillarum was the sole member of the Antillesomatidae family (Kawauchi *et al*., 2012), but recently a second species, *A. mexicanum* (Silva-Morales *et al*., 2019), was described for the genus on the eastern coast of Mexico. *Antillesoma antillarum* is known for its trunk that can reach up to 85 mm in length with prominent dark papillae covering its surface (Cutler & Cutler, 1983). The introvert, lacking hooks, exhibits small papillae, has a distinctive collar, and numerous purplish tentacles surrounding the nuchal organ dorsally to the mouth (Gerould, 1913). Internally, notable features include two pairs of introvert

retractors and a contractile vessel covered in digitiform villi (Stephen & Edmonds, 1972; Rice, 1970). *Antillesoma mexicanum*, differs from the congener in terms of size, and coloration pattern of the trunk (Silva-Morales et al., 2019).

The species *A. antillarum* involves the synonymization of twelve species, amalgamating species previously described from different parts of the world into a singular cosmopolitan entity (Cutler & Cutler, 1983; Cutler, 1994). However, Cutler's lumping approach has been contested in other studies, revealing that this supposed cosmopolitanism often conceals species complexes that are cryptic or pseudocryptic (Staton & Rice, 1999; Kawauchi & Giribet, 2010; 2014; Schulze *et al*., 2012; Johnson *et al*., 2016; Silva-Morales *et al.*, 2019).

Adopting an integrative approach that combines morphological and molecular data, this study primarily aims to investigate the boundaries of the *Antillesoma*. Consequently, we intend to generate fresh insights into differentiating and delimiting cryptic and pseudocryptic species in the context of Sipuncula.

5.3 MATERIAL AND METHODS

5.3.1 Obtaining Biological Material

Antillesoma antillarum specimens were borrowed from the Museu de Zoologia da Universidade de São Paulo (MZUSP), Laboratório de Invertebrados Paulo Young from Universidade Federal da Paraíba, Invertebrate Collection of the Museum of Comparative Zoology from Harvard (MCZ). Fresh specimens were collected in Itapuã (12°57'03" S 38°22'00" W) and Pituba (13°00'23"S 38°27'28"W) beach in Salvador, BA, Brazil, and Paraiso beach (8°18'18"S 34°56'46"W) in Cabo de Santo Agostinho, PE, Brazil. The Brazilian specimens were deposited at the Centro de Coleções Taxonômicas at Universidade Federal de Minas Gerais (CCT-UFMG). Additional paratypes and other specimens from *A. mexicanum* were borrowed from the Laboratory of Marine Invertebrate Systematics at Universidad del Mar (UMAR). Sampling locations are depicted in Figure 1 (Tab. 1–3).

Specimens were collected from overturning rocks, and broken open sandstone reefs using a chisel and hammer. Each fragment was broken into smaller pieces until the animals were exposed, allowing them to be removed. The animals were anesthetized whenever possible using methodologies described in the specialized literature (Stephen & Edmonds,

1972; Ditadi & Migotto, 1982). After anesthesia, specimens were fixed in 96% ethanol for molecular analyses and 70% ethanol for morphological analyses.

5.3.2 DNA Extraction and Molecular Analysis

Specimens of *A. antillarum* were dissected, and a piece of the introvert retractor muscle, measuring approximately 0.5 cm^2, was removed and used for DNA extraction. For specimens smaller than two centimeters in length, a piece of the tissue of the same size for larger specimens was removed from the body wall. The genetic material was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., California), following the manufacturer's instructions. The genes cytochrome oxidase subunit I (COI;), 16S rRNA (16S), and histone 3 (H3) were amplified using the TopTaq Master Mix kit (Qiagen Inc., California), with a total volume of 25 µl. The PCR program began with an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at specific temperatures for 30 seconds (35–38°C for COI, 47–48°C for 16S rRNA) or 35 seconds (56–57°C for H3), and elongation at 72°C for 1 minute (for CO1 and 16S rRNA) or 3 minutes (for H3). A final elongation step at 72°C for 7 minutes (CO1 and 16S rRNA) or 3 minutes (H3) was performed, followed by a rapid thermal ramp to 4°C to complete the process. The primers used (Tab. 4) were: for CO1 PolyLCO/PolyHCO (Carr *et al.* 2011), LCO1490 (Folmer *et al.*, 1994), and HCO2198 (Folmer *et al*., 1994) or HCOoutout (Prendini *et al*. (1998); for 16S rRNA were 16Sa (Xiong & Kocher, 1991) and 16Sbr (Xiandong *et al*., 2008); for H3 were H3F/H3R (Colgan *et al.*, 1998).

The PCR product was visualized on a 1.5% agarose gel by electrophoresis and purified using ExoProStar 1 step (GE Healthcare, UK), which was diluted in a 2 µl H2O to 0.5ml ratio. A 2 µl aliquot of this solution was used for every 23 µl of PCR product, and the temperature cycling followed the manufacturer's instructions. Sequencing reactions were performed using the BigDye V3.1 kit (Applied Biosystems Inc.) and analyzed on an ABI 3730 DNA Analyzer (Life Technologies/Thermo Fisher Scientific). Chromatograms were visualized and edited using Geneious software version 2020 1.2 (Biomatters, geneious.com). The trimmed sequences were aligned with MEGA6 (Tamura *et al.,* 2013), using the ClustalW algorithm (Thompson *et al.,* 1994).

5.3.2.1 Phylogenetic Reconstruction

To generate the phylogenies between species from Antillesomatidae, we used a set of multilocus datasets including newly generated sequences for two mitochondrial markers (COI and 16S rRNA) and one nuclear marker (H3) of 47 specimens from *A. antillarum* and 2 from *A. mexicanum*. Sequences from two additional samples from *A. mexicanum* were obtained from GenBank and added to the analysis. The mitochondrial genes (CO1 and 16S rRNA) were analyzed separately, and the three genes (CO1, 16S rRNA and H3) were concatenated for phylogenetic inference with Maximum Likelihood (ML) and Bayesian Inference (BI) approach. Outgroup taxa were chosen among five Sipuncula families, Sipunculidae (1), Phascolosomatidae (1), Siphonosomatidae (1), Golfingiidae (2), and Aspidosiphonidae (1). The relationship between the sequences, gene markers, and location are described in Table 5.

The ML analysis was carried out with the default settings of IQ-TREE ver. 2.0 (Minh *et al.*, 2020), including ultrafast bootstrap (Hoang *et al.*, 2018) with 1 million replicates with the following substitution models defined by ModelFinder (Kalyaanamoorthy *et al.*, 2017) implemented on IQ-TREE: HKY+F+I+G4 for CO1; TIM2+F+G4 for 16 and H3. Bayesian Inference (BI) was performed using MrBayes ver. 3.2.7a (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Ronquist et al., 2012) with four chains, which were run for 1 million generations with trees sampled every 100 generations, following the same substitution models also defined by ModelFinder (Kalyaanamoorthy *et al.*, 2017). The first 25% of sampled trees were discarded as burn-in. The convergence of the runs was evaluated using Tracer ver1.7.1 (Rambaut *et al*., 2018). The resulting trees were visualized and edited using FigTree ver. 1.4.4 (Rambaut, 2010).

5.3.2.2 Species Delimitation analyses

For species delimitation analyses, three species discovery methods were employed to assess the number of putative species within the dataset: the Automatic Barcoding Gap Discovery (ABGD) (Puillandre *et al.*, 2012), the Poisson Tree Process (PTP) (Zhang *et al.*, 2013), and Bayesian implementation of the PTP (bPTP) (Zhang et al., 2013). ABGD analysis was conducted using the output file of the CO1, 16S rRNA, and H3 concatenated distance matrix generated by MEGA 6 (Tamura *et al.,* 2013), and it was performed on the online server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with the Kimura2-parameter distance and default settings. The PTP and bPTP methods were executed using the online

server (https://species.h-its.org/) with default settings. For PTP and bPTP analyses, the input file consisted of ML and BI trees of the CO1, 16S rRNA, and H3 concatenated.

5.3.2.3 Population genetic

The CO1 and 16S rRNA haplotypes were estimated separately using the PHASE (Stephens and Donnelly, 2003) implemented in DnaSP ver 6 (Rozas *et al*., 2017). Statistical parsimony haplotype networks (TCS; Clement *et al*., 2002) were estimated using PopART ver. 1.7 (Leigh & Bryant, 2015) for the two separate genes (CO1, 16S rRNA). Diversity indices within populations were assessed by calculating various parameters for CO1 and 16S rRNA genes using DnaSP ver. 6 (Rozas et al., 2017), including the number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π), number of polymorphic sites (S), and average number of pairwise differences (k). These parameters were calculated for the CO1 gene between individuals from the Western Atlantic, Thailand, and Eastern Pacific. For 16S rRNA calculations were limited to the Western Atlantic and Thailand due to the availability of fewer than four sequences for these genes. The population pairwise F_{ST} between populations was calculated using Arlequin ver. 3.5.2.2 (Excoffier *et al.*, 2005), with significance assessed through 1,000 permutations. The analysis was conducted in two parts: first, considering three groups (Thailand, Western Atlantic, and Eastern Pacific), and second, focusing on Western Atlantic populations (Texas, Caribbean, and Brazil). Genetic distances were calculated for the CO1 markers using the Kimura 2-parameter model on the MEGA 6 (Tamura *et al.,* 2013).

5.3.2 Obtaining and Analysis of Morphological Data

A total of 88 individuals of *Antillesoma* were analyzed from the Western Atlantic, Eastern Pacific, and Indian Ocean (Fig.1; Tab. 2-3).

Specimens were grouped and considered for each analysis as Brazil (SAL-1, SAL-2, COR, POR, BOI, CE, ALA, PAR, RGN, GUA, and TRI), Caribe (BAR, BAH, and BEL), Texas (TEX), Eastern Pacific (MEX, and COS), and Thailand (THA) (Tab. 2-3).

Morphological data collection involved examining each individual under a stereomicroscope. The external measurements (Fig. 5A) were taken for each individual for: 1) the trunk length, 2) introvert length; 3) the distance from the anus to the posterior end. Internally we measured/counted/: 4) the distance from the nephridiopore to the posterior end

(Fig. 5C); 5) nephridial length (Fig. 5C); 6) nephridium attachment to the body wall (Fig. 5C); 7) the distance from the posterior end to attachment of introvert retractor muscles (Fig. 5D); 8) the distance from the posterior end at which the anastomosis of the muscle bands begins (Fig. 5E); 9) the number of longitudinal muscle bands (LMBs) counted at three levels: anterior (C1), introvert base (C2), and posterior region (C3) (Fig. 5F).

The morphological analyses were divided into four steps, using GraphPad Prism ver 8.0.0. The four steps are: **Step I:** correlation analyses were performed to understand the relationship between trunk length and the following measurements: 1) introvert length; 2) the distance from the anus to the posterior end; 3) the distance from the nephridiopore to the posterior end. Internally we measured/counted; 4) nephridium length; 5) the starting point of muscular band anastomosis; 6) introvert retractor insertion; 7) the Longitudinal Muscle bands (LMBs) number on three levels (C1, C2, C3). The analysis was conducted, followed by a simple linear regression to assess the level of correlation among these variables and trunk length for all individuals sampled. Correlation between trunk and introvert length was also analyzed with individuals relaxed and contracted separately. **Step II:** aiming to determine if the contraction state of the individuals influences the analyzed variable, we evaluated the variations in the ratio between individuals relaxed and contracted. Normality tests were performed using the Shapiro-Wilk test, followed by an unpaired T-student test for normally distributed variables, and Mann-Whitney tests for non-normal variables. **Step III:** assessing the differences across different populations in these measurements: 1) trunk length; 2) introvert and trunk length ratio; 3) anus position (distance from the posterior end) and trunk length ratio; 4) nephridiopore position (distance from the posterior end) and trunk length ratio; 5) nephridium and trunk length ratio; 7) Nephridia attachment ratio; 8) the starting point where the LMBs anastomosis and trunk length ratio; 9) introvert retractor insertion and trunk length ratio; 10) The LMBs number on three levels (C1, C2 and C3). Normality was tested, and the ordinary One-Way ANOVA was used for parametric variables and the Kruskal-Wallis test for non-parametric variables. Post-hoc tests were conducted using unpaired t-tests (normal distribution) or Mann-Whitney test (non-normal distribution) to compare variable proportions between specific locations. **Step IV:** statistical analyses of papillae along the trunk; the areas of papillae were measured using ImageJ software within a specific quadrant that represented 10% of the individual's total length. To address the influence of animal size, papilla areas were normalized by dividing each papilla's area by the corresponding individual length. The statistical analysis was performed using Prism ver. 8.0.0

and involved identifying potential outliers using the ROUT method and assessing data normality using the Shapiro-Wilk test. For comparisons between two independent groups, non-parametric Mann-Whitney tests were employed, while parametric t-tests were used when appropriate. To compare multiple groups, the non-parametric Kruskal-Wallis test was applied. We analyzed the difference in papillae area between contracted and relaxed states within the same locality, focusing on three body regions (anterior, mid, and posterior). Specifically, we examined individuals from Boipeba in Brazil and Barbados. Given the availability of individuals in both contracted and relaxed stages within the same location, we selected specimens exclusively from these two locations for comparison. Additionally, analyses of different populations (Brazil, Barbados, Texas, Mexico, and Thailand) of each body region were made using only contracted individuals.

5.4 RESULTS

5.4.1 Data set for molecular analysis

The final dataset comprised 51 CO1 sequences, 45 16S rRNA sequences, and 44 H3 sequences with 541 bp length, 477 bp length 327 bp length, respectively. The concatenate (CO1, 16S rRNA and H3) matrix included 30 taxa and 1439 bp length.

5.4.2 Phylogenetic Reconstruction

Phylogenetic reconstructions using ML and BI for the CO1 gene yielded congruent tree topologies (Fig. 2A). In both analyses, the monophyly of the Antillesomatidae family was supported [bootstrap (BP) = 90, posterior probability (PP) = 80%]. Four major clades were identified: Thailand individuals formed a distinct group (III; BP= 98, PP = 90%), sister to Eastern Pacific (II) + Western Atlantic (I; BP = 91, PP= 80%). Eastern Pacific individuals of *A. mexicanum*, from Mexico and Costa Rica (II; BP = 99, PP = 100%), is sister to Western Atlantic individuals (I; BP = 54, PP= 70%). Finally, Western Atlantic (I) clade show a well supported clade (BP = 99, PP = 90%) including a Texas clade (BP=71, PP=100%) sister to all the other Western Atlantic samples (BP=88, PP=88). (BP = 99, PP = 90%)].

For the 16S rRNA gene (Fig. 2B), both ML and BI analyses were congruent. Three main lineages were recovered: Thailand (III), Mexico (II), and Western Atlantic (I; BP = 92, PP = 100%). Thailand formed a well supported clade (I; BP = 100, PP = 90%), sister to

Mexico (II) + Western Atlantic (I; BP= 70, PP= 74%). Western Atlantic individuals (I), in turns, are not well supported (BP = 70, PP = 77%).

The final concatenated tree was congruent with the CO1 topology (Fig. 3). A significant clade encompassing the four groups of *Antillesoma* was recovered (BP= 99, PP = 100%). The group composed of individuals from Thailand (BP = 100, PP= 100%) was sister to the clade formed by *A. mexicanum* and individuals from the Western Atlantic (BP = 70, PP = 80%). The individuals from the Western Atlantic were grouped into a separate clade (BP= 70, PP= 80%). Within the Western Atlantic clade, Texas clade also appers isolated (BP = 97, PP = 100%), similar to COI phylogeny, and sister group to species all ather species from Western Atlantic (BP= 100, PP = 90%).

5.4.3 Species Delimitation

To evaluate the species boundary within *Antillesoma* and ascertain if it constitutes a complex of distinct species, we utilized the concatenated ML and BI tree and applied three species delimitation methods: ABGD, PTP, and bPTP. All three methods yielded consistent results (Fig. 3), identifying three putative species: individuals from Thailand, individuals from Mexico (*A. mexicanum*), and individuals from the Western Atlantic.

5.4.4 Population genetics

The CO1 network had a total of 39 haplotypes with 35 being singletons and 4 shared between two or more individuals. (Fig. 4A; Tab.5). The haplotype 4, which was significantly more prevalent in the Western Atlantic samples, was shared among three individuals from Barbados and one from Florida, USA (in red in Table 5). Additionally, three other haplotypes were observed, each shared by two individuals: haplotype 7, found in individuals from Bahamas and Venezuela (in pink in Table 5); haplotype 13, present in individuals from Barbados (in blue, Table 5); and haplotype 26, identified among individuals from Brazil (Bahia and Trindade) (in orange in Table 5). Three distinct lineages were identified (Fig 4A): lineage I, from the Western Atlantic, accomplished individuals from the USA (Florida and Texas), Brazil, Barbados, Venezuela, and Belize; lineage II from the Eastern Pacific (Mexico and Costa Rica), and lineage III from Thailand. These lineages were separated by multiple mutation steps: 73 (lineages: I from II), 85 (lineages: I and III), and 86 (lineages: II and III). In the lineage I, haplotypes were interconnected by one to four mutational steps, except for

samples from Texas, which showed 10 mutation steps between them and the rest of the Western Atlantic individuals.

The 16S rRNA network revealed a total of 22 haplotypes, with 18 being singletons and 4 being shared between two or more individuals (Fig. 4B). In the Thailand population, haplotype "t" (in purple Tab. 5) was the most prevalent, being shared by five individuals. Following closely, haplotype "d" (in dark red) stood out as the most frequent, shared by three individuals from Barbados, two from Florida (USA), and one from the Bahamas. Haplotype "u" (in green, Tab. 5) emerged as the third most common, shared among three individuals. Haplotype "e" was shared by two individuals (in light blue, Tab. 5), one from the Bahamas and another from Belize. As observed in the CO1 network, the same three distinct lineages were discernible. Lineage I showed two connection points with lineages II and III, characterized by several mutation steps (lineage I–II: 24 and 28 mutations; lineage I–III: 51 and 49 mutations; lineage II–III: 53 mutations). Within Lineage I, similar to the CO1 haploweb, differentiation was observed among haplotypes from Texas that were separated from the other Western Atlantic individuals by 4 mutation steps.

The haplotypic diversity (h) between Thailand, the Western Atlantic, and the Eastern Pacific demonstrated higher diversity, exceeding 0.80 for both CO1 and 16S rRNA (Tab. 6). Regarding the number of haplotypes (N_h) the CO1 gene exhibited variation ranging from 4 to 27 and the 16S rRNA gene from 4 to 18 (Tab. 6). Additionally, the number of polymorphic sites (S) on CO1 vary from 13 to 42 and on 16S rRNA from 4 to 20. Examining pairwise F_{ST} values (Tab. 7), there were high and significant values ($F_{ST} > 0.90$) between all three lineages. Furthermore, the F_{ST} calculated specifically for the Western Atlantic (Tab. 8) revealed moderate significant F_{ST} values ($F_{ST} > 0.70$; p value < 0,5) between the populations of Texas and the Caribbean, as well as Texas and Brazil. In contrast, other population comparisons yielded low and nonsignificant F_{ST} values (Tab. 8).

The genetic distance for CO1 (Tab. 9), between all three lineages was notably high, above 18% . Within the Western Atlantic populations, the genetic distance was generally low, staying below 1%. However, the Texas population stood out (Tab. 10), displaying a higher genetic distance of approximately 5% compared to the Caribbean, Florida, and Brazil populations .

5.4.5 Morphological analysis

5.4.5.1 Correlation Analysis

A strong positive correlation (Pearson r ≈1) was observed between trunk and introvert length (N=77) (Fig. 7A), particularly pronounced in contracted individuals (N=37) (Fig. 7 B-C). Strong positive correlation was also found between trunk length and anus position (N=88) (Fig. 7D), as well as between trunk length and nephridium length (N=68) (Fig. 7E), trunk length and the beginning point of the muscular band anastomosis (N=53), trunk length and introvert retractor insertion (N=59) (Fig. 7F), trunk length and nephridiopore position (N=53) (Fig. 7G), and introvert retractors and trunk length (N=59) (Fig. H). However, no correlation was found between trunk length and the number of longitudinal muscle bands (LMBs) at levels C1 (N=57) and C2 (N=56) (Suppl. mat. Fig. 1A-B). A weak positive correlation was observed with C3 LMBs (N=54) (Suppl. mat. Fig. 1C).

5.4.5.2 Body states significance

Differences in the variable ratio between individuals in relaxed and contracted states were only statistically significant for Introvert and Trunk Length Ratio [Mann Whitney test, $p < 0.01$; contracted ratio median = 0.28 (N=37); relaxed = 0.35 (N=38) (Fig.8)]. For other variables, the differences were not significant (Suppl. mat. Fig. 2).

5.4.5.3 Differences between populations

The correlation analysis results demonstrate that when comparing individuals of various sizes, variables exhibiting a strong positive correlation should be based on proportional measurements. This methodology allows for a meaningful comparison of these variables across individuals of different sizes.

The analysis of trunk length (Tab. 11; Fig. 9A) revealed significant variation among populations (Kruskal-Wallis test, $p < 0.05$). Mann-Whitney tests indicated significant differences ($p < 0.05$) in trunk length between Brazil and Thailand, Brazil and Eastern Pacific. Specifically, the Brazilian population had a significantly longer trunk compared to Thailand and Eastern Pacific populations, showing a notable range of 0.9 cm to 5.2 cm (Tab. 12). Significant variations were also observed between Caribbean and Eastern Pacific, and Caribbean and Thailand, with the Caribbean population exhibiting a greater trunk length. Similarly, the Texas population consistently showed a longer trunk length compared to the

Eastern Pacific and Thailand, despite its small sample size. Notably, the Eastern Pacific individuals displayed a smaller trunk size (Tab. 12)

In assessing the introvert/trunk length ratio (Fig. 9B; Tab. 11-12), we focused on contracted individuals due to significant differences observed between contracted and relaxed states. Employing a One-way ANOVA, we found statistically significant variations across the groups ($p < 0.05$. Subsequent unpaired t-tests revealed significant differences ($p < 0.05$) in the introvert/trunk ratio between Brazil and Thailand, Eastern Pacific and Caribbean, and Eastern Pacific and Thailand (Fig. 9B). Notably, Brazilian, Caribbean, and Texas populations had a higher mean introvert/trunk length ratio, while Thailand showed a lower mean. Additionally, the Eastern Pacific population exhibited an intermediated mean ratio between Western Atlantic and Thailand (Tab. 12).

Regarding the anus position and trunk length ratio (Fig. 9C. Tab. 11-12), no significant differences were observed between contracted and relaxed states, permitting the inclusion of individuals from both states for analysis. One-way ANOVA revealed notable differences across population groups ($p < 0.05$). Unpaired t-tests revealed significant differences ($p < 0.05$) in this ratio between the Caribbean and Brazil, Caribbean and Texas, Caribbean and Eastern Pacific, as well as Caribbean and Thailand (Fig. 9C). The variation in this ratio within populations was substantial. Notably, the Caribbean population exhibited the highest mean ratio, distinguishing it from all other populations. Conversely, the populations of Brazil, Texas, Eastern Pacific, and Thailand demonstrated similar mean ratios of anus position.

In the examination of the nephridium and trunk length ratio (Fig. 9D; Tab. 11-12), no significant differences were discerned between contracted and relaxed states, justifying the amalgamation of data from both conditions. Utilizing One-way ANOVA, significant variations ($p < 0.05$) were observed across the distinct population groups. Unpaired t-tests highlighted significant differences ($p < 0.05$) between specific pairs, emphasizing the highest mean ratio in the Texas population (Fig. 9D). Notably, the Caribbean population displayed considerable variability (0.5–1) in ratios (Suppl. mat. Tab. 3), resulting in a slightly lower mean than Texas. This result underscored the significant distinction between Texas and Thailand populations, with the latter demonstrating the lowest mean ratio. Interestingly, mean ratios for Brazil, Eastern Pacific, and Thailand were comparable, showcasing a broad range of variation (Brazil: 0.24–1, Thailand: 0.35–0.83)(Tab. 12). Ultimately, the Caribbean

population stood out with a significant proportion of individuals exhibiting elevated ratios, significantly different from all populations except Texas.

In the analysis pertaining to the nephridia attachment ratio (Fig. 9E; Tab. 11-12), significant variations ($p < 0.05$) were observed among the groups, using Kruskal-Wallis. Mann-Whitney test revealed significant differences ($p < 0.05$) between the Eastern Pacific group and all other groups. Notably, all individuals in the Eastern Pacific group exhibited nephridia completely attached to the body, distinguishing them from all other populations.

In examining the number of LMBs across three levels C1, C2 and C3 (Fig. 9F-H; Tab. 11 and 13), significant differences were identified. The Kruskal-Wallis tests conducted for each LMBs level revealed variations among the analyzed groups ($p < 0.05$). For C1 (Fig. 9F; Tab. 13), Brazil exhibited LMBs ranging from 12 to 23 (N=34), Caribbean displayed 13 to 19 LMBs (N=18), Texas had 15 LMBs (N=2), Eastern Pacific showed LMBs varying from 13 to 18 (N=5), and Thailand presented LMBs within the range of 13 to 15 (N=11). Mann-Whitney tests underscored significant differences ($p < 0.05$) between Brazil and Caribbean, as well as Brazil and Thailand for C1. Moving to C2 (Fig. 9G), Brazil displayed a range of 21 to 36 LMBs (N=33), Caribbean ranged from 18 to 35 LMBs (N=14) Texas exhibited 20 LMBs (N=2), Eastern Pacific displayed LMBs between 24 to 30 (N=3), and Thailand showcased a range of 18 to 32 LMBs (N=7+. Notably, a significant difference ($p < 0.05$) was observed between Brazil and Texas for C2. Finally, in the case of C3 (Fig. 9H; Tab. 13), Brazil demonstrated LMBs varying from 19 to 35 (N=33), Caribbean ranged from 16 to 33 LMBs (N=13), Texas exhibited 24 LMBs (N=1), Eastern Pacific displayed LMBs ranging from 27 to 28 (N=2), and Thailand showed a range of 18 to 25 LMBs (N=12). Significant differences ($p < 0.05$) were notable between Brazil and Thailand, Caribbean and Thailand, as well as Eastern Pacific and Thailand for C3.

In the investigation of nephridiopore position and trunk length ratio (Suppl. mat. Fig. 3A; Tab. 11-12), anastomosis and trunk ratio (Suppl. mat. Fig. 3B; Suppl. mat. Tab. 3-4); and Introvert Retractor and trunk ratio (Suppl. mat. Fig. 3C; Suppl. mat. Tab. 3-4) our analysis did not yield statistically significant differences ($p > 0.05$) between the different populations.

5.4.3 Papillae area analysis

To assess the difference in papillate area with respect to the contraction state of the individual, we analyzed two distinct populations (Boipeba/Brazil and Barbados).The

Mann-Whitney test revealed significant differences in papillae area between contracted and relaxed individuals in Barbados (N=10; Fig. 10A; Tab. 14) and Brazilian (N=6; Fig. 10B) populations across three trunk regions (p-value < 0.05; Fig.10). In Barbados samples, contracted individuals (N=5) exhibited slightly larger mean anterior papilla areas(0.0002±0.00001 cm²; npap=270) compared to relaxed individuals (0.0001±0.0001 cm²; npap=394). However, it is noteworthy that the relaxed individuals demonstrated a higher variation in papillate areas. Similarly, the mean mid papilla areas were also larger in contracted individuals (0.00012±0.00007 cm²; npap=253) than in relaxed individuals (0.00007±0.00005 cm²; npap=466), but in this case, the variation was smaller in relaxed individuals. Likewise, the mean posterior papilla areas were slightly larger in contracted individuals (0.00025±0.00001 cm²; npap=245) compared to relaxed individuals (0.00016±0.00001 cm²; npap=352), with both groups showing comparable variation. In contrast, within the Brazilian population (Fig. 10B; Tab. 14), the mean anterior papillae area was higher in relaxed individuals (0.0002±0.0001 cm²; npap=85) than in contracted individuals (0,00017±0,00005cm²; npap=120). However, the range of variation remained consistent, a distinct concentration of papillae exhibiting areas between 0.00025 and 0.0003 was observed in the relaxed group. The mid papillae areas showed a notable difference between contracted and relaxed samples, wherein contracted individuals exhibited a smaller papillae area (0.00005±0.00002 cm²; npap=80) than relaxed individuals (0.00020±0.0001 cm²; npap=62). We observed a consistent range in both contracted and relaxed individuals, highlighting similar maximum and minimum areas. However, the average area for relaxed individuals (0.0001±0.00005 cm²; npap=140) surpassed that of contracted individuals (0.00009±0.000055 cm²; npap=139), because a clustering of values below the mean for contracted individuals and a clustering of values above the mean for relaxed individuals.

The one-way ANOVA test revealed significant differences in trunk papillae area (p-value < 0.05) across the anterior, mid, and posterior regions among populations, considering only contracted individuals, as all Thailand samples were contracted (Fig. 11; Tab. 15). In the anterior region (Fig. 11A; Tab. 15), Brazil (0.00017±0.00005 cm²; npap=120; N=3) and the Caribbean (0.0002±0.00001 cm²; npap=464; N=7) exhibited distinct averages, with Brazil having a smaller mean anterior papillae area compared to the Caribbean. Similarly, for the mid papillae area (Fig. 11B; Tab. 15), Brazil (0.00008±0.00006 cm²; npap=118) and the Caribbean (0.00011±0.00007 cm²; npap=417) presented varying averages, and Brazil again showed a smaller mean. However, in the posterior papillae area (Fig. 11C;

Tab. 15), the difference between Brazil (0.00009±0.0005 cm²; npap=139) and the Caribbean (0.0002±0.0005 cm²; npap=386) was more pronounced, with the Caribbean having a notably larger mean posterior papillae area. Comparing these populations with Texas (N=2), we found that Texas had a larger mean anterior papillae area (0.0002002±0.0001027 cm²; npap=71) compared to the Caribbean. The mid papillae area in Texas (0.0001±0.00003 cm²; npap=35) was smaller than the Caribbean but larger than Brazil, showcasing an intermediate value. Interestingly, the posterior papillae area in Texas (0.00027±0.0001 cm²; npap=41) exceeded that of both Brazil and the Caribbean. Concerning the Eastern Pacific samples (N=2), their anterior papillae areas (0.0001727±0.00008 cm²; npap=23) were generally smaller than those in Brazil, the Caribbean, and Texas. The mid papillae (0.00008±0.00002 cm²; npap=21) were smaller than the Caribbean and Texas but larger than Brazil. The posterior papillae area (0.00006±0.00003 cm²; npap=31) exhibited a similar difference. Lastly, examining Thailand samples (N=3), the mean papillae areas of the three regions of the trunk were generally smaller compared to Brazil and the Caribbean but larger than the Eastern Pacific. Thailand's anterior, mid, and posterior trunk papillae areas were 0.00016±0.0003 cm² (npap=279), 0.00006±0.00002 cm² (npap=243), and 0.0001±0.00005 cm² (npap=225), respectively.

5.5 DISCUSSIONS

5.1 Molecular conclusions

The ideal strategy for species delimitation involves an integrative approach, encompassing mitochondrial and nuclear genes along with morphological, behavioral, and ecological data (Álvarez-Padilla *et al.*, 2009; Huber & Astrin, 2009; Cartens, *et al.,* 2013). The CO1 marker, as a mitochondrial gene is maternally inherited and highly conserved, and provides valuable phylogenetic insights with their mix of conserved and variable regions, aiding species discrimination across metazoan phyla (Saito *et al*., 2000; Hebert *et al*., 2003). In Sipuncula, the utilization of CO1 and 16S rRNA has shown effectiveness in detecting putative species within the *Phascolosoma perlucens* complex in a previous study (Kawauchi & Giribet, 2010). Also, the H3 gene, conserved at the amino acid level, shows notable nucleotide sequence variation, aiding differentiation of Sipuncula families, genera (Maxmen *et al.,* 2003; Schulze *et al*., 2007; Kawauchi *et al*., 2012) and some cases in species levels (Schulze et al., 2018). Despite our dataset is constrained by the limited availability of samples per population (Tab. 1) and gene markers (CO1, 16S rRNAand H3) necessary for a

comprehensive and exhaustive analysis, our results of species delimitation and population genetics indicate a high level of genetic differentiation among *Antillesoma* lineages from the Eastern Pacific, Western Atlantic, and Thailand. The F_{st} values above 0.9 and a genetic distance exceeding 18% among these three lineages support this distinction.

Moreover, these three lineages were supported through analyses of the CO1 and 16S rRNA gene and concatenated dataset (CO1, 16S rRNA and H3). As shown in our phylogenetic reconstruction using both concatenated data and individual gene markers, exhibited a consistent pattern, with well supported clades, except for a significant discrepancy regarding the positioning of the Texas population. These population stood apart from the Western Atlantic individuals, while in the 16S rRNA analysis, it clustered within the Western Atlantic clade. Anticipated discordances between gene trees were observed, considering that each gene evolves differently, manifesting distinct evolution rates (Maddison, 1997; Degnan & Rosenberg, 2009). Additionally, the relatively low support on certain branches in both CO1 and 16S rRNA trees could be attributed to their construction based on a single gene (Rokas & Carrol, 2005). However, when analyzing concatenated data, the branch support increased, enhancing the confidence in the clades.

The haplotype network generated shows a substantial number of unique haplotypes interconnected in the CO1 gene with a diffuse pattern, possibly attributed to sampling discrepancies among the analyzed localities (Allcock & Strugnel, 2012). Nevertheless, this haplotype network exhibits some signs of “parochial” pattern (Allcock & Strugnel, 2012), with distinct separates three distinct lineages by several mutations. However, the lack of additional collections from intermediate populations between the sample sites prevents us from establishing species delimitations with more certainty.

It is known that 16S rRNA is more conserved than CO1, and despite of the fact that we had a limited sample size for *A. mexicanum* individuals (only one sequence), the 16S rRNA haplotype network corroborate the tree lineages found in COI network. The Western Atlantic population of *A. antillarum* showcases a pattern of panmixia, featuring low and statistically insignificant pairwise F_{ST} values (Kawauchi & Giribet, 2010).

Among marine invertebrates, many groups are regarded as cosmopolitan due to their extensive distribution across all oceans (Spellerberg & Sawyer, 1999). The transatlantic dispersion of certain marine species has long been ascribed to planktotrophic pelagic larvae,

explaining their cosmopolitan status (Scheltema, 1968; Rice, 1978, 1981). It was believed that the planktotrophic pelagosphere larva of Sipuncula, which could be maintained in the laboratory for up to seven months without metamorphosis, was responsible for the dispersion and connection of geographically distant populations (Rice, 1976; 1978; 1981). However, the relationship between the duration of pelagic larvae and population connectivity has been a subject of debate in various taxa, demonstrating that one has little influence over the other (Cowen & Sponaugle, 2009; Shanks, 2009; Schulze *et al.*, 2012). In light of this, transoceanic dispersal capable of maintaining cohesion among globally distributed populations appears improbable (Kawauchi & Giribet, 2010; Schulze *et al*., 2012; Kawauchi & Giribet, 2014). Nevertheless, a dispersal at smaller scales as observed in *A. antillarum* in the Western Atlantic could be explained by this extended larval duration. In a larval dispersal study of the deep-sea Sipuncula species *Phascolosoma turnerae* in the North Atlantic, employing trajectory simulations (Young *et al*., 2012), it was suggested that planktotrophic Sipuncula larvae from the northern Gulf of Mexico displayed a remarkable ability to traverse vast distances, reaching the mid-Atlantic off Newfoundland, with an extensive journey estimated to exceed 3000 km, covering a maximum estimated larval life of 13 months. In the case of *A. antillarum*, larvae from the Caribbean were held under laboratory conditions for a month without metamorphosing into adults (Rice, 1975), suggesting a long planktonic period and a high potential for dispersion on Northwest Atlantic. Additionally, numerous marine species display extensive populations with minimal genetic differentiation, interconnected through gene flow. This is evident in *Clibanarius antillensis*, the Hermit Crab, which demonstrates a lack of population structure along the Western Atlantic (Nishikawa *et al*., 2021), as well as within the Western Atlantic lineages of the *Phascolosoma perlucens* complex (Kawauchi & Giribet, 2010) and *Sipunculus nudus* complex (Kawauchi & Giribet, 2014).

Among Western Atlantic lineages, particular attention is drawn to the Texas population. As shown previously in the phylogenetic analysis, these populations display a genetic distinction with a moderate F_{ST} value (>0.70) compared to other Western Atlantic localities. Moreover, both the CO1 and 16S rRNA haplotype networks reveal an apparent structuring among these individuals. Texas, is situated in the ecoregion of the Northern Gulf of Mexico, classified as Warm Temperate Northwest Atlantic (Spalding *et al.*, 2007). Previous research has highlighted a genetic break between populations in the northern Gulf of Mexico and the broader Western Atlantic, revealing subpopulation structuring in shrimp *Callichirus islagrande* (Staton & Felder 1995), and the squid *Doryteuthis plei* (Herke & Foltz, 2002;

Sales *et al*., 2016). In the study challenging the cosmopolitanism of *Phascolosoma agassizii*, *Thysanocardia nigra*, and *Themiste pyroide*, Schulze *et al*. (2012) suggest that 3% of intra-specific divergence in COI is generally accepted as a threshold value. The genetic distance in CO1 between the Texas population and other Western Atlantic localities exceeding 4% is slightly above the suggested threshold suggested by Schulze *et al. (*2012). However, to derive comprehensive conclusions regarding the connection and boundaries between the *A. antillarum* population in Texas and other Western Atlantic localities, extensive sampling and analysis of others molecular markers is required.

5.2 Morphological conclusions

Initially, our correlation results among variables [(1) introvert length ($\overline{x} \pm$ SD); (2) anus position; (3) nephridium length; (4) nephridiopore position; (5) the starting point of muscular band anastomosis; (6) introvert retractor insertion] underscore the importance of treating them as proportions to enable comparisons across individuals of varying sizes. Moreover, our findings also highlight that variables such as introvert/trunk ratio are influenced by the contraction state of individuals, as previously suggested by Cutler (1994). This demonstrated the necessity for future descriptions, to include the contraction state of individuals, distinguishing contracted from relaxed individuals for possible comparative morphological population analyses.

Our comparative morphological analysis, between populations, corroborates the distinction of the major three lineages indicated by molecular analyses: Western Atlantic, Eastern Pacific (*A. mexicanum*), and Thailand. The main distinguishing feature is the introvert/trunk ratio, which stands out significantly in the Thailand lineage, showing a notably lower introvert/trunk ratio at approximately 0.19 ± 0.07. This unique characteristic is observed exclusively in the Thailand lineage and sets it apart from the other analyzed populations. Historically, some species [*Phascolosoma (Antillesoma) asser* (Selenka & de Man, 1883); *Phascolosoma (Antillesoma) pelmum* (Selenka & de Man, 1883); *Phascolosoma schmidt* Murina, 1975; *Phascolosoma (Ruppellisoma) gaudens* (Lanchester, 1905); *Phascolosoma (Ruppellisoma) onomichianum (Ikeda, 1904); Phascolosoma (Ruppellisoma) simile* (Chen & Yeh, 1958)] that were synonymized into *A. antillarum*, had their type localities in the Indo-Pacific Ocean. Among these, the species *P. asser* (type locality: Indonesia) and *P. pelmum* (type locality: Philippines) were described by Selenka & de Man (1883) as having a short introvert, constituting approximately 16-20% of the trunk, aligning

with the observed introvert size in the specimens from Thailand population. Additionally, the species *P. pelmum* is described as representative of the American species of *A. antillarum* named in the Indian seas (Selenka & Man, 1883). In this context, it is crucial to examine the type specimens of these species. Furthermore, morphological analysis of more samples from the same localities are necessary for an accurate delineation of these linaege.

Another distinguishing factor among *Antillesoma* lineages is the nephridium attachment. In *A. mexicanum*, the nephridium is observed to be completely attached to the trunk wall, while in other populations, a small portion remains free. Cutler & Cutler (1983) provide a redescription of *A. antillarum*, indicating that 75–90% of the nephridium length is attached to the body wall, occasionally showing a lesser percentage. On the other hand, Stephen & Edmonds (1972) describe *Phascolosoma (Antillesoma) antillarum* with the nephridium fixed throughout their whole length. When we refer to the original description of this species, we encounter a significant challenge, the type locality is twofold, encompassing Puntarenas (Costa Rica) and St. Croix (Virgin Islands), representing both the Eastern Pacific and Western Atlantic. The presence of this ambiguous type locality makes it challenging to definitively determine the true *A. antillarum*. Given the description of *A. mexicanum* for the Eastern Pacific, we currently tentatively place *A. antillarum* in the Western Atlantic. However, considering that *A. mexicanum* was described without a comparison to the designated syntypes, the validity of this species remains somewhat uncertain. In the description of the species *A. mexicanum*, Silva-Morales *et al.* (2019) did not specify whether the nephridium is entirely fixed or if some part is free. For more conclusive insights that would allow us to assert this as a diagnostic characteristic, further analysis of individuals from the Eastern Pacific, along with the syntypes of *A. antillaru*m, is necessary. This would enable the accurate description of the species in question and the designation of a neotype for *A. antillarum*.

The additional morphological characters analyzed, including trunk length, anus/trunk ratio, nephridium/trunk ratio, and LMB numbers, showed statistically significant differences among populations. However, it is noteworthy that these differences, although statistically significant, fall within the range of variations observed within populations and for further investigation more samples from each population were required. Silva-Morales *et al.* (2019) emphasized the size difference between individuals of *A. mexicanum* and the *A. antillarum* population in the Caribbean as a distinguishing feature between the two species. However, in

our analyses, using trunk size as a distinguishing trait might pose ambiguity. This is because a single population, such as Brazil, displays a wide variation that encompasses all the lengths observed in *A. mexicanum* populations.

The trunk papillae area analysis revealed that the contraction state of the body could influence the papillated area. In addition, the analysis between the *Antillesoma* populations showed a large variation in the papillated areas, which could indicate an influence of the environment inhabited by that population. Papillae are secretory organs that release substances that can corrode the substrate on which the animal is located, contributing to its locomotion on or in it the substrate fixation (Rice, 1970). Thus, our results showing a significant variation between populations, and it could be explained by differences on substrate on which a particular population lives. Selenka & Man (1883) described the species *P. asser* and *P. pelmum*, highlighting mainly differences in the papillae between them and the species *P. antillarum*. However, Cutler & Cutler (1983) point out that the differences in these dermal organs would not be sufficient to distinguish two different species. As Selenka & Man (1883), Silva-Morales *et al.* (2019) use the distribution of papillae to distinguish the species *A. mexicanum* from the congener *A. antillarum*, which seems to be inappropriate since our results demonstrate a strong association of the size and concentration of these papillae being influenced by the contraction state and maybe being influenced by the habit of the individuals. Thus, we claim that the use of the pattern of papillae may not be a valid character to use in the description of *Antillesoma* species. Therefore, studies that evaluate the influence of the substrate on the shape and area of the papillae are needed to better understand this relationship and the large variation that exists in these structures.

5.6 CONCLUSIONS

According to de Queiroz's unified species concept (2007), species are considered as metapopulational lineages evolving independently. Divergent lineages gradually acquire distinct characteristics, such as morphological, immunological, ecological, and molecular traits (Flot et al., 2010). It is argued that, while a single property can suggest lineage separation, a robust hypothesis necessitates multiple lines of evidence (de Queiroz, 2007; Carstens et al., 2013).

Our study employed three distinct molecular species delimitation methods, population genetic analyses, and a comprehensive morphological analysis, providing evidence of three

distinct lineages within *Antillesoma.* In line with this, our results also shed light on three putative species: a lineage formed by interconnected populations in the Western Atlantic, a second with specimens from Thailand, and a third, *A. mexicanum* in the Eastern Pacific recently described. Moreover, our results suggest that *A. mexicanum* is a valid species, we believe that a redescription of it, including details about the external and internal anatomy as we described in this study is necessary.

In light of the morphological and molecular differences found, *A. antillarum* is not a cosmopolitan species, but rather a pseudo-cryptic species complex. To enhance our understanding of the boundaries within these three lineages, further investigation is essential. This should include comprehensive analyses involving intermediate populations, larger and more diverse population samples, and the utilization of highly variable molecular markers, such 18S rRNA, 28S rRNA, ITS and Single Nucleotide Polymorphisms (SNIPs), as well as morphological assessments on the syntypes and holotypes of all described *Antillesoma* species.

The apparent cohesion among Western Atlantic populations prompts inquiries about dispersal and connectivity, underscoring the necessity for increased sampling, particularly from locations like Texas, Florida (USA), and the Brazilian coast. Moreover, our findings shed light on intrapopulational morphological variations, stimulating an exploration of the environmental factors influencing these variations.

This study marks the initial phase of an ongoing investigation within the genus *Antillesoma*, establishing the groundwork for fundamental inquiries. Key questions regarding population dynamics, genetic boundaries, morphological variations, and environmental influences within the genus *Antillesoma* remain awaiting answers.

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5.8 REFERENCES

Allcock, A. L., & Strugnell, J. M. (2012). Southern Ocean diversity: new paradigms from molecular ecology. *Trends in ecology & evolution*, 27(9), 520–528. https://doi.org/10.1016/j.tree.2012.05.009.

Álvarez-Padilla, F., Dimitrov, D., Giribet, G., Hormiga, G. (2009) Phylogenetic relationships of the spider family Tetragnathidae (Araneae, Araneoidea) based on morphological and DNA sequence data. *Cladistics,* 25:109–146.

Augener, H. (1903) Beitrage zur Kenntnis der Gephyreen nach Untersuchung der im Gottinger zoologischen Museum befindlichen Sipunculiden und Echiuriden Archiv fur Naturgeschichte, 69:297-371.

Baird, W. B. (1868) Monograph of the species of worms belonging to the subclass Gephyrea. *Proceedings of the Zoological Society of London 1868*, 77–114.

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., *et al*. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS computational biology*, 15(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650.

Carstens, B. C.; Pelletier, T. A.; Reid, N. M.; Satler, J. D. (2013) How to fail at species delimitation. *Molecular Ecology*, v. 22, n. 17, p. 4369–4383, 2013.

Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., & Hebert, P. D. (2011). A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PloS one*, *6*(7), e22232. https://doi.org/10.1371/journal.pone.0022232.

Chen, Y., & Yeh, C. C. (1958). Notes on some Gephyrea of China with descriptions of four new species. *Acta Zoologica Sinica*, 10, 265-278.

Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: estimating gene genealogies. *Proc 16th Int Parallel Distrib Process Symp* 2:184.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G. & Gray, M.R., (1998). Histone H3 and U2 snRNA DNA

sequences and arthropod molecular evolution. *Australian Journal of Zoology*, 46, 419–437.

Collin, R., Diaz, M. C., Norenburg, J. L., Rocha, R. M., Sanchez, J. A., Schulze, A., Schwartz, M. L., & Valdes, A. (2005) Photographic Identification Guide to Some Common Marine Invertebrates of Bocas Del Toro, Panamá. *Caribbean Journal of Science*, 41(3), 638–707.

Cortés, J. (2017). Marine biodiversity baseline for Área de Conservación Guanacaste, Costa Rica: published records. *ZooKeys*, (652). https://doi.org/10.3897/zookeys.652.10427.

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. A*nnual Review of Marine Science*, 1(1), 443–466.

Cutler, E. B. (1994) *The Sipuncula. Their systematics, biology and evolution*. Cornell University Press, Ithaca, N.Y., 453pp.

Cutler, E. B., & Cutler, N. J. (1983) An examination of the *Phascolosoma* subgenera *Antillesoma*, *Rueppellisoma* and *Satonus* (Sipuncula). *Zoological Journal of the Linnean Society*, 77(2), 175–187. https://doi.org/10.1111/j.1096-3642.1983.tb00528.x

Dean, H. K. (2001) Marine Biodiversity of Costa Rica: The phyla *Sipuncula* and *Echiura*. *Revista De Biología Tropical*, 49(S2), 85–90.

Dean, H. K., Hernandez-Avila, I., & Cutler, E. B. (2007) Sipunculans of the Caribbean coast of Venezuela and Curacao. *Zootaxa*, 1431(1), 45–54. https://doi.org/10.11646/zootaxa.1431.1.2

Degnan, J. H.; Rosenberg, N. A. (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology and Evolution,* v. 24, n. 6, p. 332–340.

De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, v. 56, n. 6, p. 879–886.

Díaz-Díaz, O. (2011) Estado del conocimiento de los sipuncúlidos (Sipunculida) del Golfo de Cariaco, Venezuela. *Boletín del IOV*, 50(2), 167–171. ISSN 0798-0639.

Diesing, K. M. (1859) Revision der Rhyngodeen. Sitzungsberichte der mathematisch-naturwissen-schaftliche Klasse, Akademie der Wissenschaften in Wien 37: 719–782.

Ditadi, A. S. F., & Migotto, A. E. (1982). *O Filo Sipuncula. Guia de coleta, Identificação e manutenção em laboratório. Série Manuais de Identificação da Fauna Brasileira*. CNPq, Brasília, 43p.

Fujisawa, T., & Barraclough, T.G. (2013). Delimiting Species Using Single-Locus Data and the Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on Simulated Data Sets. *Systematic Biology*, 62, 5, 707–724. https://doi.org/10.1093/sysbio/syt033.

Fischer, W. (1922). Gephyreen des Reichsmuseums zu Stockholm. *Arkiv for Zoological,* 14 (19):1-39.

Fisher, W. K. (1952) The sipunculid worms of California and Baja California. *Proceedings of the U.S. National Museum*, 102, 371–450.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294-29.

Flot, J. F., Couloux, A., & Tillier, S. (2010). Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus Pocillopora in Clipperton. *BMC Evolutionary Biology*, 10, 372. https://doi.org/10.1186/1471-2148-10-372.

Garcia, T. M., Matthews-Cascon, H., & Franklin-Junior, W. (2010) Sipuncula Associated with Branching Fire Coral (*Millepora Alcicornis*) in A Marine Protected Area In Northeastern Brazil. *Thalassas*, 1(26), 9–12.

Gerould, J. H. (1913) The sipunculids of the eastern coast of North America. *Proceedings of the United States National Museum*, 44, 373–437.

Gomez, C., Ardila, N., & Sanjuan-Muñoz, A. (2013) Sipunculans associated with dead coral skeletons in the Santa Marta region of Colombia, south-western Caribbean. *Journal of*

the Marine Biological Association of the United Kingdom, 93(7), 1785–1793. https://doi.org/10.1017/S002531541300043X

Grube, E. & Oersted, A. S. (1858) Annulata Oerstediana. Videnskabelige Meddelelser Dansk fra naturhistorisk Forening i Kjobenhavn, 105–120.

Haldar, B. P. (1991)Sipunculans of the Indian coast. Memoirs of the Zoological Survey of India I7(4):1-169.

Hebert, P. D. N., Cywinska, A., Ball, S. L., Dewaard, J. R (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences,* v. 270, n. 1512, p. 313–321.

Herke, S.W. & Foltz, D.W.(2002). Phylogeography of two squid (*Loligo pealei* and *L . plei*) in the Gulf of Mexico and northwestern Atlantic Ocean. *Marine Biology*, 140(1), 103–115. doi:10.1007/s002270100680.

Hsueh, P. W. & Kuo, C. M. (2009). New records of sipunculan worms from Taiwan. Zootaxa, 2067(1), 51-61.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., & Vinh, L.S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518-522. https://doi.org/10.1093/molbev/msx281.

Huber B. A, Astrin JJ (2009) Increased sampling blurs morphological and molecular species limits: revision of the Hispaniolan endemic spider genus *Tainonia* (Araneae: Pholcidae). *Invertebr Syst,* 23:281–300

Huelsenbeck, J.P.,& F. Ronquist.(2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics,* 17:754-755.

Hylleberg, J. (2013). Classification and Identification of Sipunculans from Thailand, with description of new species and a new subgenus. *Phuket Marine Biological Center Special Publication*, *32*, 53–82.

Ikeda, I. (1904) *The Gephyrea of Japan. Journal of the College of Science*, Imperial University of Tokyo 20: 1–87.

Jaeckle, W., & Rice, M. E. (2002). Phylum Sipuncula. Em *Atlas of Marine Invertebrate larvae* (p. 375–396). Academic Press.

Johnson, N. D., Sanders, C., Maiorova, A., & Schulze, A. (2016). Cryptic species in Pacific sipunculans (Sipuncula: Phascolosomatidae): east‐west divergence between non‐sister taxa. *Zoologica Scripta*, 45(4), 455-463. https://doi.org/10.1111/zsc.12158

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler & A., Jermiin, L.S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods*, 14:587-589. https://doi.org/10.1038/nmeth.4285.

Kawauchi, G. Y., & Giribet, G. (2010). Are there true cosmopolitan sipunculan worms? A genetic variation study within *Phascolosoma perlucens* (Sipuncula, Phascolosomatidae). *Marine Biology*, *157*(7), 1417–1431. https://doi.org/10.1007/s00227-010-1402-z.

Kawauchi, G. Y., & Giribet, G. (2014). *Sipunculus nudus* Linnaeus, 1766 (Sipuncula): Cosmopolitan or a group of pseudo-cryptic species? An integrated molecular and morphological approach. *Marine Ecology*, *35*(4), 478–491.

Kawauchi, G. Y., & Rice, M. E. (2009). Two New Species of *Nephasoma* (Sipuncula: Golfingiidae) from the Western Atlantic Ocean. *Proceedings of the Biological Society of Washington*, *122*(1), 1–13.

Kawauchi, G. Y., Sharma, P. P., & Giribet, G. (2012) Sipunculan phylogeny based on six genes, with a new classification and the descriptions of two new families. *Zoologica Scripta*, *41*(2), 186–210. https://doi.org/10.1111/j.1463-6409.2011.00507.x

Keferstein, W. (1865) Beitrage zur anatomischen und systematischen Kenntniss der Sipunculiden. *Zeitschrift fiir Wissenschaftliche Zoologie* 15:404-445.

Klautau, M., Russo, C. A. M., Lazoski, C., Boury-Esnault, N., Thorpe, J. P., & Solé-Cava, A. M. (1999). Does Cosmopolitanism Result From Overconservative Systematics? A Case Study Using The Marine Sponge *Chondrilla Nucula*. *Evolution*, *53*(5), 1414–1422.

Leigh, J.W., Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods Ecol Evo*l 6(9):1110–1116.

Leroy, P. (1936) Les sipunculiens du Museum National d'Histoire Naturelle de Paris. *Bulletin du Museum National d'Histoire Naturelle de Paris*, ser. 2, 8:423-426.

Longhurst, A.R. (1958) *An Ecological Survey of the West African Marine Benthos*. London: Colonial Office Fisheries Publication, 101pp.

Maddison, W. P (1997). Gene trees in species trees. *Systematic Biology,* v. 46, n. 3, p. 523–536,

Maxmen, A. B., King, B. F., Cutler, E. B., & Giribet, G. (2003). Evolutionary relationships within the protostome phylum Sipuncula: A molecular analysis of ribosomal genes and histone H3 sequence data. *Molecular Phylogenetics and Evolution*, *27*(3), 489–503.

Matthewes-Cascon H., & Lotufo, T. M. C. (2006) *Biota marinha da costa oeste do Ceará* - Biodiversidade 24,. Ministério do Meio Ambiente, Brasília, 248 pp.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution,* 37, 1530-1534. https://doi.org/10.1093/molbev/msaa015

Nishikawa, K. S., Negri, M., & Mantelatto, F. L. (2021). Unexpected Absence of Population Structure and High Genetic Diversity of the Western Atlantic Hermit Crab *Clibanarius antillensis* Stimpson, 1859 (Decapoda: Diogenidae) Based on Mitochondrial Markers and Morphological Data. *Diversity*, 13(2), 56. https://doi.org/10.3390/d13020056

Pan-Wen, H., & Siang, T. K. (2016). New records of peanut worms (Sipuncula) from Singapore. *Raffles Bulletin of Zoology*, 40, 235–240.

Prendini, L., Weygoldt, P. & Wheeler, W. C. (2005). Systematics of the Damon variegatus group of african whip spiders (Chelicerata : Amblypygi): evidence from behaviour, morphology and DNA. *Organisms, Diversity & Evolution*, 5, 203–236.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular ecology,* 21(8), 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x.

Quirós-Rodríguez, J. A., Santafé-Patiño, G., & Nisperuza-Pérez, C. (2021) On the occurrence of *Antillesoma antillarum* (Grube, 1858) (Sipuncula, Antillesomatidae) on the south-central part of the Colombian Caribbean coast. *Check List*, 17(5), 1199–1204. https://doi.org/10.15560/17.5.1199

Rambaut, A. (2010). FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A, Drummond, A.J., Xie, D., Baele, G. & Suchard. M.A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology.* syy032. doi:10.1093/sysbio/syy032

Rice, M. E. (1970) Survey of the Sipuncula of the coral and beach-rock communities of the Caribbean Sea. In: Rice, M.E. & Todorović, M. (Eds), *Proceedings of the International Symposium on the Biology of Sipuncula and Echiura*. Naučno Delo Press, Belgrade, 35–49.

Rice, M. E. (1975) Observations on the development of six species of Caribbean Sipuncula with a review of development in the phylum. In: Rice ME, Todorovic M, editors. *Proceedings of the International Symposium of Sipuncula and Echiura.* Belgrade: Naucno Delo Press, p 141–60.

Rice, M. E. (1976) Larval development and metamorphosis in Sipuncula. *American Zoologist*, 16:563–71.doi:10.1093/ icb/16.3.563

Rice, M. E. (1978). Morphological And Behavioral Changes At Metamorphosis In The Sipuncula. *In: Proceedings of the Symposium on Settlement and Metamorphosis of Marine Invertebrate Larvae* (p. 83–102).

Rice, M. E. (1981). Larvae Adrift: Patterns and Problems in Life Histories of Sipunculans. *American Zoologist*, 21(3), 605–619.

Rice, M. E. (1985). Description of a wood-dwelling sipunculan, Phascolosoma turnerae, new species. *Proceedings of the Biological Society of Washington*. 98 (1):54–60.

Rice, M. E., and Macintyre I. G. (1972) A preliminary study of sipunculan burrows in rock thin sections. *Caribbean Journal of Science*, I2(I–2), 41–44.

Rokas, A., Carroll, S. B. (2005). More Genes or More Taxa? The Relative Contribution of Gene Number and Taxon Number to Phylogenetic Accuracy. *Molecular Biology and Evolution*, 22(5), 1337–1344. https://doi.org/10.1093/molbev/msi121

Ronquist, F., & Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19, 1572-1574.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., & Huelsenbeck, J.P. (2012). MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology*, 61, 539-542.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. *Molecular Biology Evolution*, 34: 3299-3302. doi: 10.1093/molbev/msx248.

Saiz Salinas, J. I., Bustamante, M., Tajadura, J., Vijapure, T., & Sukumaran, S. (2015). A new subspecies of *Phascolion Théel*, 1875 (Sipuncula: Golfingiidae) from Indian waters. *Zootaxa*, 3931(3), 433.

Sales, J. B. L., Rodrigues-Filho, L. F. D. S., Ferreira, Y. D. S., Carneiro, J., Asp, N. E., Shaw, P. W., Haimovici, M., Markaida, U., Ready, J., Schneider, H., & Sampaio, I. (2017). Divergence of cryptic species of *Doryteuthis plei* Blainville, 1823 (Loliginidae, Cephalopoda) in the Western Atlantic Ocean is associated with the formation of the Caribbean Sea. *Molecular phylogenetics and evolution,* 106, 44–54. https://doi.org/10.1016/j.ympev.2016.09.014.

Saito, M.; Kojima, S.; Endo, K. (2000) Mitochondrial COI sequences of brachiopods: Genetic code shared with protostomes and limits of utility for phylogenetic reconstruction. *Molecular Phylogenetics and Evolution*, v. 15, n. 3, p. 331–344.

Selenka, E. (1883) *Die Sipunculiden, eine systematische Monographie. Reisen in Archipel Phillippinen von Dr. C. Semper. C.W. Kreidel,* Wiesbaden, 131 pp.

Scheltema, R. S. (1968). Dispersal of Larvae by Equatorial Ocean Currents and Its Importance to the Zoogeography of Shoal- Water Tropical Species. *Nature*, 217, 1159–1162.

Scheltema, R.S. (1975) The frequency of long-distance larval dispersal and the rate of genes of gene-Xow between widely separated populations of sipunculans. *In: Rice ME, Todorovic M (eds) Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*. Kotor, Yugoslavia, pp 199–210

Scheltema, R.S., Hall, J.R. (1975) The dispersal of pelahosphaera larvae by ocean currents and the geographical distribution of sipunculans. *In: Rice EM, Tororovic M (eds) Proceedingns of the International Symposium on the Biology of the Sipuncula and Echiura.* Kotor, Yugoslavia, pp 103–115

Schulze, A., Cutler, E. B., & Giribet, G. (2007). Phylogeny of sipunculan worms: A combined analysis of four gene regions and morphology. *Molecular Phylogenetics and Evolution*, 42(1), 171–192.

Schulze, A. & Rice, M. E. (2004) Sipunculan diversity at Twin Cays, Belize with a key to the species. *Atoll Research Bulletin*, 502, 1–9. https://doi.org/10.5479/si.00775630.521.1-9.

Schulze, A., & Kawauchi, G. Y. (2021). How Many Sipunculan Species Are Hiding in Our Oceans? *Diversity*, 13(2), 43.

Schulze, A., Maiorova, A., Timm, L. E., & Rice, M. E. (2012). Sipunculan Larvae and "Cosmopolitan" Species. *Integrative and Comparative Biology,* 52(4), 497–510.

Schulze, A., Hipes, J., Borda, E., & Rice, M. E. (2018). Who's Who in the Sipuncula: Matching Larvae and Adults Using DNA. S*mithsonian Contributions to the Marine Sciences*, 42, 83–93.

Shipley, A. E. (1903) Report on the *Gephyrea* collected by Prof. Herdman Ceylon in 1902. C*eylon Pearl Oyster Fisheries Supplementary Repository,* 3:169-176.

Silva-Morales, I., López-Aquino, M. J., Islas-Villanueva, V., Ruiz-Escobar, F., & Bastida-Zavala, J. (2019). Morphological and molecular differences between the Amphiamerican populations of *Antillesoma* (Sipuncula: Antillesomatidae), with the

description of a new species. *Revista de Biología Tropical*, 67, 101-109. https://doi.org/ 10.15517/rbt.v67iS5.38934.

Shanks, A. (2009). Pelagic Larval Duration and Dispersal Distance Revisited. *The Biological bulletin,* 216, 373–385.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Robertson, J. (2007). Marine eco regions of the world: A bioregionalization of coastal and shelf areas. *BioScience*, 57, 573–583. https://doi.org/10.1641/B570707.

Spellerberg, I. F., & Sawyer, J. W. D. (1999). *An introduction to applied biogeography*. Cambridge University Press.

Staton, J. L., & Felder, D. L. (1995). Genetic Variation in Populations of the Ghost Shrimp Genus *Callichirus* (Crustacea: Decapoda: Thalassinoidea) in the Western Atlantic and Gulf of Mexico. *Bulletin of Marine Science*, 56(2), 523–536.

Staton, J. L., & Rice, M. E. (1999). Genetic differentiation despite teleplanic larval dispersal: allozyme variation in sipunculans of the *Apionsoma misakianum* species-complex. *Bulletin of Marine Science*, 65(2), 467-480.

Stephen, A. C. & Edmonds, S. J. (1972) *The phyla Sipuncula and Echiura*. Trustees British Museum (Natural History), London, 528 pp.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). Mega6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology Evolution*, 30: 2725-2729.

Thompson, J.D., Higgins, D.G., & Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucl Acid Res* 22: 4673-4680.

Varela, C. & A. Schulze. (2008) An updated checklist of the sipunculans (Phylum Sipuncula) of Cuba. *Cocuyo*, 17, 9–11.

Weigert, A., & Bleidorn, C. (2016). Current status of annelid phylogeny. O*rganisms Diversity & Evolution*, 16(2), 345–362.

Wesenberg-Lund, E. (1959) Sipunculoidea and Echiuroidea from Mauritius. Viddenskabelige Meddelelser fra Dansk naturhistorisk Forening I København, 121, 53–73.

Xiong, B. & Kocher, T. D. (1991). Comparison of mitochondrial- DNA sequences of 7 morphospecies of black flies (Diptera, Simuliidae). *Genome*, 34, 306–311.

Xiandong, D., Chen, Z., Deng, Y. W., Wang, G. H. & Huang, R. L. (2008). Genetic diversity and population structure of the peanut worm (Sipunculus nudus) in southern China as inferred from mitochondrial 16S rRNA sequences. *Israeli Journal of Aquaculture: Bamidgeh*, 60, 237–242.

Young, C. M., He, R., Emlet, R. B., Li, Y., Qian, H., Arellano, S. M., Van Gaest, A., Bennett, K. C., Wolf, M., Smart, T. I., & Rice, M. E. (2012). Dispersal of Deep-Sea Larvae from the Intra-American Seas: Simulations of Trajectories using Ocean Models. *Integrative and Comparative Biology*, 52(4), 483–496. https://doi.org/10.1093/icb/ics090.

Young, P. S. (1986) Análise Qualitativa E Quantitativa Da Fauna Associada A Corais Hermatípicos (*Coelenterata*, *Scleractinia*) Nos Recifes De João Pessoa. *Revista Brasileira de Biologia*, 46(1), 99–126.

Zhang, Jiajie, Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A General Species Delimitation Method with Applications to Phylogenetic Placements. *Bioinformatics*, 29(22), 2869-2876.

5.9 FIGURES AND TABLES

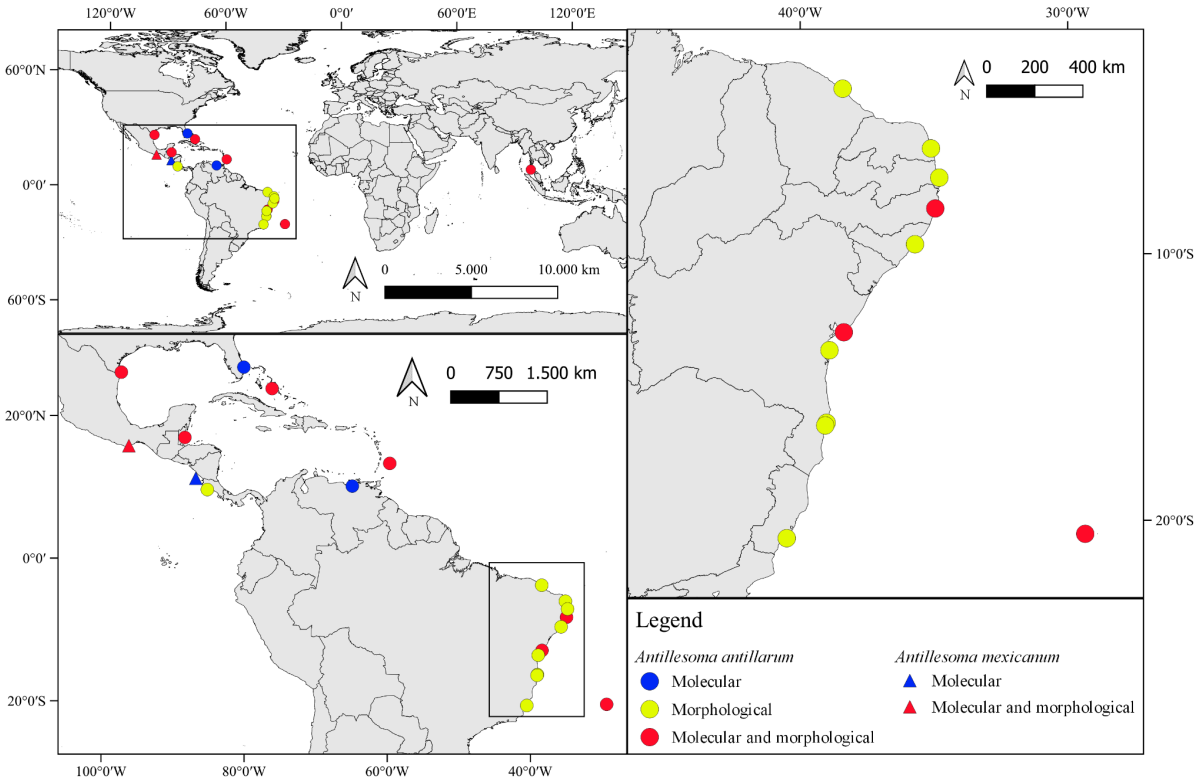

Figure 1. Geographic distribution of sampling locations. and comprehensive *Antillesoma* data analyzed in this study (molecular, morphological or a combination of both).

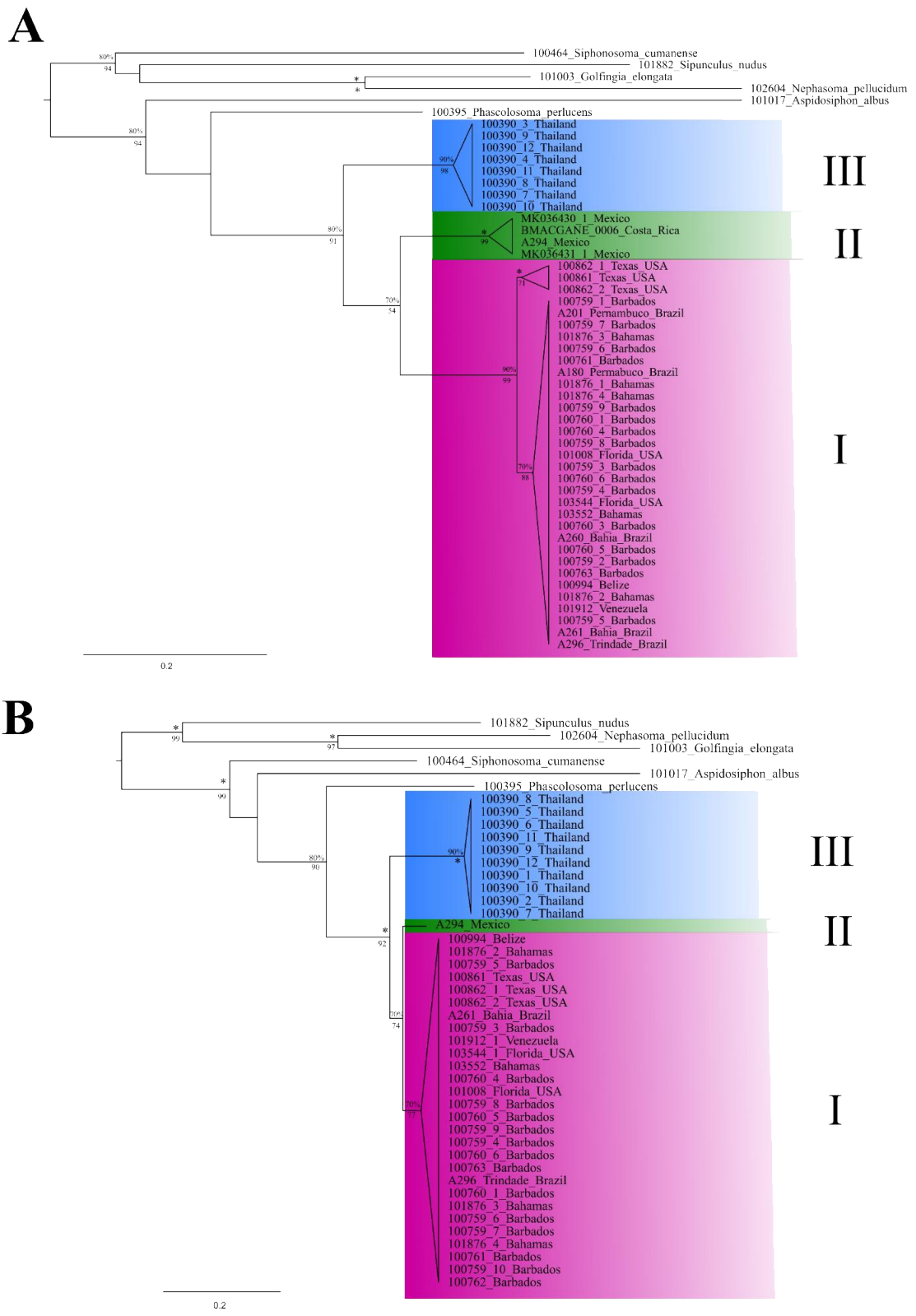

Figure 2. Maximum Likelihood (ML) and Bayesian Inference trees (BI) (A) CO1 gene tree. (B) 16SsrRNA gene tree. Posterior probability values (BI) above 70% are indicated above branches, and bootstrap values (ML) above 70 are indicated below branches. Support values equal to 100 are denoted by *. I. Western Atlantic; II Eastern Pacific; III. Thailand.

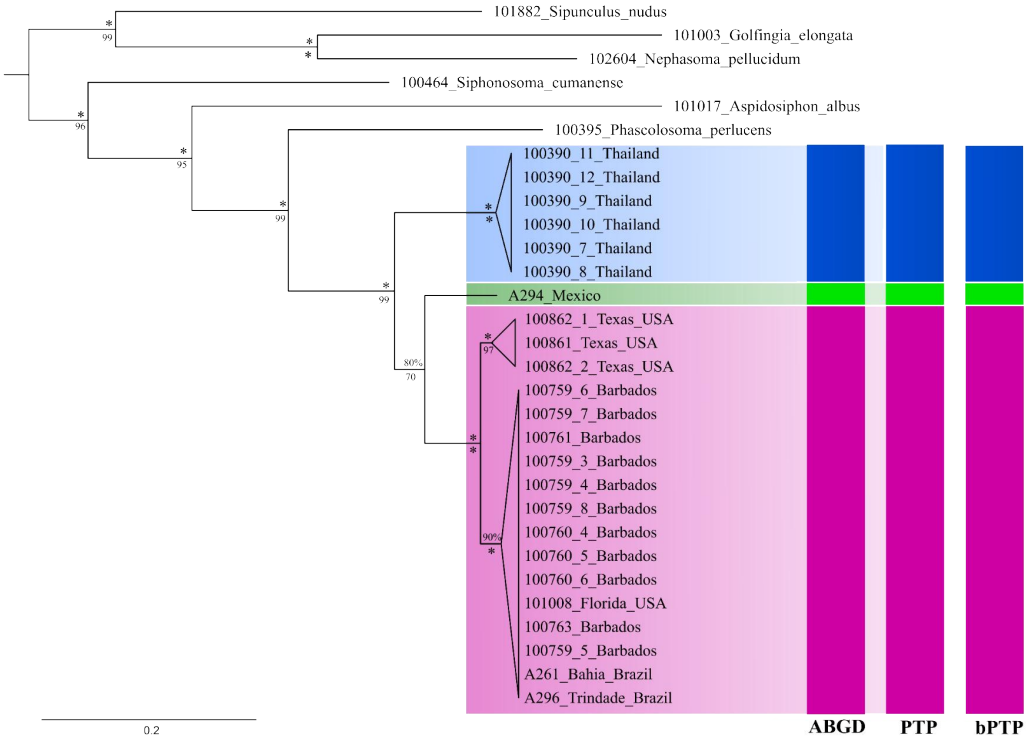

Figure. 3. *Antillesoma* species delimitation with ABG, PTP and bPTP for concatenate CO1, 16S rRNA and H3 dataset. Phylogenetics Reconstruction with Maximum Likelihood using concatenated data. Posterior probability values (Bayesian inference) above 70% are indicated above branches, and bootstrap values (Maximum Likelihood) above 70 are indicated below branches. Support values equal to 100 are denoted by *.

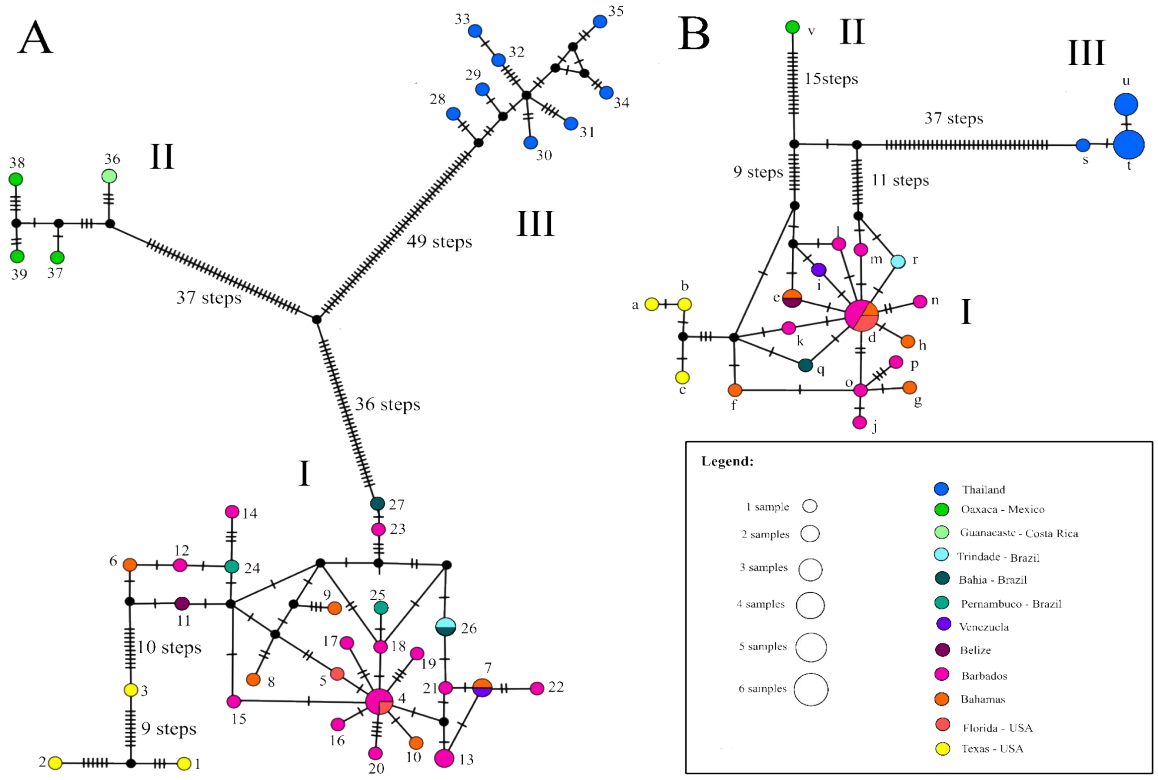

Figure 4. Statistical Parsimony haplotype networks (TCS) of CO1 (A); 16S rRNA (B) data.. Roman numbers represent separate lineages: I (Western Atlantic); II (Eastern Pacific); III (Thailand). Alphanumeric names were designated for each haplotype: numbers next to colored circles correspond to COI sequences, lowercase letters to 16S rRNA sequences. Haplotypes sampled are indicated by colored circles; unlabeled connections between haplotypes represent a single mutational step; inferred haplotypes are indicated by a black dot or by bars with the exact value of mutational steps.

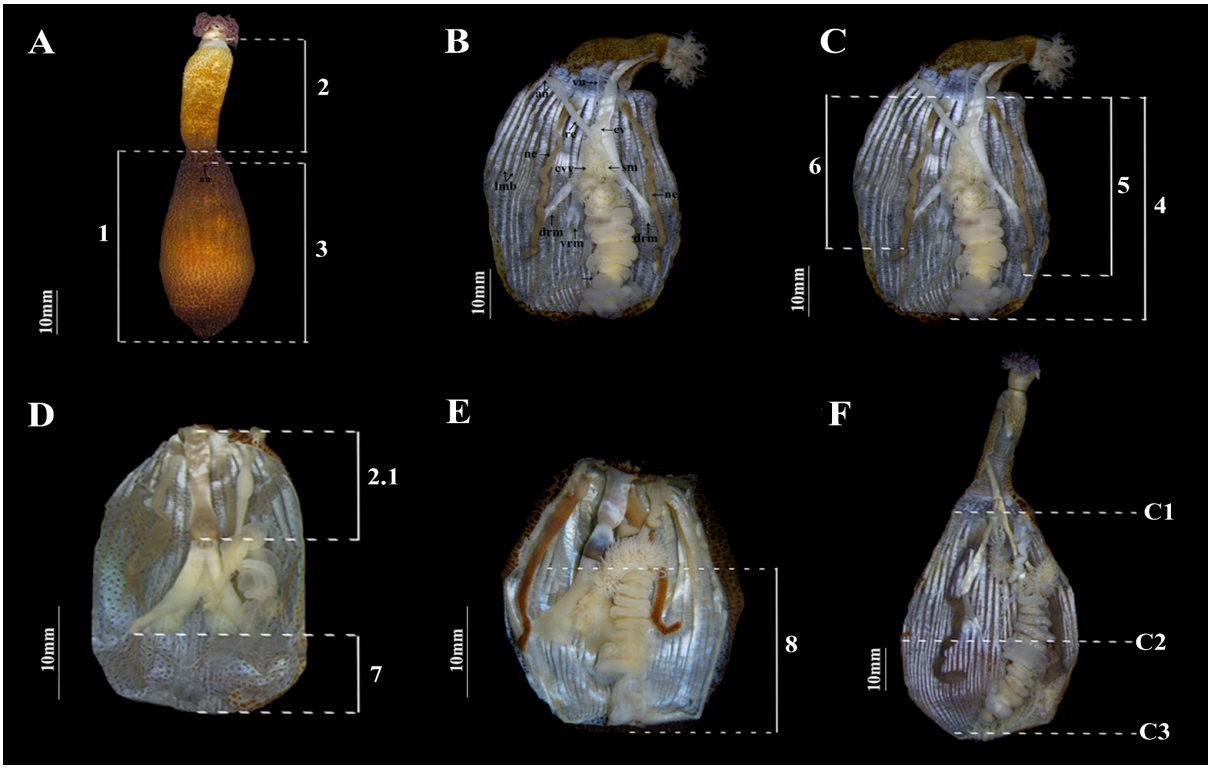

Figure. 5 ***Antillesoma*** **morphological measurements**. (A) External view: 1. trunk length; 2. introvert length; 3. distance from the anus to the posterior end. (B) Internal view with the structures pointed. (C--F) Internal view. (C) 4. distance from the nephridiopore to the posterior end; 5. nephridium length; 6. nephridium attachment to the body wall. (D): 2.1. contracted introvert length; 7. distance from base of the introvert retractor muscles to the posterior end of the trunk. (E): 8. distance from the posterior end to the anterior point where anastomosis of the LMBs begins. (F) number of LMBs counted at three levels: anterior (C1), introvert base (C2), and posterior region (C3). Abbreviations: an = anus, cv = contractile vessel, cvv = contractile vessel villi, drm = dorsal retractor muscle, in = intestine, lmb = longitudinal muscle band, ne = nephridia, re = rectum, sm = spindle muscle, vn = ventral nerve cord, vrm = ventral retractor muscle.

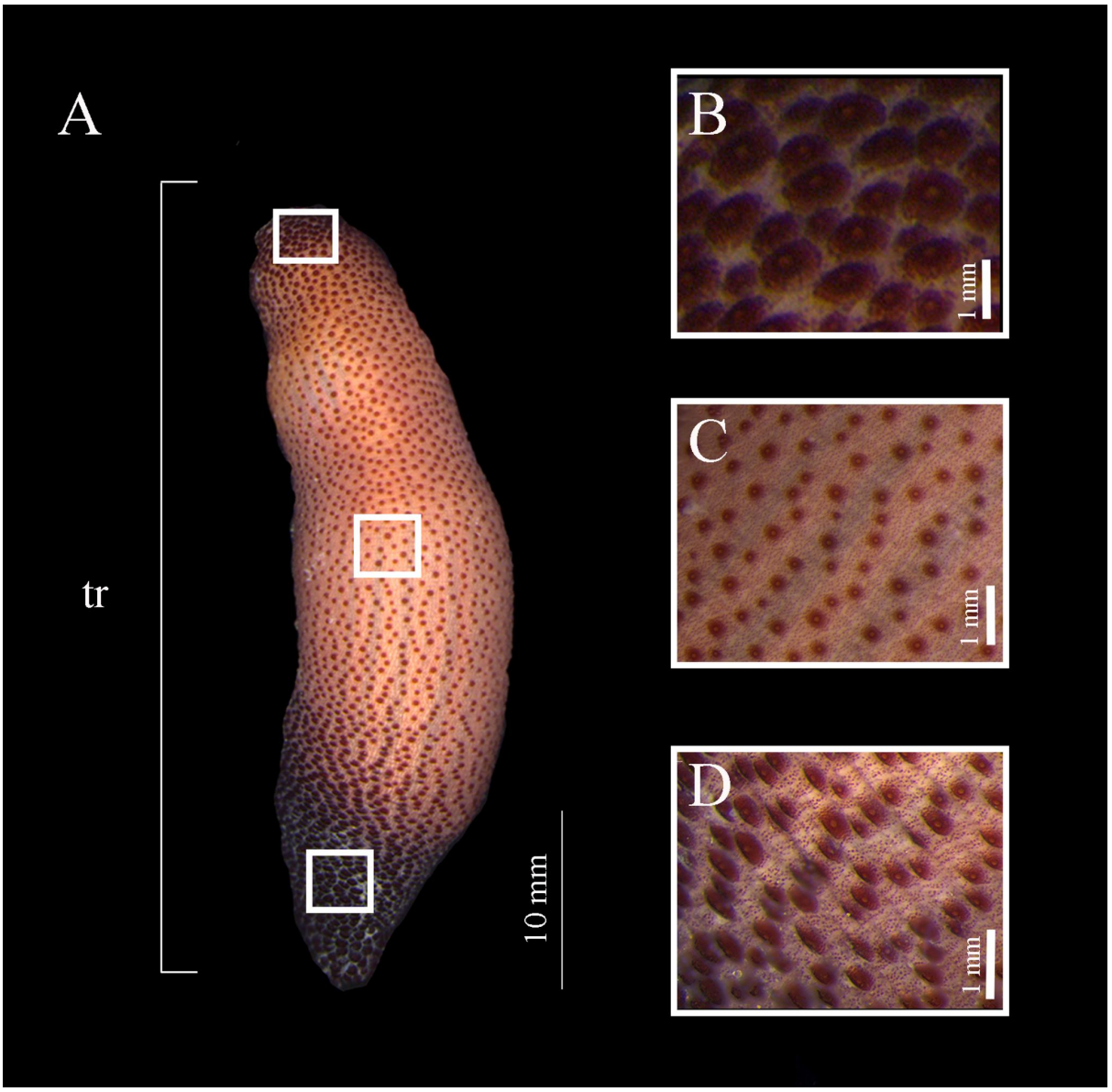

Figure 6. Trunk papillae. (A) trunk papillae regions; (B) anterior trunk papillae; (C) mid trunk papillae; (D) posterior trunk papillae. Abbreviation: tr= trunk.

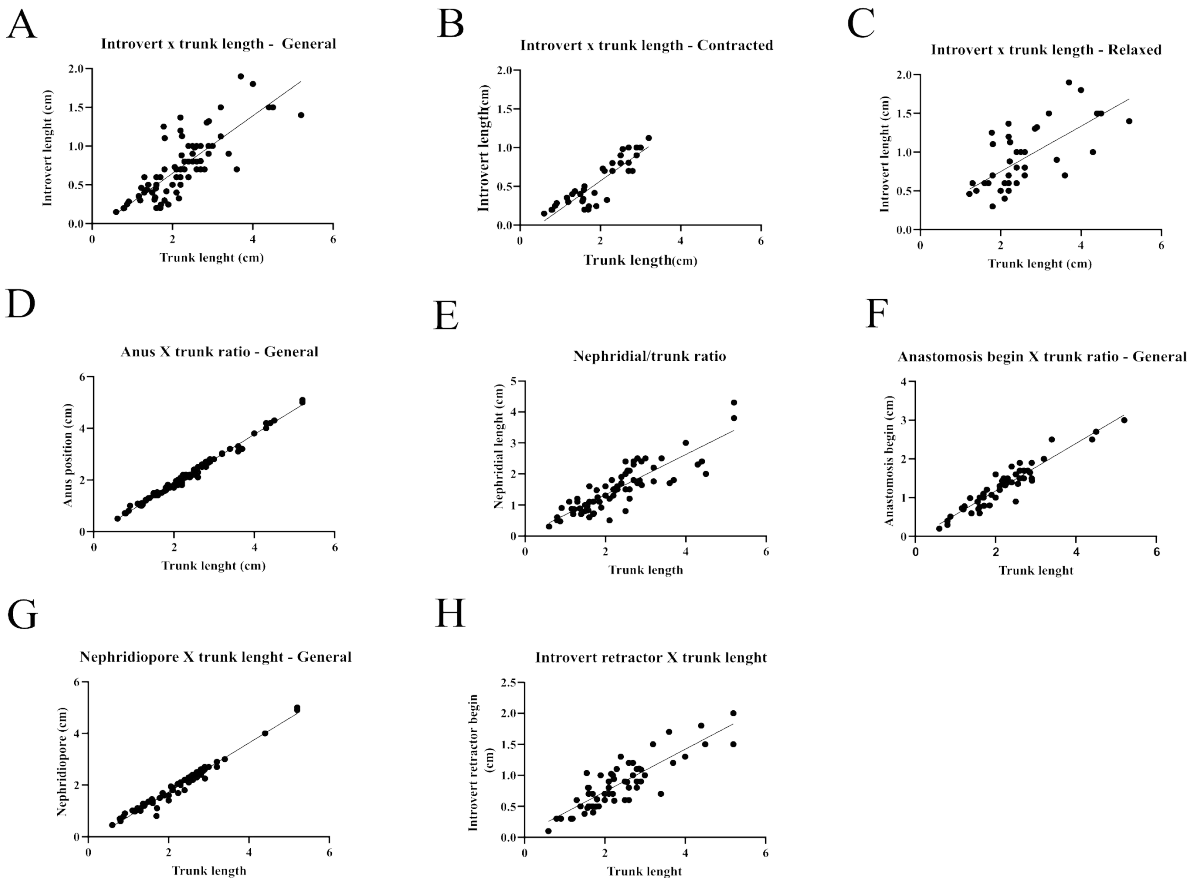

Figure 7. Morphological correlation analysis. (A) Correlation between Introvert and trunk length (N=77); (B) Correlation between Introvert and trunk length on contracted individuals(N=37); (C) Correlation between Introvert and trunk length on relaxed individuals (N=40); (D) Correlation between anus position and trunk length (N=88); (E) Correlation between nephridia and trunk length (N=68); (F) Correlation between anastomosis begin and trunk length (N=53); (G) Correlation between Nephridiopore position and trunk length (N=53); (H) Correlation between introvert retractors and trunk length (N=59).

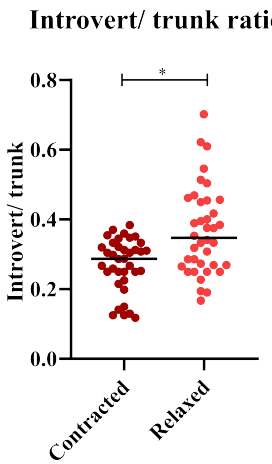

Figure 8. Difference between contracted (N=37) and relaxed (N=38) states on the introvert and trunk ratio. Significant differences ($P < 0.05$) indicated with *.

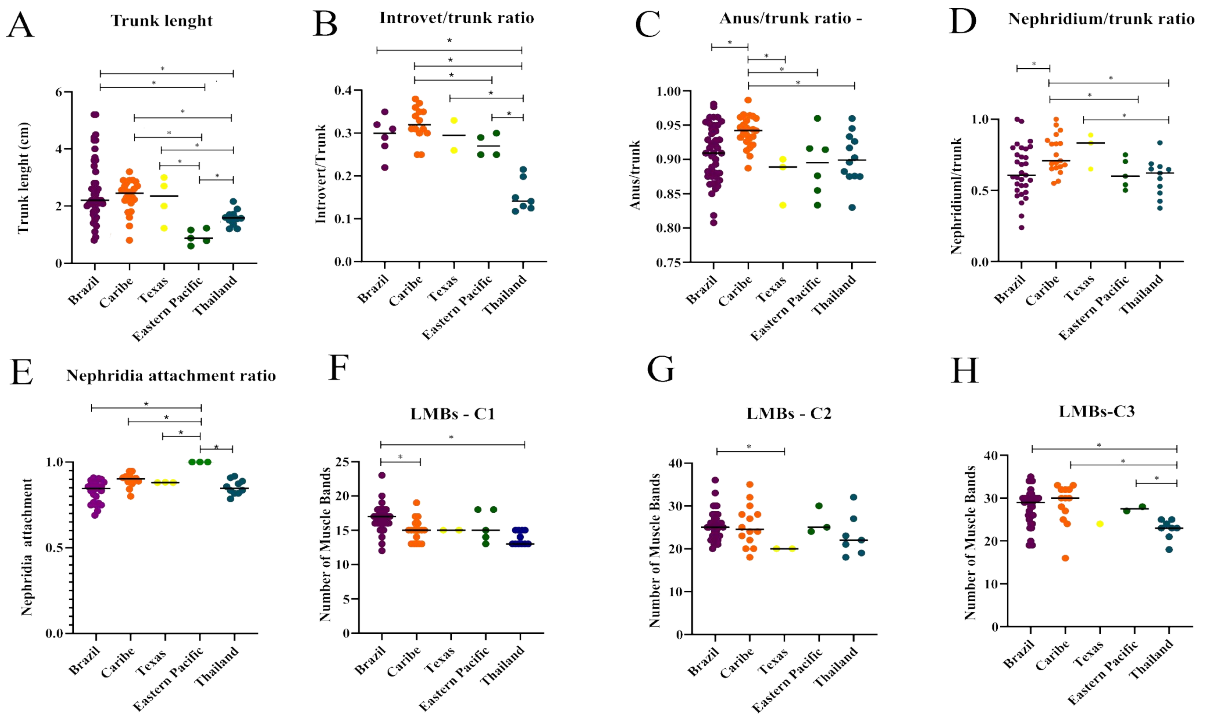

Figure 9. Statistical morphological analysis between populations. (A) Trunk length; (B) Introvert and Trunk ratio; (C) Anus and trunk ratio; (D) Nephridial and trunk ratio; (E) Nephridial attachment ratio; (F) LMBs on anterior region; (G) LMBs on introvert retractor base; (H) LMBs on posterior end. Significant differences between two populations ($P < 0.05$) indicated with a bar and *.

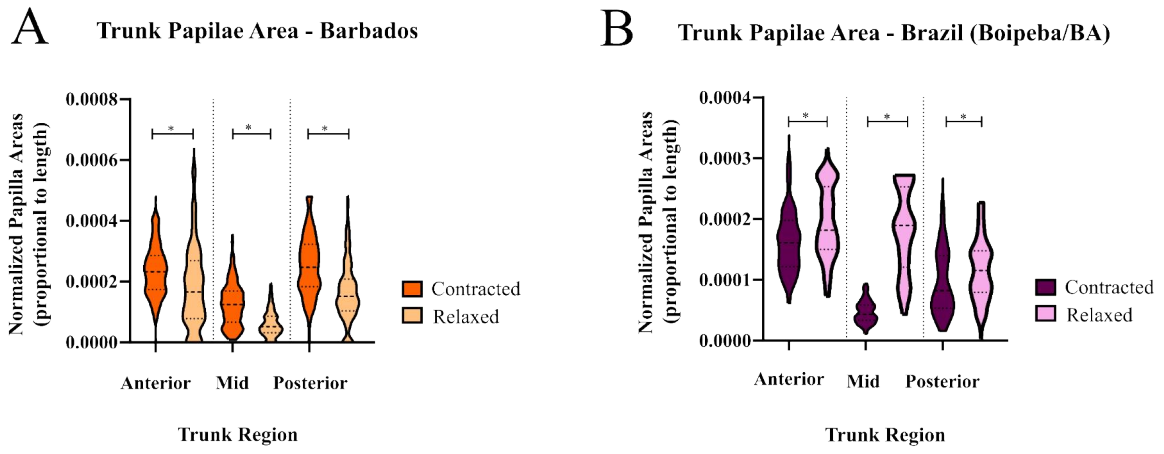

Figure 10. Trunk papillae area difference between contracted and relaxed states (cm²). (A) Barbados individuals (N=10). (B) Brazil (Boipeba/BA; N=6) individuals. Significant differences ($P < 0.05$) indicated with a bar and *.

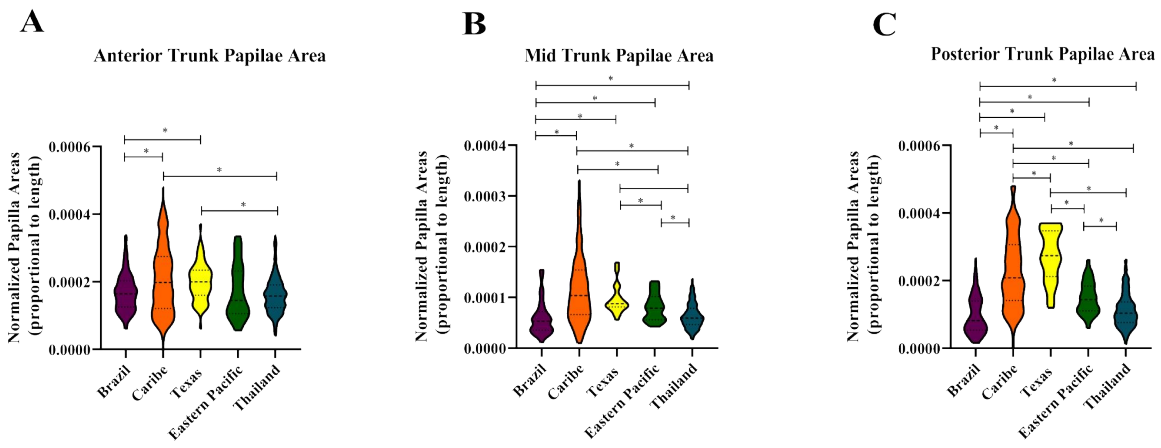

Figure 11. Trunk papillae area difference between populations. (A) Anterior trunk papillae area (B) Mid trunk papillae area; (C) Posterior Trunk Papila area. Significant differences between two populations ($P < 0.05$) indicated with a bar and *.

Table 1. List of specimens and respectively DNA code and collection locality used for molecular analyzed.

Species	DNA code	Location	N
Antillesoma antillarum	DNA100861, DNA100862	South Padre Island, Texas,USA	3
Antillesoma antillarum	DNA101008	Ft. Pierce, Florida, USA	1
Antillesoma antillarum	DNA103544	Peanut Island, Florida, USA	1
Antillesoma antillarum	DNA101876, 103552	Lee Stocking Island, Bahamas	5
Antillesoma antillarum	DNA100994_1	Tobacco Reef, Belize	1
Antillesoma antillarum	DNA101912	Cubagua Island, Venezuela	1
Antillesoma antillarum	DNA100759_1, DNA100760, DNA100761	Six Men's Bay, Barbados	16
Antillesoma antillarum	DNA100763	Martin's Bay, Barbados	1
Antillesoma antillarum	A180, A201	Pernambuco, Brazil	2
Antillesoma antillarum	A260, A261	Salvador, Bahia, Brazil	2
Antillesoma antillarum	A296, A300	Trindade, ES, Brazil	2
Antillesoma antillarum	DNA100390	Phuket, Thailand	12
Antillesoma mexicanum	BMACGANE00060	Guanacaste, Costa Rica	1
Antillesoma mexicanum	MK036430, MK036431_1, A294	Oaxaca, Mexico	3

Table 2. List of collection sites and respectively acronyms.

Location	Coordinates	Acronym
Phuket, Thailand	7°52'55"N 98°26'26"E	THA
South Padre Island, Texas, USA	26°04'09"N 97°09'24"W	TEX
Lee Stocking Island, Bahamas	23°46'04"N 76°05'18"W	BAH
Belize	16°54'56"N 88°16'51"W	BEL
Six Men's Bay, Barbados	13°16'22"N 59°38'48"W	BAR
Ceará, Brazil	3°48'35"S 38°24'35"W	CEA
Rio grande do Norte, Brazil	6°02'57"S 35°06'32"W	RGN
Paraíba, Brazil	7°01'56"S 34°49'52"W	PAR
Cabo de Santo Agostinho, Pernambuco, Brazil	8°18'18"S 34°56'46"W	PER
Alagoas, Brazil	9°39'55.0"S 35°41'48"W	ALA
Praia de Itapoã, Salvador, Bahia, Brazil	12°57'03"S 38°22'00"W	SAL-1
Praia da Pituba, Bahia, Brazil	13°00'23"S 38°27'28"W	SAL-2
Boipeba, Bahia, Brazil	13°34'59"S 38°54'50"W	BOI
Coroa Vermelha, Bahia, Brazil	16°20'53"S 39°00'40"W	COR
Porto Seguro, Bahia, Brazil	16°26'55"S 39°03'41"W	POR
Guarapari, Espírito Santo, Brazil	20°40'33"S 40°29'46"W	GUA
Arquipélago de Trindade, Espírito Santo, Brazil	20°31'29"S 29°18'57"W	TRI
Oaxaca, Mexico	15°45'33"N 96°06'12"W	MEX
Mal pais, Costa Rica	9°36'02"N 85°08'34"W	COS

Table 3. List of specimens used for morphological analysis, number of analyzed individuals (N), and locality acronym.

Species	Collection Number	N	Local
Antillesoma antillarum	MCZ130181	1	TEX
Antillesoma antillarum	MCZ130182	2	TEX
Antillesoma antillarum	MCZ130184	4	BAH
Antillesoma antillarum	MZC130182	1	BEL
Antillesoma antillarum	MCZ130176	10	BAR
Antillesoma antillarum	MCZ130177	7	BAR
Antillesoma antillarum	MCZ130178	2	BAR
Antillesoma antillarum	GYK174– GYK180	7	PER
Antillesoma antillarum	UFMG-INV2300021– 2300023	3	SAL1
Antillesoma antillarum	UFMG-INV2300024 – 2300036	13	SAL2
Antillesoma antillarum	MZUSP5366 and MZUSP5374	2	COR
Antillesoma antillarum	MZUSP5379	1	POR
Antillesoma antillarum	MZUSP5370, MZUSP5375 – 5378, MZUSP5380	6	BOI
Antillesoma antillarum	GYK114	1	CEA
Antillesoma antillarum	MZUSP5372	1	ALA
Antillesoma antillarum	MZUSP5373	1	PAR
Antillesoma antillarum	UFPB-SIP88	1	RGN
Antillesoma antillarum	MZUSP5369	1	GUA
Antillesoma antillarum	MZUSP5476, MZUSP5478, MZUSP5481, MZUSP5484, MZUSP5491	5	TRI
Antillesoma mexicanum	UMAR-SIPU112	2	MEX
Antillesoma mexicanum	UMAR-SIPU018	3	MEX
*Antillesoma mexicanum**	MCZ33452	1	COS

* Indicated specimen previously identified as *A. antillarum.*

Table 4. List of primer sequences used for PCR amplification and sequencing, along with their original references. Abbreviations: F: forward, R: reverse.

Molecular Marker	Primer	Primer Sequence	Reference
CO1	F: PolyLCO	5'–GAYTATWTTCAACAAATCATAAAGATATTGG – 3'	Carr *et al.* (2011)
	R: PolyHCO	5'-GAYTATWTTCAACAAATCATAAAGATATTGG – 3'	Carr *et al.* (2011)
	F: LCO1490	5' – GGTCAACAAATCATAAAGATATTGG – 3'	Folmer *et al*. (1994)
	R: HCOoutout	5' – GTAAATATATGRTGDGCT C – 3'	Prendini *et al*. (1998)
	R: HCO2198	5' - TAAACTTCAGGG TGACCAAAAAATCA - 3'	Folmer *et al*. (1994)
16S rRNA	F: 16S Sa	5'- CGCCTGTTTATCAAAAACAT –3'	Xiong & Kocher (1991)
	R:16Sbr	5'- CCGGTCTGAACTCAGATCAGGT-3'	Xiandong *et al*. (2008)
H3	F: H3F	5'– ATGGCTCGTACCAAGCAGACVGC – 3	Colgan *et al*. (1998)
	R: H3R	5'– ATATCCTTRGGCATRATRGTGAC – 3	Colgan *et al.* (1998)

Table 5. List of DNA vouchers and GenBank accession number for the sequenced fragments. Bold letters indicate individuals sequenced for the three genes. Haplotype name: numbers correspond to COI sequences, and lowercase letters to 16S rRNA.

					Haplotype name	
Species	**DNA code**	**CO1**	**16S rRNA**	**H3**	CO1	16S
Antillesoma antillarum	**DNA100861**	**X**	**X**	**X**	**1**	**a**
Antillesoma antillarum	**DNA100862_1**	**X**	**X**	**X**	**2**	**b**
Antillesoma antillarum	**DNA100862_2**	**X**	**X**	**X**	**3**	**c**
Antillesoma antillarum	**DNA101008**	**X**	**X**	**X**	**4**	**d**
Antillesoma antillarum	**DNA103544**	**X**	**X**	**X**	**5**	**d**
Antillesoma antillarum	DNA101876_1	X	X		6	e
Antillesoma antillarum	DNA101876_2	X	X		7	f
Antillesoma antillarum	DNA101876_3	X	X		8	g
Antillesoma antillarum	DNA101876_4	X	X		9	h
Antillesoma antillarum	DNA103552	X	X		10	d
Antillesoma antillarum	DNA100994_1	X	X		11	e
Antillesoma antillarum	DNA101912	X	X		7	i
Antillesoma antillarum	DNA100759_1	X		X	12	-
Antillesoma antillarum	DNA100759_2	X		X	13	-
Antillesoma antillarum	**DNA100759_3**	**X**	**X**	**X**	**4**	**d**
Antillesoma antillarum	**DNA100759_4**	**X**	**X**	**X**	**14**	**-**
Antillesoma antillarum	**DNA100759_5**	**X**	**X**	**X**	**15**	**-**
Antillesoma antillarum	**DNA100759_6**	**X**	**X**	**X**	**16**	**j**
Antillesoma antillarum	**DNA100759_7**	**X**	**X**	**X**	**17**	**k**
Antillesoma antillarum	DNA100759_8	X			4	-
Antillesoma antillarum	**DNA100759_9**	**X**	**X**	**X**	**18**	**l**
Antillesoma antillarum	DNA100759_10		X			m
Antillesoma antillarum	**DNA100760_1**	**X**	**X**	**X**	**19**	n
Antillesoma antillarum	DNA100760_3	X			20	-

Antillesoma antillarum	**DNA100760_4**	**X**	**X**	**X**	**21**	**o**

CONTINUATION

TABLE 5

					Haplotype name	
Species	**DNA code**	**CO1**	**16S rRNA**	**H3**	CO1	16S
Antillesoma antillarum	DNA100763	X		X	13	-
Antillesoma antillarum	A180	X		X	24	-
Antillesoma antillarum	A201	X			25	-
Antillesoma antillarum	**A261**	**X**	**X**	**X**	**26**	**q**
Antillesoma antillarum	A260	X			27	-
Antillesoma antillarum	A300			X	-	-
Antillesoma antillarum	**A296**	**X**	**X**	**X**	**26**	**r**
Antillesoma antillarum	DNA100390_1		X	X	-	s
Antillesoma antillarum	DNA100390_2		X		-	t
Antillesoma antillarum	DNA100390_3	X		X	28	-
Antillesoma antillarum	DNA100390_4	X		X	29	-
Antillesoma antillarum	DNA100390_5			X	-	-
Antillesoma antillarum	DNA100390_6		X	X	-	t
Antillesoma antillarum	**DNA100390_7**	**X**	**X**	**X**	**30**	**u**
Antillesoma antillarum	**DNA100390_8**	**X**	**X**	**X**	**31**	**u**
Antillesoma antillarum	**DNA100390_9**	**X**	**X**	**X**	**32**	**t**
Antillesoma antillarum	**DNA100390_10**	**X**	**X**	**X**	**33**	**t**
Antillesoma antillarum	**DNA100390_11**	**X**	**X**	**X**	**34**	**u**
Antillesoma antillarum	**DNA100390_12**	**X**	**X**	**X**	**35**	**t**
Antillesoma mexicanum	BMACGANE00060	X			36	-
Antillesoma mexicanum	MK036430_1	X			37	-
Antillesoma mexicanum	MK036431_1	X			38	-
Antillesoma mexicanum	**A294**	**X**	**X**	**X**	**39**	**v**
Outgroups						
Golfingiida						
Golfingia elongata	DNA101003	DQ300123.1	JN864985.1	JN865156.1	-	-
Sipunculidae						

Sipunculus nudus	DNA 101882	JN865108	JN865000.1	JN865128	-	-

CONTINUATION

TABLE 5

					Haplotype name	
Species	**DNA code**	**CO1**	**16S rRNA**	**H3**	CO1	16S rRI
Aspidosiphonidae						
Aspidosiphon albus	DNA 101017	DQ300105	JN864990.1	DQ300053	-	-
Phascolosomatidae						
Phascolosoma perlucens	DNA100395	GU190249	GU190305	DQ300082	-	-
Siphonosomatidae						
Siphonosoma cumanense	DNA100464	DQ300155	JN864971	DQ300088	-	-

Numbers in color show shared haplotypes in COI and 16S rRNA (COI: haplotype 4 in red, haplotype 7 in pink, haplotype 13 in blue, haplotype 26 in orange; 16S rRNA: haplotype "t" in purple, haplotype "d" in dark red, haplotype "u" in green, and haplotype "e" in light blue).

Table 6. Demography parameters and standard deviations for each lineage of *Antillesoma*.

Marker	Colections sites	N	N_h	h	S	π	k
CO1	TH	8	8	1 ± 0,063	18	0,0124± 0,001	6,714±0,0061
	WA	33	27	0,983 ± 0,014	42	0,01354± 0,006	7,326±-0,00156
	EP	4	4	1 ± 0,031	13	0,01263±0,001	6,833±0,0002
16S rRNA	TH	6	4	0,8667 ± 0,023	4	0,00345±0,000018	1,533±0,5663
	WA	24	18	0,942 ± 0,040	20	0,0081±0.00004	0,00810±0,01204

Collections sites: TH (Thailand), WA (Western Atlantic), EP (Eastern Pacific). N = number of sampled individuals, N_h = number of haplotypes, h = haplotypic diversity, S = number of polymorphic sites, π = nucleotide diversity, k = mean number of pairwise differences. Significant values ($P < 0.05$) indicated in bold type.

Table 7. CO1 F_{st} values for *Antillesoma* lineages.

	TH	EP
TH	0	
EP	**0,92542**	0
WA	**0,92639**	**0,91375**

TH Thailand, WA Western Atlantic, EP Eastern Pacific. Significant values ($P < 0.05$) indicated in bold type.

Table 8. F_{st} values for Western Atlantic populations.

	Caribe	Florida	Texas
Caribe	0		
Florida	0,05234	0	
Texas	**0,77379**	0,73711	0
Brazil	0,06485	0,11326	**0,74173**

Significant values ($P < 0.05$) indicated in bold type.

Table 9. Genetic distance Kimura-2 parameters between *Antillesoma* lineages.

	TH	WA
TH	0	
WA	0,209669	0
EP	0,196128	0,181399

TH Thailand, WA Western Atlantic, EP Eastern Pacific.

Table 10. Western Atlantic populations genetic distance Kimura-2 parameters.

	Caribe	Florida	Texas
Caribe	0		
Florida	0,009113	0	
Texas	0,047563	0,045610	0
Brazil	0,011261	0,008425	0,048960

Table 11. Number of individuals analyzed for each morphological character.

Location	Trunk length	Introvert/trunk	Anus/trunk	Nephridium/ trunk	Nephrideopore/ trunk	Nephridia attachment	Anastomosis/ trunk	Introvert Retractor	C1	C2	C3
Brazil	44	5	44	31	7	25	22	31	34	33	33
Caribe	24	14	23	19	14	12	15	12	18	14	13
Texas	4	2	3	3	2	3	2	3	2	2	1
Eastern Pacific	5	4	6	5	3	3	4	3	5	3	2
Thailand	12	7	12	11	5	10	9	9	11	7	12

Table. 12. Mean and standard deviation ($\bar{x}$ ± SD) of morphological measurements with minimum and maximum values.

Location	Trunk length		Introvert/trunk		Anus/trunk		Nephridium/ trunk		Nephrideoopore/ trunk		Nephridia attachment		Anastomosis/ trunk		Introvert Retractor	
	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max	$\bar{x}$ ± SD	Min–Max
Brazil	2.5±1.1cm	0,9–5,2 cm	0.29±0.04	0,27–0,35	0.91±004	0,8–0,9	0.64±0.18	0.24–1	0.92±0.04	0.85–0.98	0.83±0.06	0.7–0.9	0.56±0.16	0.16–0.8	0.34±0.07	0.2–0.5
Caribe	2.3±0.5cm	0.8–3.2 cm	0.32±0.04	0.25–0.38	0.94±0.02	0.88–0.98	0.75±0.13	0.55–1	0.87±0.05	0.75–0.93	0.90±0.04	0.78–0.94	0,56±0.09	0.36–0.67	0.33±0.05	0.28–0.47
Texas	2.8± 0.21cm	1.2–3 cm	0.30±0.04	0.26–0.33	0.87±0.04	0.8–0.9	0.80±0.12	0.65–0.88	0.91±0.02	0.90–0.92	0.88±0.001	0.88	0.52±0.03	0.5–0.5	0.35±0.01	0.33–0.37
Eastern Pacific	0.9±0.23cm	0.6–1.2 cm	0.27±0.02	0.25–0.30	0.89±0.04	0.8–0.96	0.59±0.10	0.5–0.75	0.87±0.03	0.83–0.89	1	1	0.54±0.14	0.3–0.65	0.35±0.004	0.36–0.37
Thailand	1.6±0.3cm	1.2–2.1 cm	0.15± 007	0.11–0.21	0.90±0.04	0.8–0.95	0.61±0.13	0.35–0.83	0.82±0.11	0.64–0.93	0.85 ± 0.04	0.78–0.91	0.57±0.09	0.45–0.72	0.39±0.12	0.23–0.58

Table 13. Longitudinal Muscle bands minimum and maximum counts (C).

Location	C1	C2	C3
Brazil	12–23	21–36	19–35
Caribe	13–19	18–35	16–33
Texas	15	20	24
Eastern Pacific	13–18	24–30	27–28
Thailand	13–15	18–32	18–25

Table 14. Mean and standard deviation ($\bar{x}$ ± SD) of contracted and relaxed trunk papillae area (in cm²).

Location	Contracted				Relaxed			
	N	Anterior	Mid	Posterior	N	Anterior	Mid	Posterior
Barbados	5	0.0002± 0,00001 (npap=270)	0.00012± 0.0007 (npap=253)	0.00025± 0,00001 (npap=245)	5	0.0001± 0,0001 (npap=394)	0.00007 ±0,000062 (npap=466)	0.00016 ± 0,00001 (npap=352)
Brazil	3	0,00017 ±0,00005 (npap=120)	0.00005± 0,00002 (npap=80)	0,00009± 0,000055 (npap=139)	3	0.0002± 0.0001 (npap=85)	0.00020± 0.0001 (npap=62)	0.0001± 0,00005 (npap=140)

N= number of analyzed individuals; npap= number of analyzed papillae.

Table 15. Mean and standard deviation ($\bar{x}$ ± SD) of contracted trunk papillae area (in cm²) across locations.

Location	N	Anterior	Mid	Posterior
Brazil	3	0,00017±0,00005 (npap=120)	0,00008±0,00006 (npap=118)	0,00009±0.0005 (npap=139)
Caribe	7	0.0002083±0,00001(npap=464)	0,00011±0,00007 (npap=417)	0,0002±0.0005 (npap=386)
Texas	2	0,0002002±0,00005(npap=71)	0,0001± 0,00003 (npap=35	0,00027±0,0001(npap=41)
Eastern Pacific	2	0,0001727± 0,00008(npap=23)	0.00008±0,00002 (npap=21)	0,0001±0.00003 (npap=31)
Thailand	3	0,00016± 0,00005 (npap=279)	0.00006±0.00002 (npap=243)	0,0001±0.00002 (npap=225)

N= number of analyzed individuals; npap= number of analyzed papillae.

9 SUPLEMENTAR MATERIAL

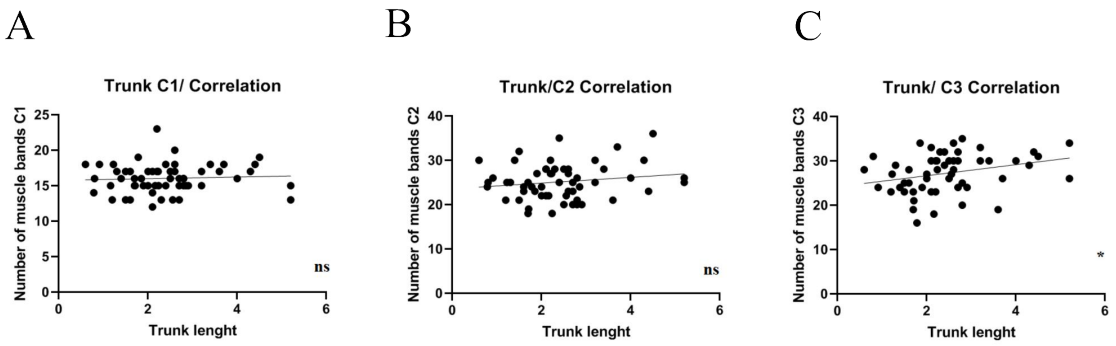

Figure 1. **Morphological correlation analysis**. (A) Correlation between LMBs from anterior region and trunk length (N=57). (B) Correlation between LMBs from introvert base and trunk length (N=56). (C) Correlation between LMBs from posterior region and trunk length (N=54).

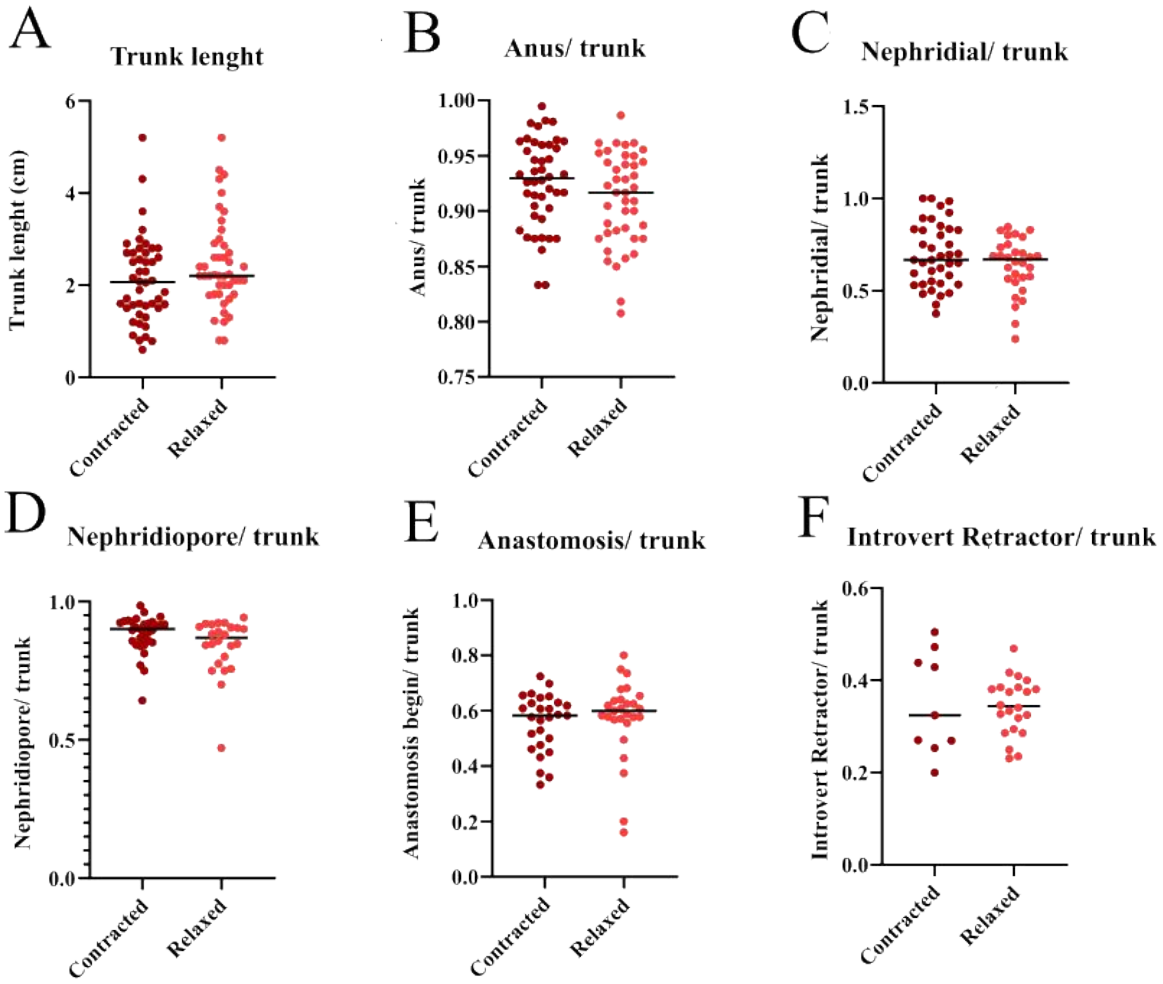

Figure 2. Body states significance within (A) Trunk length (contracted N =46; relaxed N=45). (B) Anus and trunk ratio(contracted N =44; relaxed N=42). (C) Nephridial and trunk ratio(contracted N =39; relaxed N=30). (D) Nephridiopore and trunk ratio (contracted N =31; relaxed N=24). (E) Anastomosis begin and trunk ratio (contracted N =27; relaxed N=27). (F) Introvert Retractor and trunk ratio(contracted N =9; relaxed N=21). This analysis does not show significant differences ($P < 0.05$) between the contracted and relaxed states.

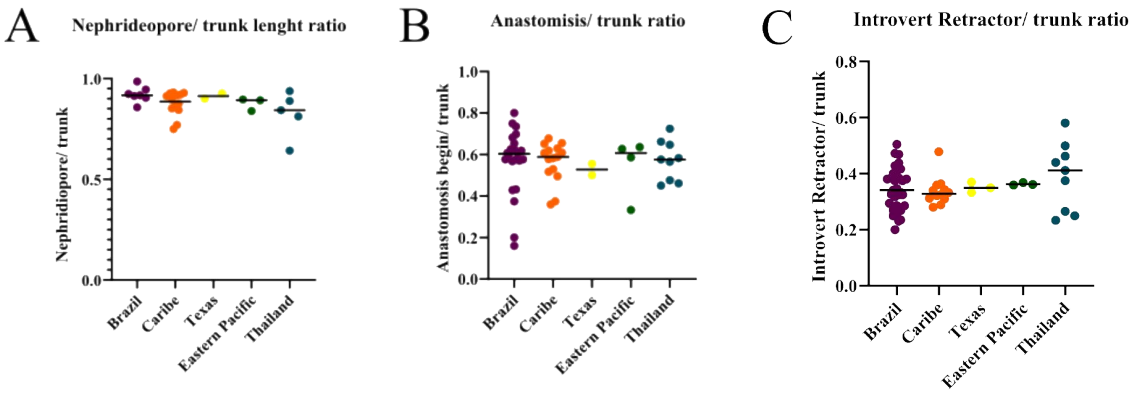

Figure 3. **Statistical morphological analysis between populations.** (A) Nephridiopore and trunk ratio; (B) Anastomosis begin and Trunk ratio; (C) Introvert retractor and trunk ratio. This analysis does not show significant differences ($P < 0.05$) between populations.

Table 1. CO1 haplotype distributions

		Location											
Lineage	Haplotype	1	2	3	4	5	6	7	8	9	10	11	12
1	1	1											
	2	1											
	3	1											
	4		1				3						
	5		1										
	6			1									
	7			1		1							
	8			1									
	9			1									
	10			1									
	11				1								
	12						1						
	13						2						
	14						1						
	15						11						
	16						1						
	17						1						
	18						1						
	19						1						
	20						1						
	21						1						
	22						1						
	23						1						
	24							1					
	25								1				
	26								1	1			
	27									1			
2	36											1	
	37												1
	38												1
	39												1
3	28										1		
	29										1		
	30										1		

CONTINUATION Table 1

Lineage	Haplotype	Location											
		1	2	3	4	5	6	7	8	9	10	11	12
3	31										1		
	32										1		
	33										1		
	34										1		
	35										1		

Location: 1 Texas, USA, 2 Florida, USA, 3 Bahamas, 4 Belize, 5 Venezuela, 6 Barbados, 7 Pernambuco, Brazil, 8 Bahia, Brazil, 9 Trindade, Brazil, 10 Thailand, 11 Guanacaste, Costa Rica, 12 Oaxaca, Mexico

Table 2. 16S rRNA haplotype distributions

		Locations									
Lineage	Haplotype	1	2	3	4	5	6	7	8	9	10
	a	1									
	b	1									
	c	1									
	d		2	1			3				
	e		1		1						
	f			1							
	g			1		1					
	h			1							
	i						1				
1	j						1				
	k						1				
	l						1				
	m						1				
	n						1				
	o						1				
	p										
	q							1			
	r						1		1		
2	v										1
	s									1	
3	t									5	
	u									3	

Location: 1 Texas, USA, 2 Florida, USA, 3 Bahamas, 4 Belize, 5 Venezuela, 6 Barbados, 7 Bahia, Brazil, 8 Trindade, Brazil, 9 Thailand, 10 Oaxaca, Mexico.

5 CONSIDERAÇÕES FINAIS

Em conclusão, este estudo apresenta uma revisão abrangente sobre a distribuição da espécie *Antillesoma antillarum* no Brasil, revelando novas ocorrências em três estados brasileiros (Alagoas, Bahia e Espírito Santo). Nossos resultados instigam a reflexão acerca do limite sul dessa espécie no Atlântico Ocidental, sublinhando a importância de investigar os fatores que influenciam sua distribuição. Ademais, ressalta-se a relevância e a necessidade das coleções científicas biológicas, sendo grande parte do material utilizado oriundo de museus e coleções.

Os resultados desta dissertação revelam a presença de espécies pseudo-crípticas em *Antillesoma antillarum*, um cenário em que o suposto status cosmopolita mascara uma diversidade de complexos de espécies. Esse fato é evidenciado pela identificação morfológica e molecular de duas linhagens distintas dentro de *A. antillarum*: uma linhagem amplamente distribuída pelo Atlântico Ocidental (desde a Flórida, EUA, até o Espírito Santo, Brasil) e outra na Tailândia. Além disso, o estudo não apenas corrobora, mas também salienta novas características morfológicas que possibilitam a distinção entre *A. mexicanum* e *A. antillarum*.

Adicionalmente, este trabalho esclarece a análise de certos caracteres morfológicos em *Antillesoma*, revelando padrões de correlação e a influência do estado de relaxamento na análise desses caracteres. Isso destaca a necessidade de investigações semelhantes para outros gêneros em Sipuncula, visando tornar a taxonomia do grupo menos subjetiva.

Diante das limitações deste estudo, torna-se imperativo incluir populações intermediárias entre as linhagens do Atlântico Ocidental e Tailândia, bem como realizar o sequenciamento e análise de outros marcadores moleculares. Nesse sentido, ressalta-se a importância da análise de holótipos e sintipos para possibilitar a descrição de novas espécies dentro da família Antillesomatidae.

Em síntese, este estudo representa o ponto de partida para uma investigação mais profunda, levantando questões essenciais: A população do Texas, EUA, está realmente conectada às demais populações do Atlântico Ocidental? Como ocorrem as conexões e dispersões entre as distintas populações no Atlântico Ocidental? Qual é a dinâmica genética dessas populações diversas? Quais os limites de distribuição de *A. antillarum* no Brasil e quais fatores influenciam isso? Qual é a extensão da distribuição de *A. mexicanum* no Pacífico Oriental? Existem características morfológicas adicionais capazes de distinguir

espécies dentro do gênero *Antillesoma*? As distintas características apresentadas neste trabalho podem ser observadas em um conjunto mais amplo de amostras? Qual é a amplitude de variação nos caracteres observados ao incorporar mais amostras de *A. mexicanum* e da linhagem tailandesa? Além disso, de que maneira fatores ambientais, como o tipo de substrato, influenciam características como o comprimento do tronco e a forma e tamanho das papilas do tronco, e qual é a extensão dessa influência? Nesse contexto, muitas indagações acerca do gênero *Antillesoma* ainda necessitam ser respondidas.